Immunointervention in Autoimmune Diseases

Papers based on an international meeting in Paris, France, in June 1988

Edited by

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Introduction

Some years ago, I was asked to present before the French Academy of Sciences—l'Académie des Sciences—the concept of autoimmunity. I explained why my laboratory's work on graft rejection quite naturally led us to take an interest in autoimmune diseases. Autoimmunity, I said, means that one of our tissues or organs is mistakenly regarded by our immunological system as an allograft. Hence the hope of mastering these diseases with the same immunosuppressive techniques which are so effective in preventing graft rejection. I was wrong. Immunosuppression is far less effective in curing autoimmune disease than it is in the practice of organ transplantation. The question is: can we now explain this difference?

This question arises in the context of an everlasting discussion: what is the primary cause of an autoimmune disease, especially in the case of organ- or tissue-specific autoimmunity? Is it an abnormal antigenicity of the target, with a normal immune response similar to that induced by an allograft? Is it, on the contrary, an abnormal immune response to a normal target, in other words a primary immunological defect? This was, I believe, the central question raised in this meeting. Data have been presented in favour of one or the other assumption.

On the one hand, evidence for modified autoantigens has been clearly obtained in some models. Abnormal immunoglobulin with an irregular glycosylation pattern has been found in patients with rheumatoid arthritis [1]. An abnormal cell membrane protein has been found in mice or patients with lupus [2]. Abnormal expression of Class II HLA antigens has been described in patients with Grave's disease or other endocrine autoimmune disorders [3]. The role of genetically-determined alterations of the target organ has been shown in the autoimmune thyroiditis of obese strain chickens [4]. And this list is not exhaustive.

On the other hand, evidence for an innate imbalance of the immune system is now demonstrated in a large number of cases. The fact alone that patients with particular HLA patterns are more likely to develop autoimmune diseases than other subjects, as proved by Jean Dausset and others, suggests that they may have an immune response different from that of the general population. And we now have much more specific evidence of abnormal balance of T or B cells in animal models and patients. A loss of suppressor T cells has been found in lupus mice [5] and in patients with myasthenia gravis and other autoimmune diseases. [6]. Abnormal thymus function is proved in several models [7]. Autoimmune diabetes is prevented in BB rats or NOD mice by transfusing normal T cells [8], while lymphocytes from Type I diabetic patients suppress insulin release from normal pancreatic cells [9]. Many papers presented during this meeting add other pieces of evidence to support the idea that an abnormal immunological system is the primary cause of most autoimmune diseases.

Finally there is no reason to reject a third assumption, that the genetic anomaly could theoretically concern the couple formed by the target and the corresponding immune response.

From a practical viewpoint these various possibilities imply quite different expectations. If an abnormal organ or tissue autoantigen is responsible for the disease, there is little hope that we may ever change its antigenicity. Let us, on the contrary, consider the other possibility: that is, a primary immunological defect. This would elucidate perfectly the question raised at the beginning of this introduction: why do immunosuppressive agents less regularly cure autoimmune diseases than they prevent allograft rejection? When we treat a graft recipient, we treat a subject whose immune system is normal or subnormal. Some uremic patients are spontaneously immunodepressed, but there is no innate abnormality of their immune system. If the immune response is abnormal in autoimmune diseases, we should expect to be less effective when we give immunosuppressive agents since we do not correct the primary imbalance of their immune system. All our efforts should then be directed toward a better understanding of that primary immune defect, so that we might be able to correct it and restore a normal immune response preventing the effect of the autoaggressive reaction. This would be the price to pay in order to acquire a final cure for autoimmune diseases. The papers in this supplement will probably allow us to make some progress along this difficult road.

JEAN HAMBURGER

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The Role of Antigen in Autoimmune Responses with Special Reference to Changes in Carbohydrate Structure of IgG in Rheumatoid Arthritis

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Evidence indicating an important role for antigen in the provocation of autoimmune responses is presented. Attention is especially focused on carbohydrate abnormalities in IgG in rheumatoid arthritis, since autosensitization to this molecule is thought to be of central importance in the pathogenesis of this disease.

A higher percentage of Fcγ oligosaccharide chains in the serum IgG of patients with rheumatoid arthritis lack terminal galactose residues relative to age-matched controls. This does not appear to be a characteristic feature of chronic inflammatory diseases in general. A new, more rapid assay for agalactosyl chains is described and shown to give results comparable to the more conventional biochemical analysis. The defect probably arises from a reduction in activity of B-cell galactosyltransferases. The galactose changes may contribute to the autoantigenicity of IgG and could facilitate the self-association of IgG rheumatoid factors.

Introduction

For many years, studies on the mechanisms underlying the development of autoimmune diseases were directed largely at the identification of abnormalities in the immune response. Recently, however, there has been a greater awareness of the possible role played by antigen and its presentation (see for example Roitt, [1]).

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Thus, the importance of thyroid antigen in the induction of the autoantithyroglobulin response in obese strain chickens was shown clearly by the effects of neonatal thyroidectomy [2]. We have also reported on the relevance of epitopes dependent upon iodination of thyroglobulin in creating pathogenetic T-cell effectors [3]. Furthermore, it is difficult to avoid linking the particular clusters of antibodies associated with specific diseases (e.g. anti-DNA and Sm in SLE, anti-SS-A and SS-B with Sjögren's syndrome and so on) with some antigen-directed process rather than simply a non-antigen-specific polyclonal activation mechanism. Although a variety of IgM autoantibodies appear to be produced spontaneously in normal individuals [4], it is unlikely that the high affinity IgG autoantibodies characteristic of most autoimmune disorders could be generated without an antigen-driven response involving somatic mutation and selection.

The other area of research in this field which has burgeoned over the last few years concerns the role of Class II MHC expression. The nature of T-cell recognition implies that potential autoantigens on the surface of cells which do not express Class II will be unable to stimulate the corresponding autoreactive T cells. Thus, the finding that thyroid cells from patients with thyrotoxicosis 'inappropriately' expressed Class II molecules on their surface [5] was most intriguing and stimulated a great deal of interest. It was shown subsequently that the T cell lymphokine γ-interferon could induce Class II expression on normal thyroid cells [6] and on the pancreatic β-cells of diabetes-prone but not the diabetes-resistant strain of BB rat [7]. These findings raise the still unresolved question of whether Class II expression is a prerequisite for the induction of autoimmunity or is restricted to a role in maintaining the pathogenetic effect of the autoimmune response.

The present paper focuses on abnormal features of the antigen generally recognized to be of central importance in provoking the immunological reactivity ultimately responsible for joint erosion in rheumatoid arthritis, namely IgG itself.

Serum IgG in patients with rheumatoid arthritis (RA) shows defective N-glycosylation

The oligosaccharides in the Fc region of IgG are N-linked and predominantly have the structures shown in Figure 1, each sugar having either two terminal galactose residues [G(2)], one galactose and one N-acetylglucosamine (GlcNAc) [G(1)], or two terminal GlcNAcs [G(0)] (Figure 1). The two oligosaccharides, one originating from each Cγ2 domain, form a bridge to hold the Cγ2 domains apart as shown in Figure 2, while the terminal galactose residues on the α (1–6) arms sit in a pocket on the surface of each domain. In 1985 [8] the striking finding was reported that the oligosaccharides in the IgG from patients with RA were deficient in galactose, with an increase in structures of the G(0) type. Later studies with a further group of patients, confirmed these findings, taking into account the natural change in IgG galactosylation with age [9]. It was also found that a similar defect occurred in the IgG of patients with juvenile RA so strengthening the view [10] that both juvenile and adult forms shared common pathogenetic mechanisms. Within the rheumatological disorders, the defect showed quite considerable disease specificity. For example, primary systemic lupus erythematosus (SLE), primary Sjögren's disease, ankylosing spondylitis and polymyositis, had normally galactosylated IgG oligosaccharide structures. Only in

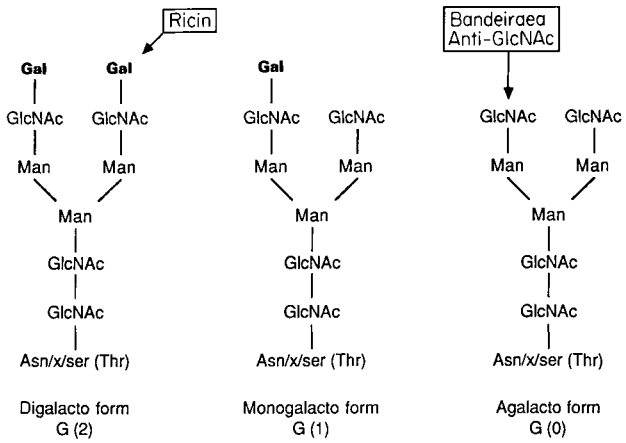

Figure 1. The predominant IgG Fc oligosaccharides. Gal, galactose; GlcNAc, N-acetylglucosamine; Man, mannose. Terminal galactose residues bind the lectin ricin and N-acetylglucosamine the monoclonal antibody or the lectin bandeiraea.

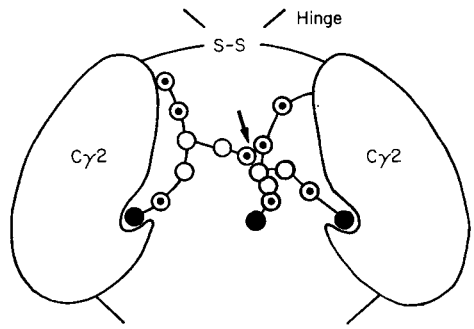

Figure 2. IgG structures. The second heavy chain domains in IgG (Cγ2) are kept apart by the two N-linked biantennary sugars. One of the α (1–3) arms must terminate in an N-acetylglucosamine to form a bridge (arrowed) with the oligosaccharide on the opposing Cγ2 domain. The terminal galactose residues on the α (1–6) arms fit snugly into pockets on the protein domains as shown. ○, Mannose; ⊙, N-acetylglucosamine; ●, Galactose.

the case of secondary Sjögren's disease with SLE was defective galactosylation similar to that in RA observed.

Of the chronic inflammatory disorders studied, the majority of chronic infections, such as leprosy and klebsiella were normal. Quite surprisingly, however, patients with pulmonary tuberculosis (TB) had galactose defects similar to those seen in RA.

A new assay for agalactosyl-IgG

Since the strict biochemical analysis of the structure of the IgG sugars is time-consuming and sophisticated, we have developed a new, more simplified assay which gives an approximate measure of the degree of galactosylation. Purified IgG is dot-blotted onto nitrocellulose and on separate aliquots of the same sample, probes for terminal galactose and terminal GlcNAc are applied. Biotinylated ricin is used to

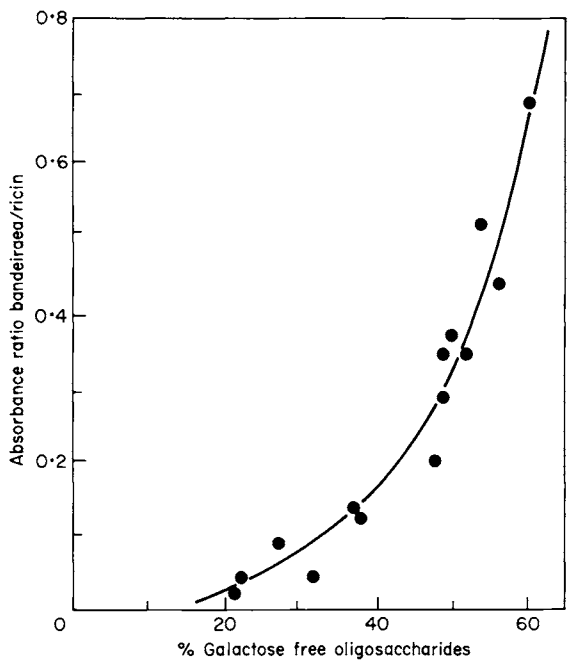

Figure 3. Correlation of assay ratios with G(0). Correlation of the ratio of bandeiraea/ricin using the dot blot assay against the percentage of galactose-free chains in IgG obtained by the biochemical method.

detect galactose, and either biotinylated bandeiraea lectin or monoclonal anti-GlcNAc are employed for the detection of terminal N-acetylglucosamine. The blots are developed by addition of streptavidin horseradish peroxidase conjugates and the colour reaction formed on the nitrocellulose. The colour intensity is recorded by transmitted colorimetry, and the ratio of the two stains gives a measure of the galactose: N-acetylglucosamine ratio. This ratio correlates closely with the percentage of galactose-free chains [G(0)] determined by conventional biochemical techniques (Figure 3).

This faster assay gives comparable results, as may be seen from measurements made on the serum IgG from patients with RA (Figure 4). The majority of the IgG galactose-free values lie above the mean ± 2 standard deviations for the age-matched controls. The few sera which lie within the normal range are from patients with inactive disease. The elevated incidence of [G(0)] in patients with pulmonary TB is confirmed and may be clearly seen in Figure 5.

Preliminary studies on the families of patients with RA indicated a clustering of relatives with the galactose defect in two out of seven such families. In one of these cases the unrelated spouse had an elevated galactose-free value. Further studies clearly are required to see whether this finding can be substantiated.

Implications of this structural galactose defect

The ability of IgG rheumatoid factors to form self-associated complexes capable of stimulating the chronic inflammatory change giving rise to the erosive pannus in RA,

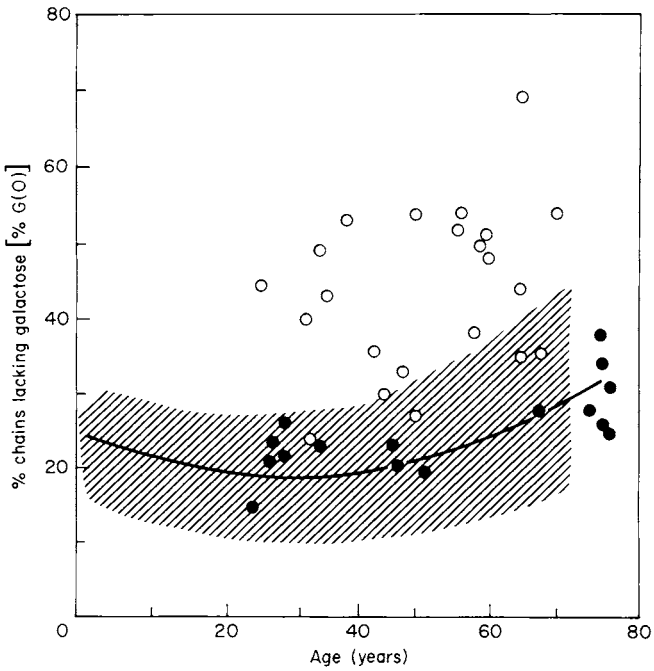

Figure 4. Analysis of RA using new assay. Galactose defect in IgG patients with rheumatoid arthritis analysed by the dot blot assay. The percentage G(0) values are plotted against age. The outer bounds of the hatched lines depict the regression functions for ± 2 (SD) of the mean. ●, controls; ○, rheumatoid patients.

has long been recognized. While it has been assumed generally that the binding between the Fab on one rheumatoid factor molecule, and the Fc of another, was due to conventional antigen/antibody links based upon the hypervariable region of the combining site, the present studies suggest a further possible mechanism by which the self-association could be strengthened. Since it has been established that the Fab oligosaccharides which occur on approximately one in three different immunoglobulin molecules, are not defective with respect to glycosylation in RA, a Fab galactose region could become inserted in the Fc pocket left vacant by a galactose-deficient $C\gamma 2$ oligosaccharide (Figure 6).

This suggests a new possibility for therapeutic intervention, since if the carbohydrate moieties contribute significantly to the formation of the self-associated complex, then it might be possible using the appropriate sugars, to interfere with aggregate formation. Early experiments have been encouraging in this respect.

The defect may arise from low activity of galactosyltransferase

In order to examine the possibility that the change in N-glycosylation of serum IgG in RA was the result of impaired activity of the galactosyltransferase enzyme responsible for addition of the galactose residue to terminal N-acetylglucosamine, we measured the activity of this enzyme in peripheral B- and T-lymphocytes of patients with RA, using ovalbumin as the receptor glycoprotein [11]. Enzyme activity in B

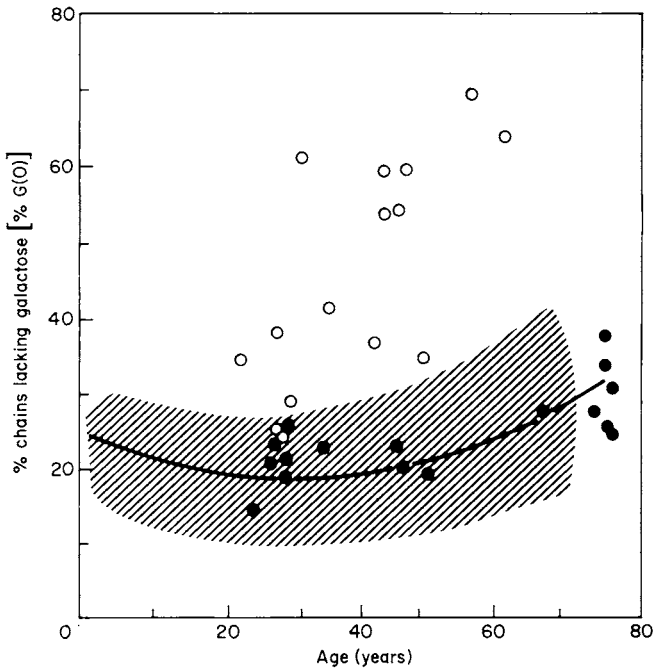

Figure 5. Analysis of pulmonary tuberculosis using new assay. Galactose defect in IgG patients with tuberculosis analysed by the dot blot assay. The percentage G(0) values are plotted against age. The outer bounds of the hatched lines depict the regression functions for ± 2 (SD) of the mean ●, controls; ○, patients with tuberculosis.

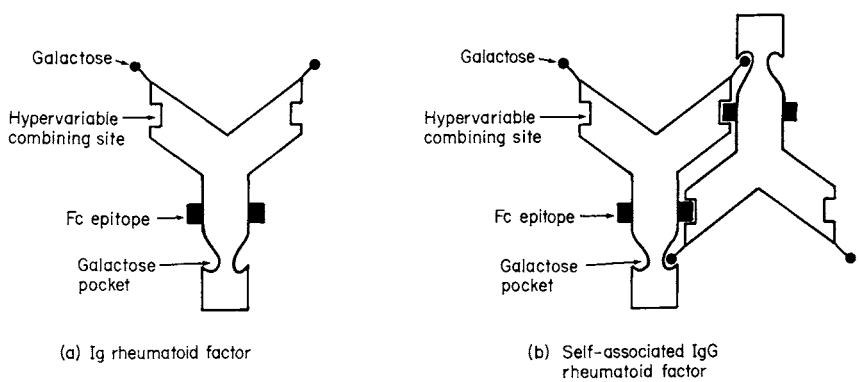

Figure 6. Self-association of IgG rheumatoid factor. (a) Ig rheumatoid factor; (b) Self-associated IgG rheumatoid factor.

cells from patients was considerably lower than that observed in control subjects using ovalbumin and a variety of other high molecular weight glycoprotein receptors as well as GlcNAc itself. A lesser but still significant diminution in activity was seen in the T cells (Figure 7). We have mentioned earlier that the IgG from untreated pulmonary TB subjects had raised G(0) levels, and it was of considerable interest that both B- and T-lymphocytes from the peripheral blood of such patients showed

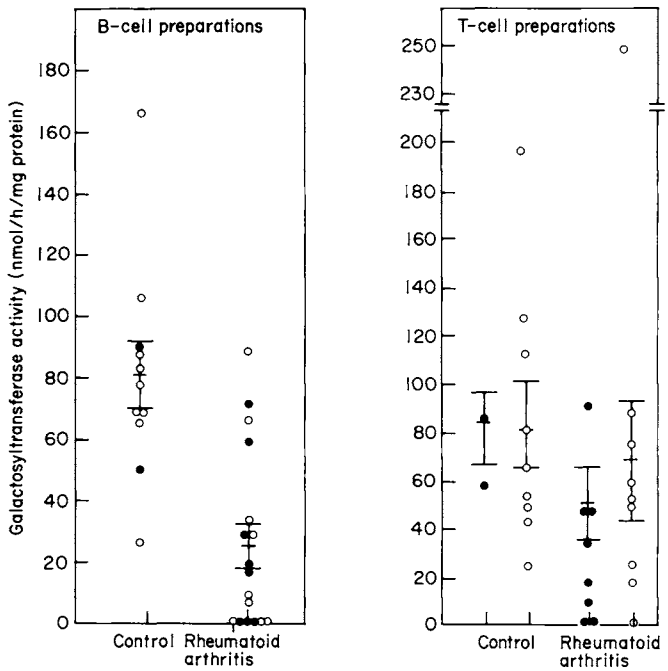

Figure 7. Reduced galactosyltransferase activity in T- and B-lymphocytes from patients with rheumatoid arthritis. ○, age-matched; ●, not age-matched. For T-cell preparations, control means (SEM) are $n = 9$ for ○ and on left $n = 11$ for ○ + ●; and rheumatoid arthritis means are $n = 9$ for ○ and on left $n = 17$ for ○ + ●.

grossly lowered galactosyltransferase activity. It has been postulated that Crohn's disease is mediated by some abnormal type of mycobacteria, and it may thus be relevant to note that in the patients with active as distinct from inactive Crohn's disease, serum IgG shows a raised G(0), and peripheral blood B cells and T cells a lowered galactosyltransferase.

Conclusions

Similarities in galactose deficiency in the IgG from patients with RA and primary TB, point rather tantalizingly to some possible connection between an infective agent and RA. Even more provocative in this respect, is the production of adjuvant arthritis in rats by T-cell clones sensitized to the 65 kD protein of *Mycobacterium tuberculosis*. This molecule belongs to the group of substances collectively termed 'heat shock proteins'. Should injection of such clones induce changes in the galactosylation of serum IgG, one's interest must turn to the possibility that interaction of homologous human heat shock proteins with the immune system (perhaps as a result of a cross-reaction induced by sensitization with some microbial product), might trigger the IgG galactose defect so leading to IgG autosensitization and possibly the train of pathogenic events which lead finally to joint erosion. Recent results by Peter Lydyard and his colleagues (Tsoulfa *et al.* in preparation) confirmed by a study of a further group of patients in Kuwait (Barr *et al.* in preparation) suggest that patients

with RA have abnormally high levels of IgG and IgA antibodies to heat shock proteins of human as well as mycobacterial origin. These new insights into the puzzling nature of RA should provide exciting prospects for future study.

Acknowledgements

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Autoimmunity: the Moving Boundaries Between Physiology and Pathology

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This paper considers current concepts of autoimmunity and concludes with a discussion on the need for viable alternatives. It is argued that, if a century of 'horror autotoxicus' and over 30 years of active research based on 'clonal deletion' models have failed to contribute solutions to the problem, these notions are probably inadequate. Instead, it is proposed that pathological states of autoimmunity should be considered as deviations from normal autoreactivity which is a central property of the immune system. It follows that the study of autoimmune *physiology* is necessary to the understanding of *pathology*. Furthermore, the discrimination between destructive immune responses and physiological, self-directed immune activities is thought to be a systemic property based on a particular network organization, rather than the result of isolated clonal properties. These views suggest novel strategies in basic and clinical approaches to autoimmunity, more particularly the possibility of manipulating physiological autoreactivity to compensate diseases which are not of immunological origin.

Introduction

In a paper dedicated to concepts in autoimmunity, we should perhaps start with a brief consideration of current theoretical frameworks and an evaluation of their impact on the management of autoimmune diseases. This is, indeed, one of the attractive aspects of autoimmunity; aside from their interest for the fundamentalist as 'experiments of nature' that may reveal basic rules of physiology, autoimmune diseases also offer the investigator a 'problem-solving' test. This is probably more immediately rewarding for scientists than producing models to solve problems that they have, themselves, generated. Having said that, we should recall the words of

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Jacobi in a letter written in French to Legendre, where he expresses his opinion on Fourier: '. . . mais un philosophe comme lui aurait dû savoir que le but unique de la Science, c'est l'honneur de l'esprit humain, et que sous ce titre, une question de nombres (or autoimmunity) vaut autant qu'une question du système du monde'.

We were asked to deal with the question of normal versus pathological autoreactivity. We shall not address this topic lightly without referring the reader to the general problems pertaining to the definition of the limits between health and disease, physiology and pathology, or to the classification of biological systems as normal or abnormal. The classic 'textbook' of Canguilhem constitutes a good introductory reading (G. Canguilhem: *Le normal et le pathologique*, PUF, 1966).

We should also express our convictions that, more often than not, progress in clinical medicine (including autoimmunity) is empirical and arises in the absence of conceptual frameworks or basic experimental information. Finally, our own models may well be as fallacious as 'clonal deletion'. They have, for us, only three advantages: they make sense, they are new, and, in contrast with the previous ones, they have not been tested. Why not, then, give them a chance?

Evaluation of prevalent concepts in autoimmunity: the central notion

Until recently, all models of autoimmunity were mere variations on a central concept nearly as old as Immunology, and have proved inadequate. In other areas of science, especially biology, revolutions over the last century have dislodged central notions and pointed to other directions. Not so in Immunology.

Originally described by Erlich as the 'horror autotoxicus' [1], this notion has essentially banned immune autoreactivity from normality. Given that immune activities are always destructive for the 'target' organism, they are unacceptable if directed at an autologous tissue or molecule, and mechanisms must exist to prevent such responses. Since such mechanisms cannot be genetic and vertebrates can make antibodies to any antigen whatsoever [2], inactivation of potential autoreactivities must take place along with the development of the individual and its immune system. By proposing a clonal basis to lymphocyte diversity, Burnet provided the final solution: elimination of all autoreactive cells during ontogeny [3]. Later, additions to this central notion only concerned details: keeping cells secluded (in the thymus) during this 'educational' period; applying the ontogeny idea to cells (produced throughout life) rather than to the whole individual; physically eliminating the lymphocytes or keeping them uselessly 'anergic'; attributing inactivation versus induction to differential affinity thresholds, or to different kinds of 'signals' and combinations thereof; considering direct elimination of the cells by antigen or their inactivation by antigen-specific suppressor cells. Not even technological progress managed to change the level of these discussions: today's controversy debates whether lymphocytes with autoreactive receptors are killed (a drastic form of autoimmune aggression) or whether they are only deprived of some other molecules that are necessary to their functions [4–7].

Criticism of these concepts and consideration of the basis for their establishment and maintenance in Immunology have been given before [8–10]. It suffices here to underline three aspects. The starting point in the argument might have been acceptable 100 years ago, but is certainly excluded today: not all immune activities are

necessarily destructive for their target organism. First of all on qualitative terms, and it is interesting to realize that every time non-destructive immune functions were discovered (e.g. 'facilitating' antibodies, suppressor cells), they were attributed some role in 'self-tolerance'. Next, on quantitative levels: growth hormones, insulin, acute-phase proteins or tumor necrosis factors are only destructive above some concentrations and in particular conditions. Moreover, deficits in the 'normal' range of concentrations of biologically active molecules are often more deleterious than their excesses if these can be removed and excreted.

Secondly, the idea that normal individuals do not mount anti-self immune responses is far more of a conviction than a conclusion derived from experimental observations. Throughout this century, the facility to elicit responses to autologous antigens has astonished many an investigator. The multiplicity of experimental models of autoimmune diseases, by demonstrating that normal individuals harbor this potential, provides an excellent argument to exclude solutions of 'self-tolerance' based on elimination of autoreactive cells.

Finally, as pointed out before [10], there is a confusion in the levels of organization and description: toxicity of molecules, cells, or systems are often, but unduly, interchanged. Oncogene products are not toxic and yet cancers often kill their hosts. All normal cells harbor and use oncogenes, but very rarely is a cell transformed.

Variations in clonal models of autoimmunity

With autoreactive clonal inactivity as the safety mechanism, pathological autoaggression has been explained by the failure of that mechanism in a variety of ways. We recall the main types here.

(1) One is alterations in the structure, availability, concentration, presentation or 'immunogenicity' of the autoantigen. These models invoke structural modifications of antigen by external agents (chemical or microbiological); release of sequestered antigens, after tissue destruction for example; responses to external antigens which cross-react with autologous structures; heterotopic expression of MHC Class II antigens, leading to the anomalous presentation of tissue-specific antigens. Even without getting into the very unclear situations of 'partial' and 'split' tolerance, these proposals are often contradictory to the very principle which originates them. One general modification in that principle has been quite widely adopted and assumes that self-tolerance is, essentially, a T-cell property while autoreactive B lymphocytes can be left to exist in normal individuals, given that autoantigens are T-cell dependent. Although necessary to accommodate some of the above examples, this proposition was only satisfactory in the frame of T-B cell cooperation schemes limited to 'linked' recognition of two determinants in the same molecule [11]. With the discovery of a variety of other modes of T-cell dependent B-cell activation [12] and of the T-cell ability to react with short peptide sequences processed from larger molecules by the B cell itself [13], this explanation is no longer satisfactory. Furthermore, in those models it is often not clear why a given precipitating cause does not *always* lead to the autoimmune reaction.

(2) This statement leads us to the second class of models. If there is nothing wrong with the autoantigen, there must be something special about the antibody (or T-cell

receptor). Deficient sensitivity to inactivation signals or hyperreactivity (to factors or antigens) have been invoked.

(3) With the discovery of suppressor T-cell activities and autoanti-idiotypic antibodies, mechanisms of a novel kind were proposed. Here, both antigen and antibody are normal but, instead, the suppressive mechanisms which normally limit autoreactivities are deficient. We are all aware of the slightly outdated fashion of counting T4/T8 ratios in the blood from all sorts of patients, and of the present abundance of reports that studied sera from autoimmune individuals for the presence (or absence) of anti-idiotypes to autoantibodies. These models, if superficially at variance with 'clonal deletion' (as they allow for the existence of immunocompetent autoreactive lymphocytes) may be included in the same class of proposals, as they consider that such specificities are suppressed in normal individuals. It makes little difference if the autoreactive cell is killed at birth or suppressed in the periphery: its activity is absent from normal individuals in both cases. Although these models are often associated with network, both the approaches and interpretations are almost without exception perfectly clonal: instead of looking for the wrong autoreactive clone, they simply look for the wrong (absent) autoanti-idiotypic clone.

This very brief description does not do justice to all the details and intricacies of both models and experimental systems (Ir-gene effects and multigenic controls, hormonal influences, particular tissue conditions, differences between organ-specific and 'generalized' autoimmunity, the role of molecules and mechanisms participating in effector phases of immune activities). Our intention was simply to point out that, to date, *most (or all) concepts of autoimmunity do have a clonal basis and continue to share the general view that activities of autoreactive lymphocytes are (or should be) absent from normal physiology*. We argue that such principles lead us nowhere. In spite of the multiplicity of experimental and clinical observations and the progress in analytical technology, *we continue to treat autoimmune diseases by non-specific immunosuppression:* corticoids, cyclosporin A, plasmapheresis, cytostatics, irradiation. We believe, therefore, that the time has come to try alternatives to those rather uniform points of view, particularly because other general ways of considering the operation in immune systems have been developed [e.g. 8, 34], and a number of observations with normal individuals radically oppose the classical concepts. These will now be analysed.

Autoreactivity is physiological and necessary to avoid pathology

We believe the most negative influence of clonal deletion principles to progress in autoimmunity has been the general idea that autoreactivity is always pathological and, therefore, that there is no physiology of autoreactivity to study. Clinicians and experimentalists have, consequently, been trying to understand pathology while ignoring the physiological basis of the phenomena. The lack of progress is thus not astonishing. Already Auguste Comte, referring to Broussais, has pointed out: 'l'état pathologique ne diffère point radicalement de l'état physiologique, à l'égard duquel il ne saurait constituer, sous un aspect quelconque, qu'un simple prolongement plus ou moins étendu des limites de variations, soit supérieures, soit inférieures propres à chaque phénomène de l'organisme normal, sans pouvoir jamais produire de

phénomènes vraiment nouveaux qui n'auraient point à un certain degré leurs analogues purement physiologiques'.

To study autoreactivity in normal individuals should, therefore, be our first concern, and it is stimulating to find that over the last 5 years or so, a large number of observations from many independent groups show the existence of circulating autoantibodies in normal animals and humans [e.g. 14–18]. The original claims of Avrameas and colleagues have been extensively confirmed, particularly by the analysis of the reactivities of hybridomas isolated from normal, unprimed individuals, and by techniques which reveal single cell secretion of antibodies [15–17]. The very high frequency of autoreactivities detected in limited panels of autoantigens strongly suggests that natural antibodies and natural plasma cells are for the most part (if not all) producing autoantibodies. These observations demonstrate not only that autoreactive B lymphocytes exist in normal individuals, but, most importantly, that these are positively selected and activated. Formal demonstration of these indications has been obtained by the clonal analysis of autoreactivities amongst small, resting lymphocytes or naturally-activated cells from normal donors. It was shown that for a variety of autoantigens (erythrocytes, Class II MHC antigens, thyroglobulin) the majority of, if not all, B cells producing autoreactive antibodies are naturally activated blast cells, many of which are in mitotic cycle [18; Pena Rossi *et al.* to be published]. It is interesting to point out here that the small lymphocyte compartment in normal individuals appears depleted of autoreactivities, which are not deleted from repertoires but rather positively selected and activated. This rule, however, does not seem to apply to all autoantibodies: small B cells with reactivities to DNA, IgG C-region and acetylcholine receptors are found in normal individuals [Pena Rossi and Pereira, unpublished; Sundblad, Poncet, personal communications].

A normal reaction to these findings is to question the affinity of these antibodies, current convictions being that for the most part natural autoantibodies are degenerate, low-affinity, cross-reactive molecules. Whenever measured, however, the avidities of these antibodies to their respective multivalent ligands have been found quite high, perfectly in the range of what is considered as a 'good' antibody [19]. Interestingly, many such natural autoantibodies show 'multireactivity', that is, the same monoclonal antibody binds to several, often many, structurally different antigens. This property must have a structural basis and, in fact, the analysis of large collections of hybridomas obtained from normal individuals quite sharply separates classes of reactivities rather than showing a continuous dispersed pattern of cross-reactivities [20; unpublished observations; J. Stewart *et al.* to be published]. Primary sequences of these antibodies, however, have revealed no distinctive characteristic when compared to conventional antibodies [S. Avrameas, D. Holmberg, personal communications]. From the point of view of lymphocyte physiology, however, this finding is not necessarily surprising. Thus, lymphocytes in the internal environment exposed to all sorts of autoantigens, will be simultaneously selected by all the ligands to which they are exposed. It follows that those which show degenerate binding to a high number of ligands will preferentially be activated. More experimentation in this area is necessary before understanding the significance of these findings.

The positive selection and natural activation of autoreactive lymphocytes is not limited to B cells. We shall not discuss the matter at length here, but simply recall the repeated observations in autologous mixed leukocyte reactions and the recent results

of Bandeira, Pereira and colleagues [to be published]. These authors have compared in normal individuals, small, resting CD4 or CD8 lymphocytes with their naturally-activated counterpart for their ability to directly help or suppress, respectively, B lymphocyte responses. When such T-cell populations were assayed on syngeneic versus allogeneic target B cells, they observed a clear bias of naturally activated T cells to interact with syngeneic targets, and a complete depletion of autoreactivities from the small lymphocyte pools. Furthermore, we have demonstrated that the natural activation (and expansion) of autoreactive B lymphocytes is T-cell dependent, at least in the particular systems analysed [21]. Most interestingly, the T cells from normal donors that could reconstitute positive selection and natural activation of autoreactive B cells when transferred into athymic nude mice, were already naturally activated in the donors, while small, resting lymphocytes of the same phenotype could not perform at all, or very poorly in the same conditions.

It seems, therefore, that normal individuals contain a compartment of activated lymphocytes harboring autoreactive T and B cells which are accordingly depleted from the small lymphocyte pools. There are direct indications that such a 'large lymphocyte' compartment, which constitutes 10–20% of all lymphocytes in the spleen, essentially represents autoreactivity. Thus, the number of lymphocytes in this compartment is comparable in normal, conventionally bred mice, in germ-free animals, and in 'antigen-free' donors [22–23]. Even if the precise identification of the autoreactivities pertaining to this set of clones remains to be done, these observations do indicate directly that 10–20% of all lymphocytes in a normal individual are activated by their exposure to the internal environment and, therefore, must represent the level of autoreactivities in normal immune systems. If all these results demonstrate the physiology of autoreactivity, they do not imply that such activities are necessary to escape pathological autoimmunity. This possibility is derived from several observations. The first concerns the finding that selective stimulation of clonal specificities present either in the 'small' or 'large' lymphocyte pools, revealed that conventional high-titered and rapid immune responses are exclusively obtained from the 'small' lymphocyte compartment [24]. For some reason, the activated lymphocyte compartment responds poorly and slowly when clonally stimulated *in vivo*, in spite of the fact that naturally activated B cells when isolated as clones *in vitro*, develop responses as good as individual, small lymphocytes. The second observation also pertains to the dynamics of antibody production in both sets. The kinetic analysis of *natural* antibody concentrations in the serum of normal individuals has revealed dynamic patterns which are a mixture of oscillations or even a 'chaotic' regime and is sharply different from immune response kinetics [25]. Again, for some reason, immune activities ongoing in the large lymphocyte compartment do not reproduce conventional immune responses, leading to the notion that bringing autoreactive cells into natural activation is actually a way to ensure that such specificities will not participate in immune responses with characteristics as we currently define them.

Physiological versus pathological autoreactivity are systemic (not clonal) properties

From the above observations we shall now proceed to discuss possible reasons why the abundant autoantibody reactivities are not toxic to normal individuals. This conclusion has been reinforced by clinical counterparts in the lack of correlation

between symptoms of disease and autoantibody titers. All clinicians know of seronegative rheumatoid arthritis, but even in the case of diseases which can be reproduced experimentally by transfer of autoantibodies, such as in myasthenia gravis, antibody concentration does not determine severity of the disease, and there are wide differences in clinical state amongst patients with the same total concentration of anti-receptor antibodies [26]. In view of all these observations, it is tempting to classify autoantibodies into two groups (pathogenic and non-pathogenic) and correlate pathology and physiology with the presence or absence of 'pathogenic' clones. This notion has been reinforced by the observations showing structural differences between rheumatoid factors isolated from normal mice or from autoimmune donors: while the first always show germ-line sequences, the latter very often appear with extensive somatic mutations [27–29]. The conclusion that disease is caused by such altered antibodies that have become pathogenic might be unwarranted, however. Thus, it can be argued that pathology and the appearance of such mutated antibodies are both the consequence of a primary perturbation in the physiological systemic organization of the immune system. In this particular example, such an alternative explanation is compatible with the extreme monoclonality of the autoantibodies isolated from sick individuals, as compared with the extreme diversity and polyclonality of those isolated from normal individuals. For the above classification, all antibodies isolated from normal individuals in the absence of previous stimulation should be non-pathogenic. We have recently initiated screenings of pathogenic activities of natural multireactive autoantibodies with anti-acetylcholine receptor activities [Sundblad and Jacquemart, unpublished observations]. These experiments have already revealed an interesting point in our discussion: one such antibody kills adult mice, if injected in sufficient amounts, but has no effect on newborn animals if injected in proportionally even higher doses. Clearly, therefore, natural autoantibodies can be toxic to the host, but toxicity obviously depends on the context in which they exist.

In the light of the above observations concerning the recruitment of autoreactivities into the naturally activated lymphocyte pool, it is tempting to speculate on other properties of these repertoires that may be at the origin of their lack of toxicity. One may be V-region connectivity. Thus, direct measurements of connectivity amongst antibodies produced by B lymphocytes that exist as small cells in normal adult mice, have revealed very low levels of connectivity, as compared to the 10- to 100-fold higher values obtained with naturally activated B lymphocytes in the perinatal period [30–31]. Although the high connectivity values for naturally activated B lymphocytes in adults remain to be confirmed, a considerable number of observations have demonstrated the V-region-directed processes of repertoire selection amongst activated B and T lymphocytes in the adult, as well as their absence from the small lymphocyte compartment [e.g. 32, see 9]. We hypothesize, therefore, that high V-region connectivity is an essential property of autoreactive lymphocytes of normal, adult individuals. Such connectivity may, on the one hand, limit their toxicity and, on the other, impose dynamic characteristics to this set of connected cells which, as seen above, excludes them from engaging in typical immune response dynamics with extensive clonal amplifications. We know indeed that naturally activated lymphocytes divide very little [33]. In turn, these dynamic characteristics also result in a very low probability of somatic mutation and alterations in the germ-line specificities

which could obviously rescue mutated clones from connectivity. The role of multireactive antibodies and T-cell receptors in the establishment of this naturally activated network might be relevant.

This hypothesis implies an inverse correlation between pathogenicity of autoreactivities and their level of connectivity. Computer simulations based on a model recently developed [34] and on empirical data for connectivity confirms this hypothesis [J. Stewart *et al.* to be published]. Furthermore, injection of one multireactive monoclonal antibody connected with several anti-acetylcholine receptor associated antibodies resulted in suppression of the anti-receptor antibody response upon subsequent immunization [Sundblad *et al.* submitted]. Most interestingly, the often spectacular therapeutic success of the injection of normal immunoglobulin at high dose in autoimmune disease may as well be explained by increased connectivity. Thus, such preparations are, after all, collections of normal natural antibodies and the work of Kazatchkine and colleagues has shown the reactions of a variety of autoantibodies from diseased donors with, in each case, a high proportion, of the normal immunoglobulin injected [35–36].

It is obvious to us that this area of research is very much in need of further experimentation and that efforts should be concentrated here. It proposes radically different alternatives to conventional wisdom in the treatment or prevention of autoimmune diseases, as it suggests, for example, that *autoreactive clones should be activated in the appropriate context rather than suppressed.* It should be pointed out, however, that connectivity should be considered in genuine network perspectives and not, as often happens, as linear relationships between clonal specificities. From the little information available, it is already quite clear that we deal here with a densely connected network where each clonal specificity interacts with a variety of antigenic ligands and of other antibodies.

The learning of self or the ontogeny of autoreactivity: establishment of high connectivity

A system arises from the interactions amongst its components. Such connectivity, if functional, provides the conditions for the emergence of properties which are not attributable to each individual component and are, therefore, systemic. We consider that physiological versus pathological modes of autoimmune activities represent systemic, supra-clonal, behaviours, emerging from the connectivity established amongst lymphocyte clones. Obviously, connectivity is determined by the structural properties of the connected clones, but once established and again if functional, it can then operate in the recursive selection of connected repertoires from the very large diversity available. Systemic behaviours can, therefore, be 'learned', in contrast with molecular or clonal characteristics which cannot be taught. In the case of autoimmunity this constitutes another strong reason to prefer *systemic* rather than *clonal* perspectives, since we all agree that self must be 'learned' by the immune system. It follows from the discussion above that learning must represent positive selection and recruitment of autoreactive cells into the activated lymphocyte compartment, which must be accompanied by the establishment of V-region connectivity of the selected clones. This notion of learning can be made quite precise [37].

Recent experiments confirm some of these predictions and suggest how this proceeds. First of all, we have demonstrated that it does take time (up to 6 weeks) to

achieve positive selection of autoreactive B lymphocytes in the spleen, and to establish the adult repertoire of activated B cells. Positive selection through functional connectivity was further demonstrated by the transfer of adult, syngeneic, normal T cells into newborns, which already results in the establishment of the autoreactive, adult repertoire by 3 weeks of age [Huetz *et al.* to be published]. Furthermore, analysis of the reactivity patterns in large collections of neonatal monoclonal antibodies with variable regions of other antibodies in the set, established the very strong bias in those repertoires for V-region connectivity [30]. Since connected antibodies are predominantly encoded by variable regions of the most D-proximal V_H-gene families [38], which by necessity of their chromosomal position are the first to be expressed in ontogeny [39], it follows that normal immune systems *necessarily* start as a high connectivity, germ-line encoded, variable region network, the expression of which is developmentally controlled. Other than by the multireactivity of some of its components, however, this primordial repertoire is not autoreactive in regard to the 'somatic' self. These processes obviously take time and involve the participation of T lymphocytes. Much evidence has been produced for mutual repertoire selection of T and B lymphocytes, and for the functionality of variable region connectivity amongst these sets of cells [9, 40]. It should be recalled, however, that these properties only apply to the activated lymphocyte pool. Since activated lymphocytes are effector cells, they will, in turn, select the respective complementarities. Therefore, functional connectivity is recursive and readily explains one characteristic of self-learning in the establishment of activated lymphocyte repertoires: positively selected autoreactive cells will necessarily be included in a high connectivity network.

Central and peripheral immune system: the emergence of immunosomatics

These considerations led us to the development of a working model that may be useful for operational purposes. We consider a *central immune system* (CIS), composed of activated lymphocytes expressing a repertoire characterized by high levels of connectivity both with V-regions in the same set, and with other somatic structures (autoreactivity). This organization results in typical dynamics which are very different from the immune response dynamics and ensure recursivity in the continuous selection of newly arising clonal reactivities, which are connected and autoreactive. The majority of the lymphocytes in a normal individual, however, integrate the *peripheral immune system* (PIS), composed of resting cells which find no productive complementarities in the internal environment, and, therefore, embody a repertoire which is devoid of V-region connectivity and autoreactivity. Lack of a network organization in this set, however, allows it to perform clonal responses, elicited obviously by molecular patterns absent from the internal environment. Differential population dynamics and functional properties amongst the two sets of cells must be considered when discussing the dynamics of their interactions. Thus, if at any time, CIS and PIS can be distinguished by definition, it is unlikely that this separation applies as soon as alterations in either compartment come about. These may result from the dynamics in the CIS or from the continuous production of new lymphocyte reactivities as immunocompetent cells. Most importantly, alterations may also arise from modifications in the available concentrations of autologous or external antigens. Furthermore, clonal responses to external antigens are certainly

bound to alter the V-region composition of the internal environment, and, therefore, to command modifications in the previous structure and dynamics. It is likely, therefore, that interactions between CIS and PIS have to do with other systemic properties, such as immunological memory, and almost certainly with autoimmunity.

A final comment, to point out a particular development that is offered by the concepts developed here. Since autoreactivities are not excluded from normal immune systems but are activated and functional, instead of the ignorance of self proposed by classical concepts, our views suggest a physiological equilibrium of the immune system's components with all self-molecules available in the internal environment. It is, therefore, possible to envisage that manipulations of the self-referential immune network of antibodies and T-cell receptors may serve in re-establishing equilibria in any other biological system. One can consider, for example, the possibility of correcting spontaneous hypertension by rising concentrations of natural antibodies against renin, or of modifying levels of circulating insulin by raising the concentrations of the sets of antibodies connected to anti-insulin reactivites. These are only possibilities, if exciting, and much thinking and experimentation must be carried out before they can be asserted. Nevertheless, such relationships between the immune system and the rest of the body, which we refer to as *immunosomatics*, open to the field of autoimmunity a very wide area much beyond the management of autoimmune diseases.

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Cyclosporine A and the Regulation of Autoimmune Disease

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Autoreactive B cells are present in the normal individual. These cells are not normally induced to release their destructive autoantibodies; T cells regulate the responsiveness of the B-cell population, and self-reactive T cells are normally absent. Modification of an epitope on a self component, or immunization with antigens that are cross reactive with self components, can lead to autoantibody formation. This phenomenon has been explained by linked recognition of self epitopes by the autoreactive B cell and the foreign epitopes by inducive T cells. The new concept of antigen processing requires degradation of epitopes on an antigen molecule and their representation as peptides associated with the major histocompatibility complex (MHC) antigens of the antigen presenting cell (APC); the autoreactive B cell can be the APC. A revised model for autoreactive B-cell activation is presented in this paper. This model shows how linkage of foreign and self epitopes can lead to autoantibody formation in a system where antigen is degraded and presented in a peptide form by the APC.

Cyclosporine acts by blocking the function of primed T cells. In this way, the drug prevents induction of autoreactive B cells. Specifically, cyclosporine acts by blocking the production of messenger RNA for lymphokine production. The agent does not block T-cell priming *in vivo*. Thus, while cyclosporine can provide effective control of autoimmunity, drug levels must be maintained to prevent disease relapse.

Introduction

Cyclosporine is an immunosuppressive agent that was first introduced for the control of tissue rejection. In this role it has proved spectacularly successful and has largely been responsible for the expansion of organ transplantation programs around the world. The reason cyclosporine is so clearly superior to other immunosuppressive therapies lies in its selective manner of action. Over a dose range of 100–1,000 ng/ml,

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cyclosporine has the ability to eliminate lymphokine-dependent immune responses (specifically, delayed-type hypersenstivity and allograft rejection). However, at this dose the phagocytic activity of macrophages is uneffected [1]. Thus cyclosporine (Cy-A) inhibits T-cell dependent reactivity but leaves the non-specific defense systems intact. Immunosuppressive agents such as steroids inhibit both arms of the immune system. Our task here is to examine the potential of Cy-A for treating forms of immunopathology that are generally grouped under the heading 'autoimmune disease'.

Our approach will be to review the theoretical basis of autoimmunity in the light of a new understanding of MHC restriction of T-cell recognition, and antigen processing. We will then discuss the effect of Cy-A on T-cell function and examine how Cy-A might be used to influence the progression of autoimmune disease.

Cellular basis of autoimmunity

Burnet described autoimmunity as the pathological development of 'forbidden clones' that for some reason escaped the normal process of clonal deletion, a process which is applied to self-reactive elements of the immune system [2]. As our knowledge of lymphocyte biology has increased, it has become increasingly clear that self-tolerance in the T-cell compartment is something that is learned during T-cell maturation. During thymic development, clonal deletion results in a lack of self-reactive T cells in the periphery. Such cells are present in the immature T-cell population of the thymus, but do not migrate into the periphery under normal developmental conditions [3]. There are relatively few, if any, *spontaneous* autoimmune diseases that have been shown to result from the pathological function of autoreactive T cells.

There are conditions in which autoreactive T cells can be generated when animals are challenged with antigen in Freund's complete adjuvant. Experimental allergic encephalomyelitis (EAE) is an example of such a disease. Autoreactive T cells have now been isolated, cloned, and shown to transmit the disease [4]. These T cells have also been shown to be reactive to the autoantigen which is relevant to the disease process. Similar evidence is needed before we can make the statement that other forms of spontaneous autoimmunity are truly of a T-cell dependent autoimmune etiology.

Type I diabetes is sometimes said to be an autoimmune disease [5, 6]. This may be inappropriate usage of the term. Control of the disease process in clinical trials and in animal models with immunosuppressive drugs points to an immunologic etiology [7–9]. The disease in rodents has been shown to be T-cell dependent [10], probably resulting from an inflammatory process activated by the CD4 T-cell subset [10, 11]. However, not all T-cell-mediated immunopathology results from autoimmunity. The classic example is lymphocytic choriomeningitis (LCM) virus infection in rodents [12]. In carrier colonies the virus is transmitted from mother to offspring with no apparent ill effect. The virus replicates but is non-cytopathic, and the host is 'tolerant' to the agent which has been present throughout neonatal development. When animals from non-carrier colonies are infected with the virus, severe encephalitis develops and inflammatory cells are seen in the brain. The immune reaction to the virus causes this disease. This is not autoimmunity, although clearly a form of immunopathology. The term autoimmunity must be used to describe generation of

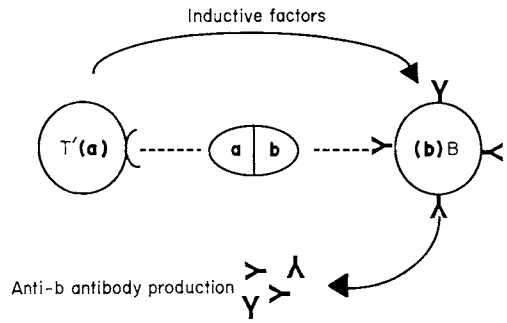

Figure 1. Mitchison's model of linked recognition. The antigen molecule **a.b** has two recognizable epitopes. The T cell specific for the **a** epitope and the B cell specific for the **b** epitope bind the antigen molecule simultaneously. The T cell then provides inductive factors to the B cell, causing it to produce anti-**b** antibody.

what Burnet called 'forbidden clones', that is, clones of T or B cells which normally would not develop, and which are specific for particular self-antigens. It is the interaction of the target cells with these 'forbidden clones' which is responsible for the pathological process that is termed autoimmunity. Strictly, a disease process cannot be classified as autoimmune until we have isolated the T cell or antibody responsible for the process and shown that one or both of these agents interacts specifically with an auto-antigen. We have not reached this point with the study of Type I diabetes, or other supposedly T-cell-mediated forms of *spontaneous* autoimmunity.

Regulation of B-cell autoimmunity

It appears that the B-lymphocyte compartment of the mature immune system contains autoreactive B cells. A small percentage of circulating B cells in normal individuals will bind autologous thyroglobulin or DNA to their surface receptors [13]. There are also reports of thyroglobulin autoantibody production by cell lines derived from normal individuals which have been induced with Epstein-Barr (EB) virus [14]. Such autoreactive B cells do not cause disease in the normal individual because their activity is under T-cell control. Tolerance in the T-cell compartment is maintained primarily by clonal deletion [3] and perhaps secondarily by some form of peripheral regulation (suppression) which is still not clearly understood [15].

On the basis of Mitchison's studies [16], linked recognition of epitopes on an antigen molecule was seen by B and T cells to be required for B-cell induction (Figure 1). The primed T cell of specificity **a**, [T'(**a**)] interacts through the antigen carrying epitopes **a** and **b** with B cells of specificity **b** [(**b**)B]. The provision of lymphokine signals by the T cell then drives the B cell to production and secretion of anti-**b** antibody [17]. If **a.b** is a self molecule, T cells of specificity T(**a**) and T(**b**) will be deleted. Thus, even if B cells of specificity anti-**a** or anti-**b** exist in the periphery they would not be induced under normal conditions.

B-cell autoimmunity could result from a bypass of normal T-cell tolerance which allows induction of the autoreactive B cell. A straightforward example is autoantibody formation during a graft-versus-host (GVH) reaction [18] in which donor T cells are potentially reactive to all host cells including the cells of the B lymphocyte

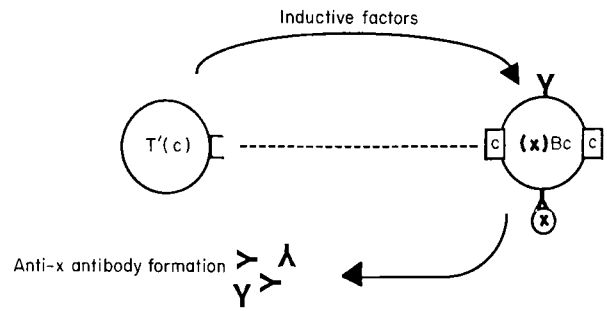

Figure 2. Autoantibody production during graft-versus-host (GVH). Antigen is provided to the T cell in the form of allogeneic MHC on the B cell itself. The activated T cell then can produce inductive factors for the B cell, as in Figure 1.

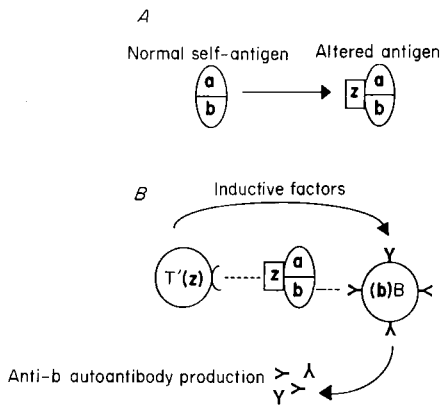

Figure 3. (A.) A self antigen **a.b** is altered to present a new epitope, **z**, in conjunction with **b**. (B.) A non-self reactive T cell specific for **z**, and a self-reactive B cell specific for **b** can now bind the linked epitopes **z.b**. Inductive factors produced by the T cell can then drive the B cell to produce autoantibody.

compartment (Figure 2). In this situation, the alloreactive T cell can provide inductive factors for the B cell capable of producing autoantibody.

In terms of classical theory, another way to bypass tolerance in the regulatory T-cell system is by modification of one or more epitopes of the autologous antigen molecule. Addition of a new recognizable epitope to the self molecule either by chemical alteration (possibly drug induced) or by the introduction of a cross-reactivity molecule into the system [Figure 3(a)] could break the normal state of B-cell tolerance [19] as shown in Figure 3(b). The self antigen, **a.b**, is altered to present a new non-self epitope, **z**, to a T cell of specificity **z**. A B cell specific for the **b** epitope in the antigen molecule binds to this molecule. Because **z** and **b** are linked, the T cell bound to **z** can supply inductive factors to the B cell specific for a self antigen, a situation that would not normally occur. This autoreactive B cell [(**b**)B] would then be able to produce self-reactive antibody, anti-**b**.

The Mitchison model of linked recognition is based on two assumptions: (1) The T-cell receptor interacts productively with an epitope on the antigen molecule. (2)

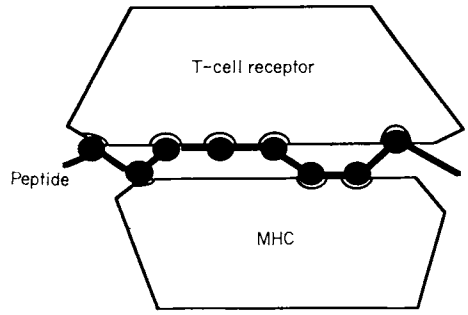

Figure 4. Ova peptide fragment residues bind both Ia of APC MHC and the T-cell receptor recognition site (Adapted from Grey *et al.* [25]).

T/B interactions are regulated by linked recognition of epitopes on the antigen molecule. Both of these assumptions are now untenable in terms of new information concerning antigen presentation and recognition within the immune system. The primed T cell produces lymphokine factors in a receptor-directed manner, preferentially secreting lymphokine in the area in which its receptors have been cross-linked [20]. However, the T cell does not recognize antigen alone, but the combination of antigen and MHC in an MHC-restricted manner [21].

Data relating to the crystal structure of Class I MHC antigen provides evidence for a molecular cleft which can be filled with peptide material, thought to be degraded and processed self components [22]. Antigen presentation can occur through both Class I and Class II MHC antigen [23, 24]. The more common form of antigen presentation is in association with Class II MHC antigen, and Grey's group has defined the characteristics of peptide presentation to the T-cell receptor (Figure 4). By engineering various single-amino acid substitutions in an immunogenic peptide derived from chicken ovalbumin, they demonstrated that different residues in the peptide fragment were capable of binding to both Ia and the T-cell receptor (Figure 4).

This concept of epitope presentation in a peptide form poses a theoretical problem for the Mitcheson model of linked associative recognition (Figure 1), a concept that is the basis of modern immunological theory [25]. Degradation of an antigen, **a.b**, into peptide epitopes **a** and **b**, of necessity breaks the linkage required by the model for antigen-directed T-cell regulation of B-cell activity.

A solution to the linkage problem

Experimental evidence suggests that a B cell, specific for a particular antigen, can use its surface immunoglobulin to internalize and concentrate antigen for presentation in association with MHC antigen [26, 23]. Theoretically the B cell can present antigen that is not homologous with its receptor. For example, consider processing of a simple antigen **a.b** made up of two epitopes **a** and **b**, by a B cell of an anti-**b** specificity [(**b**)B]. The epitopes which are ultimately presented on the B-cell surface are processed and removed from their immunoglobulin carrier after internalization, and seen on the cell surface as antigenic peptide fragments which are capable of binding both T-cell receptor and MHC (Figure 4). In this manner, epitopes **b** and **a** can both

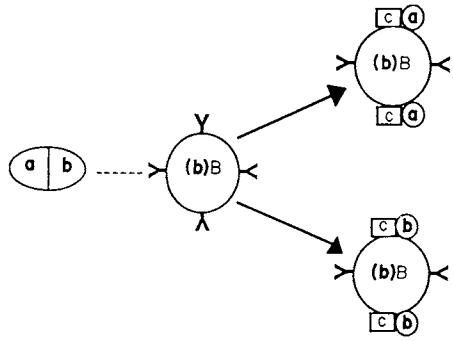

Figure 5. Antigen processing and presentation by the B cell. The antigen comprised of epitopes **a** and **b** is internalized by the **b**-specific immunoglobulin receptor on the B-cell surface, and broken into peptide fragments. These are presented on the surface of the B cell as separate antigen fragments in the context of the B-cell MHC.

be presented to the T cell by the B cell in association with MHC antigen of the B cell and these cells should be able to interact productively.

Let us make the assumption that T cells are tolerant to the set of self epitopes (**s**) seen in the context of self MHC, (c); the set of T cells defined as T(c.**s**) (that is the T cell with specificity defined by both self MHC, c, and the self epitope, **s**, are deleted during development. Any antigen, X, is made up of epitopes **a,b,c** . . . , **n**, physically linked together. Consider self tolerance to a simple antigen made up of epitopes **a** and **b**, (**a.b**). If tolerance is expressed at the level of the T cell, then the T cells of type c MHC, of which Class I or II MHC reactive cells are subsets, reactive to self plus **a** [T(c.**a**)] and self plus **b** [T(c.**b**)] will be deleted in the thymus during development [3].

Potentially autoreactive B cells: (**a**)B (a B cell which can secrete anti-**a** antibody) and (**b**)B exist in the animal. Both these B cells can use their receptor (anti-**a** or anti-**b**) to capture antigen **a.b** and process this antigen to generate the following antigen present cells: (**a**)Bc.**a**, (**a**)Bc.**b**, (**b**)Bc.**a**, and (**b**)Bc.**b**; where c.**a**, and c.**b** represent the peptide form of epitopes **a** and **b** in association with the MHC presenting structure, c (Figure 5). Because **a.b** is a self antigen, T cells of specificity (c.**a**) and (c.**b**) have been deleted. Therefore, neither an **a**-reactive, or a **b**-reactive B cell can be activated under normal conditions.

Induction of autoimmunity by cross-reactive antigen

Consider the entry into the system of a naturally occurring cross-reactive molecule of a modified self antigen, **z.a.b**, where **z** is a foreign epitope, while **a** and **b** are normal self epitopes. B cells of specificity anti-**b** can process this antigen to generate the following antigen-presenting cells: (**b**)Bc.**z**, (**b**)Bc.**b**, (**b**)Bc.**a**, (**a**)Bc.**a**, (**a**)Bc.**b**, (**a**)Bc.**z**, (**z**)Bc.**a**, (**z**)Bc.**b**, and (**z**)Bc.**z**. One set of these B cells can be induced by a *nonself*-reactive T cell, T(c.**z**). The interaction between this cell and the B-cells which present **z** on their surface [(**z**)Bc.**z**, (**a**)Bc.**z**, and (**b**)Bc.**z**] produces not only clones which make antibody to **z**, but can also induce autoreactive clones which produce anti-**a** and -**b** antibody (Figure 6). Therefore the response to the foreign epitope, **z**, results in the production of autoantibody.

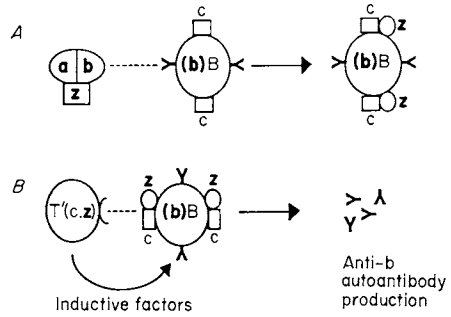

Figure 6. Induction of an autoreactive B cell by a non-self-reactive T cell. (A.) The antigen **z.a.b** is taken up by the B cell's receptor for the **b** epitope. The antigen is broken into peptide fragments and these are presented on the B-cell surface in conjunction with B-cell MHC antigen, (c.**b**). (B.) A T cell reactive to the foreign epitope **z**, T(c.**z**), is able to recognize c.**z** on the surface of the B cell and induce it to produce antibody for the self antigen it is specific for, **b**.

Linkage of epitopes is required for transport of the foreign signalling epitope into the autoreactive B cell

Thus, while antigen is presented in a fragmented form, linkage during internalization explains how modified autoantigens or cross-reactive foreign antigens can lead to autoimmunity.

Cyclosporine and the control of autoimmunity

Autoimmunity can result from the action of T cells on B cells, or directly by means of T-cell-mediated inflammatory tissue damage. Cyclosporine (Cy-A) can effect the reactivity of B cells only indirectly, through inhibition of the T cells which regulate their function. There is general agreement that Cy-A effects the function of T-lymphocytes; and that many of its *in vivo* effects stem from this modification of T-cell behaviour [27–32]. There is little agreement beyond this point.

T-cell activation

It was once thought that antigen alone, binding to the T-cell receptor, triggered the process of T-cell activation [33]. However, it is now clear that antigen does not provide a sufficient stimulus for lymphocyte activation. Two signals, delivered coordinately, are required [34–37]. One signal is provided by antigen binding to the T-cell receptor, and the second signal is provided by a molecule that expresses a co-stimulator (CoS) activity [35]. Evidence for the two signal model came originally from the study of T-cell responses to living or uv-irradiated spleen cells [34], and from the study of the *in vitro* response of T cells to cultured tumor lines [36]. The initial step, T-cell priming to produce activated T cells (T′), with receptors for IL-2 can be written as follows:

$$T \xrightarrow[(2)]{(1)} T' \qquad \text{Reaction A}$$

The process of T-cell activation has been analysed in some detail by Larsson *et al.* [35], who showed the antigen-presenting stimulator cells of the mouse to be Ia+, possibly the dendritic cell [37]. It was initially thought that IL-1 was the co-stimulator produced by the antigen-presenting cell [32, 38]. More recent results suggest

this may not be the case [39]. The activated T cell has receptors for IL-2/IL-4 [40, 41]. These molecules provide the signal required for T-cell activation, and result in clonal expansion. We can write this reaction in the following way:

$$T' \xrightarrow{\text{IL-2/IL-4}} n\,T' \qquad \text{Reaction B}$$

where T′ is the activated T cell that carries receptors for IL-2/IL-4 and can express effector function [32].

T′ cells produce a number of different lymphokines when appropriately stimulated [42]. These lymphokines include IL-2, IL-3, GMCSF, and interferon (IF). Cycling T′ cells do not secrete lymphokine in the absence of antigen. In fact, biochemical studies have shown that these cells do not contain mRNA necessary for lymphokine synthesis [43]. Within 2–4 h of triggering with either antigen or mitogen, T′ cells begin to secrete lymphokine [44]. In marked contrast to the process of T-cell activation, triggering of T′ cells and lymphokine production requires only one signal: engagement of the T-cell receptor [45]. We can write this process in the following form:

$$T' \xrightarrow{(1)} Lk \qquad \text{Reaction C}$$

where signal (1) is provided by antigen or mitogen binding to the T-cell receptor and results in lymphokine (Lk) production.

In the past there has been controversy concerning the role of T-cell subsets. One school of thought saw T cells as being divided into functionally distinct subsets, the helper subset and cytotoxic/suppressor subset, which had distinct antigenic markers and differed in function [28]. Helper T cells were required for the induction of the cytotoxic/suppressor subset. The more extreme adherents of this notion suggested that the helper subset produced growth factors for effector cells, and that the cytotoxic effector responded to, but was unable to produce such factors [46, 47]. Others did not see such a distinct division of function; they admit that T cells of both subsets respond to growth factors, but maintain that cytotoxic and helper functions were properties of different subsets [45, 48]. There is now evidence that cells belonging to different subsets defined in terms of antigenic markers (CD4/CD8) can express both cytotoxic activity and produce lymphokines when appropriately triggered [49–51].

Site of action of cyclosporine A

Two different models have been proposed to account for the biological activity of cyclosporine.

1. The subset model postulates that different T-cell subsets show differing sensitivity to cyclosporine. The drug inhibits the activation of the helper subset, and because 'help' is required for the activation of the cytotoxic subset it secondarily inhibits cytotoxic cell formation [28]. It is also proposed that cyclosporine does not inhibit the activation of suppressor cells [52]. In this framework cyclosporine has a differential effect on T-cell subsets.

2. The signalling model does not discriminate between the activities expressed by subsets. This model postulates that cyclosporine interferes with the transmission of the antigen-specific signal from the surface to the interior of the cell after binding of

antigen and T-cell receptor [50]. A variant of this model is the idea that cyclosporine interferes with signalling by preventing antigen binding to the T-cell membrane [53].

Let us consider how the known experimental information relates to each of these models. To carry out this analysis it is convenient to consider the effect of cyclosporine on each of the three steps in T-cell activation and expression of function (reactions A, B, and C above).

Clonal expansion—reaction B

There is general agreement that cyclosporine, in concentrations of up to 1 μg/ml, does not effect IL-2-dependent clonal expansion of activated T cells [27, 28, 30–32]. At concentrations higher than 4 μg/ml, the drug is inhibitory, possibly due to a toxic effect. Cyclosporine does not effect lymphokine-dependent clonal expansion of T′ cells at doses below the toxic level.

Function of activated T cells—reaction C

Cyclosporine does not inhibit the expression of cytotoxic activity by cells of the T′ class [32, 54]. Since expression of cytotoxic activity requires binding of the T cell to its target, it follows that the drug cannot inhibit antigen/MHC binding to the T′ cell. Palacios and Moller suggested that cyclosporine expresses its function by inhibiting the binding of Class II MHC antigen on the APC to the specific T-cell receptor [52]. The above evidence makes this an unlikely explanation. A close examination of the data that formed the basis of Palacious and Moller's conclusion shows that cyclosporine inhibits the *results* of Class II antigen binding to the reactive T cell, lymphokine production.

Antigen or mitogen triggered by release of lymphokine from activated T cells is inhibited by cyclosporine at the concentration range of 10 to 100 μg/ml; in this concentration range it does not inhibit binding of antigen to the T′-cell population. It follows, therefore, that the drug must interfere with the delivery of the antigen-dependent signal at some stage after antigen binding by the T-cell receptor [30, 32].

Two sets of experimental evidence allow us to focus more sharply on the point at which cyclosporine blocks lymphokine production: the 'helper function' of T′ cells. At concentrations of 1 μg/ml cyclosporine has no effect on lymphokine production by tumor lines producing IL-2 or IL-3 respectively [55]. That is, constitutive lymphokine-producing cells are insensitive to this drug. At face value this evidence suggests that cyclosporine does not inhibit the process of lymphokine synthesis and export from the cell. Also, once T′ cells have been activated to produce message for lymphokine systhesis they become insensitive to cyclosporine [56]. Thus, the agent does not block the translation of message for lymphokine once it has been formed in the cell. Cyclosporine does block new lymphokine-message formation and in this way inhibits the 'helper function' of the T′ cell [1] (Figure 7).

Primary T-cell activation—reaction A

Early *in vitro* studies from our laboratory indicated that cyclosporine blocked the process of T-cell activation by antigen [32]. It now appears that this finding was an artifact of the mouse system, in which cells die over the 5-d assay period if not

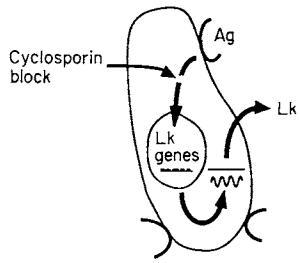

Figure 7. Mechanism of action of cyclosporine. Cyclosporine blocks delivery of the antigen-mediatd signal, thus preventing the synthesis of lymphokine message RNA by the T′ cell (primed T cell).

restimulated. Other studies indicate that cyclosporine does not inhibit the initial priming of T cells *in vivo* [57, 58], but because cyclosporine blocks the process of lymphokine production, this priming step does not have any measurable outcome providing that inhibitory cyclosporine levels are maintained.

In conclusion we can make the following points: (1) Clonal expansion of activated T cells is insensitive to cyclosporine. That is cyclosporine does not interfere with the signal delivered to T′ cells by IL-2/IL-4. (2) The response of T′ cells to antigenic triggering (lymphokine production) is very sensitive to cyclosporine, and the effect appears to result from an interference with delivery of the antigen-mediated signal to the nucleus of the cell. Cyclosporine blocks the transcription of lymphokine message, but has no effect once message is present in the cytoplasm of the T′ cell. (3) The process of primary T-cell activation is not inhibited by cyclosporine. However, since cyclosporine blocks all lymphokine-dependent T-cell function, this priming process does not lead to any observable *in vivo* reaction in the presence of inhibitory levels of cyclosporine.

Cyclosporine is not a subset-specific drug when subsets are defined in terms of antigenic markers (CD4/CD8). However, it is also clear that cyclosporine effects the helper function without interfering with T-cell effector functions, such as the expression of cytotoxic activity or clonal expansion.

There is clear *in vitro* evidence that both antigen-specific and antigen-non-specific suppressor cells can be generated in the presence of cyclosporine [59]. One question still requires resolution in relation to these studies. Are the suppressor cells generated *in vitro* relevant to tolerance mechanisms seen *in vivo*? In particular, the antigen-specific tolerance that develops in animals grafted with heart tissue under the cover of short term cyclosporine [60]. One would like to see evidence for the *in vivo* transfer of antigen-specific unresponsiveness before wholeheartedly excepting the concept that cyclosporine treatment allows the development of suppressor cells. A better understanding of how suppressor cells function would also assist in the interpretation of this aspect of T-cell biology.

Cyclosporine and autoimmunity

As was pointed out in our discussion of autoimmunity, autoreactive B cells are normally present in the immune system. The lack of autoimmunity under normal conditions results from a lack of T/B interactions involving the autoreactive B cell. Self tolerance in the T-cell compartment is the result of clonal deletion during the development of the T cell. However, autoimmunity can readily be precipitated when

an antigen which is cross-reactive with self, but contains a foreign epitope, is taken up and processed by the autoreactive B cell (Figure 3). Such cross-reactive antigens could be the product of infectious agents or result from a chemical modification (drug-induced autoimmunity) of a normal self-component. In some cases autoimmunity can result from forcing a self-reactive T-cell response to a normal antigen, such as myelin-basic protein in the case of EAE. Such reactivity requires the administration of antigen in complete Freund's adjuvant. Experimental autoimmunity of this kind may not have a naturally occurring analog.

Autoimmunity, whether it results from induction of autoreactive B cells, or T-cell-mediated inflammatory tissue damage, is dependent on lymphokine production by the T cell involved. It follows therefore that cyclosporine will be an effective agent for the control of autoimmunity. There is now strong experimental and clinical evidence that this is so [19]. However, our analysis of cyclosporine's mechanism of action points up difficulties that could arise in the clinical use of cyclosporine.

Cyclosporine does not appear to block T-cell priming *in vivo*. However, it does block T-cell-effector function by blocking lymphokine production. This latter block is exerted at the level of lymphokine message production (see Figure 7). Cy-A is ineffective once message is present in the cytoplasm of the T′-cell. This places severe restrictions on how the agent should be used clinically. First, once control of the disease process has been obtained with cyclosporine the level of drug has to be maintained to hold the T′ cells under control. If the level of cyclosporine falls, even for a relatively brief period, new lymphokine message can be generated, and the T′ cell escapes cyclosporine control. Second, because the agent blocks new lymphokine message formation, it will be rather slow in expressing its effect if we have an ongoing disease process. Cyclosporine could block new message formation and so arrest the progression of disease. However, there would be a lag phase determined by the life span of existing lymphokine message during which cyclosporine would have little or no effect on the disease process. Once control has been achieved with cyclosporine it is important to maintain drug levels so as to prevent episodes of disease relapse. If a particular condition leads to irreversible damage of the transplanted tissue, and if the disease process is sufficiently acute, cyclosporine may not be able to prevent destruction. This may be the explanation of why cyclosporine can reverse the development of clinical diabetes but has little or no effect when used to control existing disease in non-obese diabetic (NOD) mice [6, 8, 11]. In the latter situation the disease process is very acute.

In some transplantation models cyclosporine therapy can result in suppressive tolerance induction [61]. When this state is achieved the drug can be removed and the allograft is maintained. The mechanism of this process is not understood, and at present we have no evidence that in autoimmunity cyclosporine leads to the establishment of tolerance, allowing removal of the drug from the system. Thus, while cyclosporine can be an effective agent for the control of autoimmunity there are limitations, and we may not be able to withdraw therapy without accompanying disease relapse.

Acknowledgements

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Hazards of Cyclosporine A Therapy and Recommendations for its Use

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Cyclosporine nephrotoxicity represents a new type of drug-toxicity. Morphologic lesions occur in both the tubular and vascular system. Cyclosporine nephrotoxicity is dose-dependent. At therapeutic drug levels only functional changes occur consisting of a reduction of glomerular filtration rate and renal plasma flow. Superimposed on the functional toxicity are morphologic changes which may result in severe cases in acute or chronic renal failure. The threshold for the development of cyclosporine nephrotoxicity depends on drug-blood-levels, individual sensitivity to cyclosporine and the presence of concurrent risk factors.

Based on the experience with the drug the following recommendations are made for clinical use especially in patients with autoimmune diseases: the initial dose should not exceed 5 mg/kg body weight; the dose should be reduced if blood cyclosporine levels are over 400 ng/ml; in addition, a dose reduction is recommended if serum creatinine values exceed 30% of pretreatment values or if other signs of cyclosporine toxicity, such as hepatoxicity, hypertension, etc. occur. By strict adherence to these suggestions it should be possible to treat patients for a prolonged period with cyclosporine without irreversible morphologic lesions.

Introduction

Cyclosporine A (Cy-A) is an immunosuppressive drug of proven value in organ transplantation. In recent years, clinical trials with Cy-A in autoimmune disease gave encouraging results. Cy-A therapy is accompanied by several side effects, nephrotoxicity being the most important. Nephrotoxicity was first noticed by Calne (1978) [1]. During the following years the morphologic lesions of Cy-A-nephrotoxicity were defined [2–5].

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Table 1. *Classification of Cy-A-nephrotoxicity of autoimmune disease*

1.	Lesions related to Cy-A
1.1.	Functional (basic) toxicity
1.1.1.	Special form: Acute renal failure with oligo-anuria
1.2.	Tubular toxicity
1.3.	Vascular-interstitial toxicity
2.	Lesions unrelated to Cy-A, e.g. infection, glomerulonephritis, interstitial nephritis

The classification of Cy-A-nephrotoxicity as developed on the basis of kidney transplant biopsies [6, 7] proved also to be appropriate in patients with autoimmune diseases.

Classification of Cy-A-nephrotoxicity

Cy-A-nephrotoxicity can be divided into two major subgroups (Table 1): (a) functional (basic) toxicity without significant morphologic lesions; (b) morphological forms of toxicity with tubular and/or vascular-interstitial lesions.

Functional toxicity encompasses renal side effects which are not associated with distinct morphologic renal lesions and which can already be present at therapeutic trough (predose) levels and/or doses. Most patients develop signs of so-called functional (basic) toxicity at therapeutic doses of between 5–10 mg/kg body weight, corresponding to Cy-A trough levels of 200–500 ng/ml (whole blood measured by polyclonal RIA kit) or 20–60 ng/ml (serum), respectively. Functional toxicity is characterized by slight increase of serum creatinine and decreased glomerular filtration rate. At high doses and/or trough levels morphologic lesions i.e. tubular toxicity or vascular-interstitial toxicity and its clinical symptoms may be superimposed on functional toxicity. Whereas functional (basic) and tubular toxicity are reversible, vascular-interstitial toxicity may result in irreversible renal damage.

The different forms of toxicity have been reported in patients with kidney, liver, heart and bone-marrow transplants as well as in patients with autoimmune diseases.

Frequency and time of presentation

No precise data can be given for the frequency of the different morphologic lesions. In about 50% of the biopsies from renal transplant patients lesions related to Cy-A are present [7]. In kidney transplants, the frequency of vascular-interstitial toxicity decreased with the use of lower Cy-A doses from about 30 to 15% [8] and tubular toxicity became rare within the last few years in the biopsy material.

In a series of different autoimmune diseases, signs of vascular-inerstitial toxicity was found in more than 50% of the patients [9]. A dose-dependent decrease in the frequency of vascular-interstitial toxicity to less than 10% was noted in later studies [10–12].

Functional (basic) toxicity is always present, as long as the patient is treated with Cy-A. Tubular toxicity predominates within the first few months of starting Cy-A therapy, when high Cy-A doses are used but may be found at any time. Cy-A-associated arteriolopathy is rarely found before the second month but may already be present within the first week [13]. Interstitial fibrosis, striped form, is usually seen more than 6 months after commencement of Cy-A therapy [7].

Functional (basic) toxicity

Clinical findings

At therapeutic Cy-A doses, a decrease in renal function is observed in almost every patient: creatinine clearance declines on average by 20%. Thirty percent of the patients, however, have a decrease of renal function of more than 25%. The increase in serum creatinine is of the same order and is due to decreased glomerular filtration rate. Hypertension develops in about 10% of the patients with autoimmune diseases (V. Graffenried, personal communication). Hypercalcemia, mild metabolic acidosis, hypomagnesemia and hyperuricaemia are rare [14].

Morphology

Systematic biopsy studies of patients with functional toxicity are not available. From the limited knowledge it is deduced that the renal tissue is either normal, or peritubular capillary congestion [4] may be present. Peritubular capillary congestion differs from normal renal tissue only by a dilatation of peritubular capillaries containing mononuclear cells. Peritubular capillary congestion is a non-specific finding often observed in acute renal failure of any aetiology even in the absence of Cy-A treatment.

Pathogenesis and etiology

The mechanisms leading to renal failure are poorly understood [15]. The renal findings after a single intravenous administration in the absence of general hemodynamic changes suggest that Cy-A causes a rather selective preglomerular vasoconstriction. The following pathogenetic mechanisms are considered: direct effects on renal vessels, stimulation of the adrenergic system, stimulation of the tubulo-glomerular feedback mechanism, alterations of the renal prostanoid synthesis [15]. The most important pathogenetic factor is most probably a direct toxic effect on the renal vessel, although convincing experimental evidence is still lacking.

Tubular toxicity

Clinical findings

The clinical findings do not differ from those of functional toxicity. Although there is no qualitative difference there might be a quantitative one. Serum creatinine may be higher ($> 100\%$ of baseline). Interestingly enough there is only limited evidence of proximal tubular dysfunction [14].

Morphology

The morphologic changes seen in tubular toxicity [for detailed review see 3, 7] comprise inclusion bodies in tubular epithelial cells corresponding to giant mitochondria, isometric tubular vacuolization and microcalcification. Giant mitochondria predominantly occur in the convoluted part of the proximal tubule, whereas isometric vacuolization is limited to the straight part. Interstitial lesions are either minimal or absent.

Giant mitochondria, isometric vacuolization and microcalcifications are not specific for Cy-A therapy though highly characteristic, especially when the three different lesions are present in one and the same biopsy. Tubular toxicity is well documented in patients with autoimmune disease [9, 16].

Etiology and pathogenensis

An essential etiologic factor in the development of tubular toxicity is a toxic Cy-A trough level [3, 7]. Tubular toxicity must be expected in patients with Cy-A trough levels exceeding 1,000 ng/ml (whole blood), corresponding to 200 ng/ml (serum). In the presence of additional renal damage, especially ischemia, or co-medication with nephrotoxic drugs, tubular toxicity may be found (at usually) non-toxic Cy-A trough levels. Increased individual sensitivity to Cy-A may account for the occurrence of tubular toxicity at low Cy-A trough levels in the absence of additional risk factors [3, 7]. Tubular toxicity must be considered as the result of a direct tubular damage which is due to the parent compound rather than metabolites. Tubular toxicity reflects general cytotoxicity which may also occur at other target sites such as smooth muscle and endothelial cells.

Vascular-interstitial toxicity

Clinical findings

The clinical findings are superimposed on the symptoms of functional toxicity. Slow, progressive deterioration of renal function and eventually hypertension are the most important signs. Increased factor VIII and antithrombin III activity in the serum may be indicators of a vascular damage [17, 18]. Furthermore, an increased platelet deposition is found in kidney grafts with vascular-interstitial toxicity [19].

Finally, several cases of hemolytic uremic-like syndrome were reported in patients with organ transplants [20], but only once in patient treated for autoimmune disease.

Morphology

Three different morphologic lesions were observed which may occur either alone or in combination [for detailed morphological review see 3, 7, 9, 19]: glomerular and/or arteriolar thrombi, Cy-A-associated arteriolopathy and interstitial fibrosis, striped form, with tubular atrophy. Arteriolar or glomerular fibrin or platelet thrombi affect only very few glomeruli or vessels (Figure 1). In 25–50% of the patients with thrombi, Cy-A-associated arteriolopathy is present as well.

The most frequent lesions and hallmarks of vascular-interstitial toxicity are by far the Cy-A-associated arteriolopathy and interstitial fibrosis, striped form, with tubular atrophy. The vascular lesion predominates in the peripheral vascular tree: arterioles (afferent vessels) and arteries (up to two layers of smooth muscle cells). The vascular lesion extends sometimes to the vascular pole of the glomerulus, involving even some glomerular segments, and into arteries close to the branching arterioles. Proliferative arteriopathy of interlobular and arcuate arteries [21] is not a feature of Cy-A toxicity.

Cy-A-associated arteriolopathy occurs in two forms which may coexist: (a) Circular nodular protein deposits permeate the arteriolar wall and may narrow or even occlude the vascular lumen (Figure 2). Electron microscopy reveals that the protein deposits replace necrotic myocytes. The protein deposits consist of IgM and /or complement (C3/C1q). In up to 20% of the cases, fibrin may be present as well. (b) The second form is characterized by a mucoid thickening of the intima resulting in narrowing of the vascular lumen (Figure 3).

The final result of the vascular damage may be complete occlusion of the damaged arterioles (Figure 4) which finally vanish within the interstitial fibrosis.

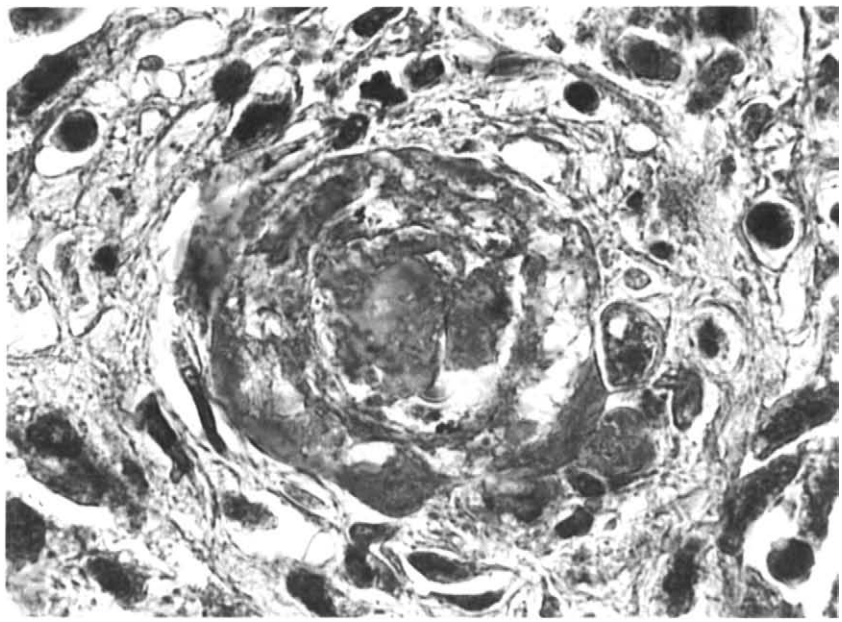

Figure 2. Arteriole with fibrin thrombus in the lumen and complete circular necrosis of smooth muscle cells. PAS-stain (× 1050).

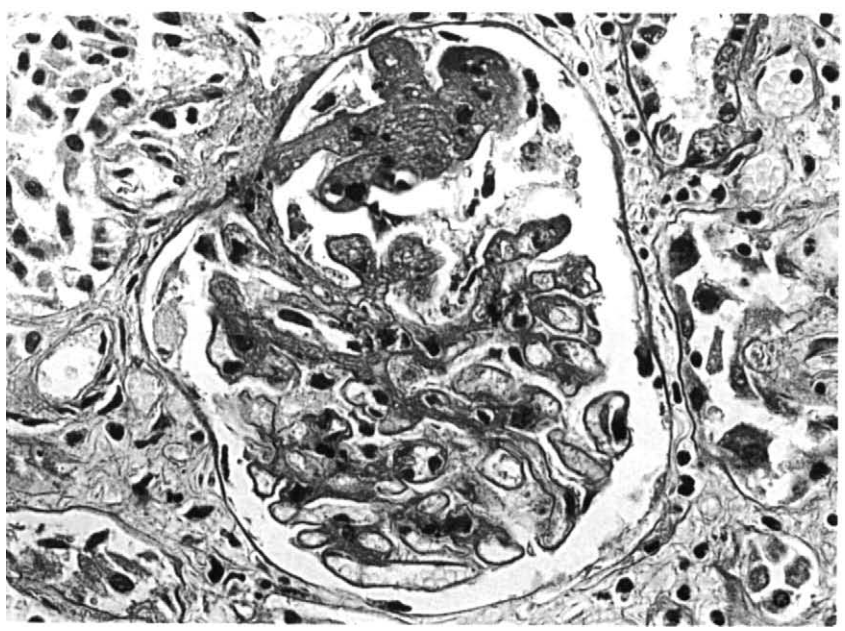

Figure 1. Fibrin thrombi in a glomerular segment PAS-stain (× 370).

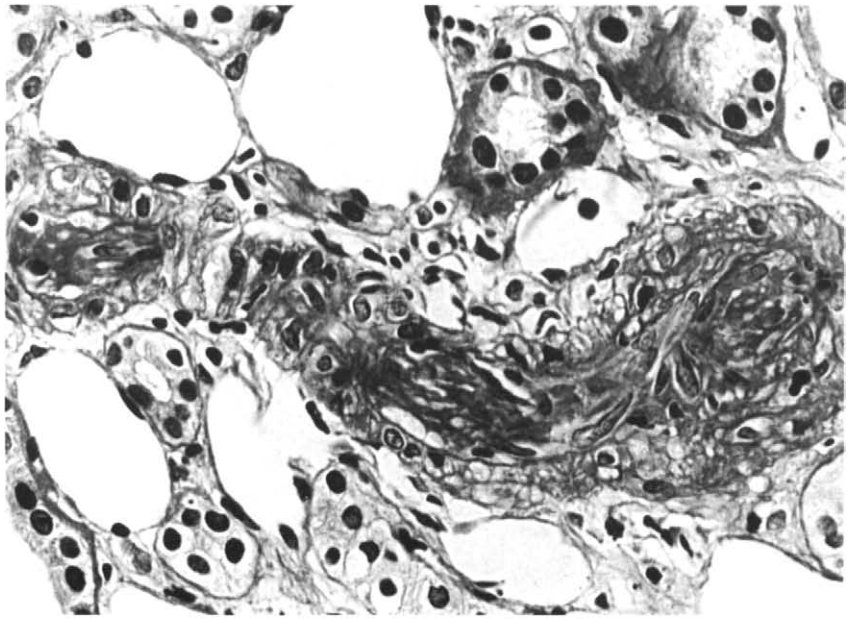

Figure 4. Complete irreversible occlusion of the arteriolar lumen. PAS-stain (× 450).

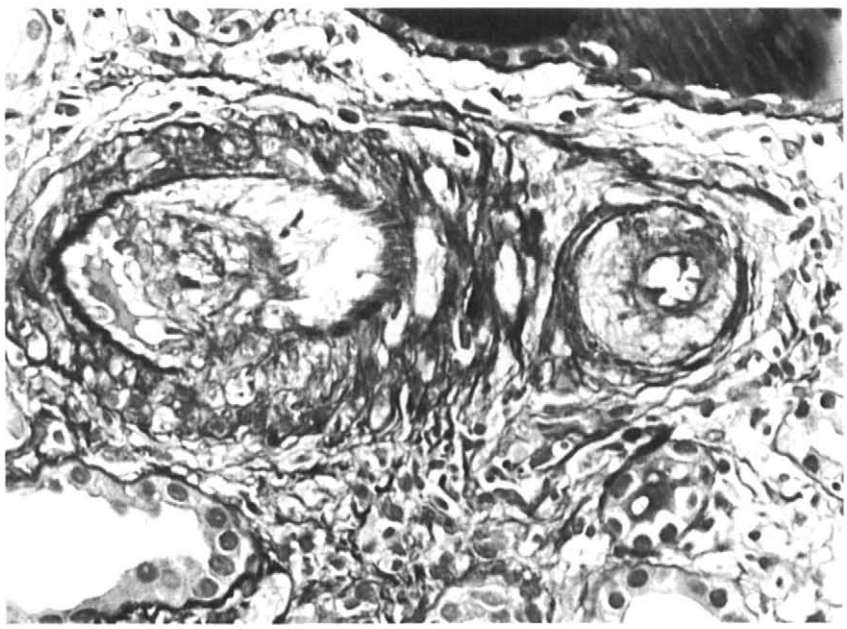

Figure 3. Mucoid thickening of the intima with severe narrowing of the vascular lumen. AFOG-stain (× 380).

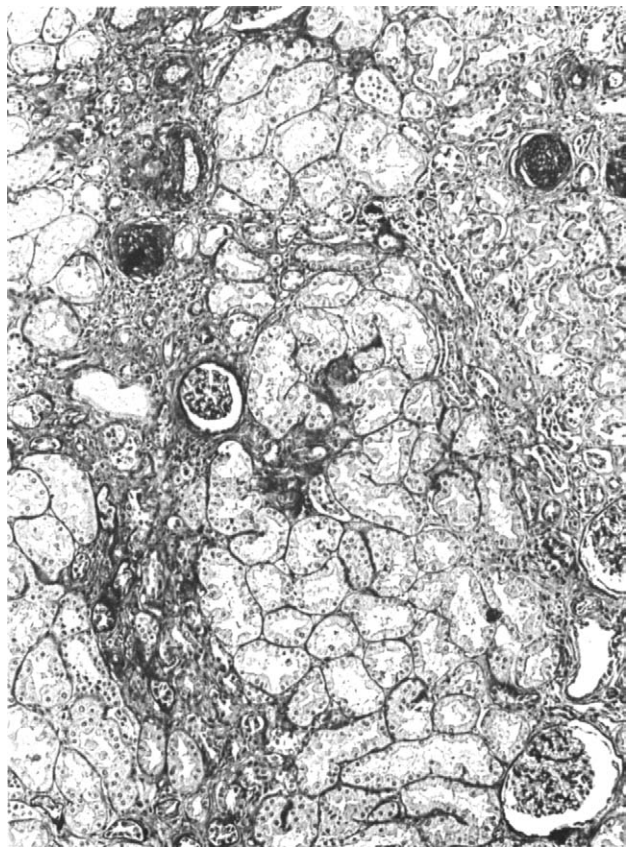

Figure 5. Striped form of interstitial fibrosis and tubular atrophy. In addition two completely obsolescent glomeruli. PAS-stain (× 70).

In both types of Cy-A-associated arteriolopathy, or even in its absence, minor changes of endothelial and smooth muscle cells may be present: vacuolization, single-cell necrosis and inclusion bodies corresponding to giant lysosomes.

Cy-A-associated arteriolopathy is accompanied or followed by interstitial fibrosis (Figure 5). Irregular foci or stripes of interstitial fibrosis with atrophic tubules are observed in the renal cortex. Tubules in other areas appear essentially normal. A sparse mononuclear cell infiltrate is often seen in the fibrotic areas.

The morphologic lesions seen in vascular-interstititial toxicity are non-specific. Cy-A-associated arteriolopathy is very similar to what may be found in cases of thrombotic microangiopathy independent of Cy-A treatment. Interstitial fibrosis should not be attributed to Cy-A therapy in the absence of Cy-A-associated arteriolopathy unless other possible pathogenic factors are excluded or highly unlikely. Vascular-interstitial toxicity is well documented in patients with autoimmune disease [9, 10].

Vascular interstitial toxicity results in most cases in irreversible renal damage and chronic renal failure even after withdrawal of the drug may develop [22].

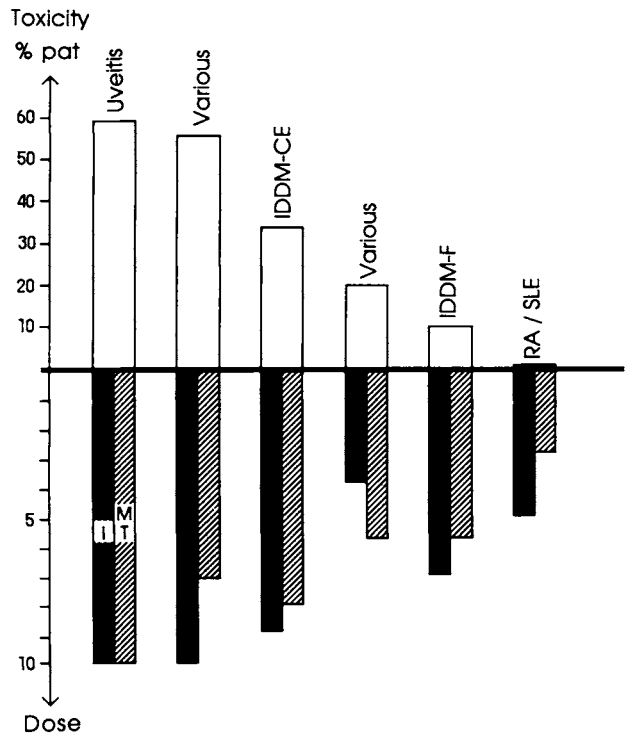

Figure 6. Relationship between nephrotoxicity and Cy-A dose in different studies in autoimmune diseases.

Pathogenesis and etiology

There are several lines of evidence that in the pathogenesis of vascular-interstitial toxicity, thrombotic microangiopathy plays a key role. Some patients treated with Cy-A develop the typical clinical picture of hemolytic uremic syndrome. The renal morphology is compatible with protracted and mild thrombotic microangiopathy. An increased consumption of platelets is found in kidney transplants with Cy-A-associated arteriolopathy. An increased excretion of thromboxane indicates platelet degradation within the kidney. Focal interstitial fibrosis with tubular atrophy is the result of Cy-A-associated arteriolopathy [7].

Vascular-interstitial toxicity is related to Cy-A trough level and/or dose [7, 23–25]. The cumulative dose of Cy-A within the first 6 months after transplantation were higher in the group of patients with focal interstitial fibrosis. Patients with signs of vascular-interstitial nephrotoxicity have significantly higher Cy-A whole blood trough levels within the first 3 months after transplantation than patients without. The Cy-A trough level/dose dependence of vascular-interstitial toxicity was confirmed in patients with bone marrow transplants [26]. The experience in patients with different autoimmune diseases further strengthened the relationship between the Cy-A trough level/dose and the vascular-interstitial toxicity (Figure 6) [9–12].

Table 2. *Proposed pathogenesis of cyclosporine toxicity*

Dose-dependent cell damage		
Tubule	Small vessels	
High levels	Low levels	High levels
Giant mitochondria, vacuoles, microcalcification ↓ Minor functional disturbance ↓ ?	Vasoconstriction due to: —direct effect? —sympath. nerve activity? —Ras? —Prostaglandins? ↓ GRF↓, RPF↓, Hypertension	Endothelial and smooth muscle cell damage ↓ Platelet aggregation ↓ Local i.v. coagulation ↓ Cy-A-arteriolopathy ↓ Interstitial fibrosis ↓ GRF↓, RPF↓, Hypertension

The risk factors for the development of vascular-interstitial toxicity in patients with autoimmune diseases are poorly defined. However, co-medication with nephrotoxic drugs resulting in acute renal failure, severe impairment of renal function [25] and old age [V. Graffenried, personal communication] may be the most important. Some individuals, however, develop vascular-interstitial toxicity despite low blood levels and in the absence of risk factors. The reason for this variable individual sensitivity is not understood.

Conclusion

Nephrotoxicity occurring after Cy-A treatment represents a new type of drug toxicity (Table 2). Morphologic lesions occur in both the tubular and vascular system. Cy-A-associated nephrotoxicity is a dose-dependent process. At low trough levels functional renal changes consisting of GFR and RPF reductions commonly occur (functional, basic toxicity). Superimposed on functional toxicity, morphologic changes e.g. tubular toxicity and/or vascular interstitial toxicity may develop and progress to chronic renal failure. The threshold for the development of morphologic renal lesions and/or chronic renal failure depends on individual patient's sensitivity and the presence of concurrent risk factors. The best possible recommendation which can be given to avoid major signs of toxicity in patients treated with Cy-A for autoimmune diseases is to maintain Cy-A trough levels definitely below 400 ng/ml (whole blood). The pathophysiology of Cy-A-nephrotoxicity is still poorly understood. It is hoped that further experimental approaches will give more insights into

the molecular events leading to functional toxicity and especially those mediating the progression to vascular-interstitial toxicity.

Recommendations for the use of Cy-A in autoimmune disease

An initial dose of Cy-A of 5 mg/kg BW or less is recommended. The Cy-A trough level should not exceed 400 ng/ml (whole blood, RIA unspecific) or 200 ng/ml resp. (whole blood, RIA specific). In case of Cy-A-toxicity (serum creatinine increase of more than 30% over baseline, hypertension or hepatotoxicity) the Cy-A dose should be reduced until overt signs of toxicity are no longer present. With this therapeutic strategy therapeutic effects may be achieved without irreversible renal side effects as seen in patients with Type I diabetes mellitus or SLE treated for years with Cy-A.

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Cancer is a Long-term Hazard of Immunosuppressive Therapy

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An increased incidence of certain cancers occurs in immunodeficiency states. The incidence in organ transplant patients is increased more than three-fold. The predominant tumors are lymphomas, carcinomas of the skin and lips, carcinomas of the vulva/perineum, *in situ* carcinomas of the uterine cervix and Kaposi's sarcoma. Tumors appear a relatively short time after transplantation (average 61 months). Unusual features of the lymphomas are the high incidence of non-Hodgkin's lymphoma; frequent involvement of extranodal sites; and marked predilection for the brain. Skin cancers present unusual features: predominance of squamous cell carcinomas; young age of the patients, and a high incidence of multiple tumors. Cancer of the vulva/perineum occur at a younger age than in the general population and may be preceded by condyloma acuminatum or herpes genitalis. Certain autoimmune diseases *per se* are associated with an increased incidence of malignancy, mainly lymphomas. Superimposed on this background incidence is an increase in lymphomas, skin cancers, primary liver cancers, leukemias and bladder cancers associated with the use of immunosuppressive or cytotoxic agents. The overall incidence is probably less than in organ transplant recipients possibly because of lower dosage of immunosuppressive therapy and the shorter duration of treatment.

Immunosuppressive therapy is being used increasingly to suppress immunity or inflammatory responses in a number of autoimmune disorders, collagen–vascular diseases, and disorders of obscure etiology [1, 2]. These include glomerulonephritis, nephrotic syndrome, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), psoriasis, chronic cold agglutinin disease, dermatomyositis, ulcerative colitis, hepatitis, idiopathic thrombocythemia, primary amyloidosis, Wegener's granulomatosis, multiple sclerosis, pemphigus and pemphigoid, allergic angiitis, polymyalgia rheumatica and other disorders. The agents used include corticosteroids, azathio-

prine, cyclophosphamide, methotrexate, chlorambucil, cyclosporine and other chemical agents, as well as heterogenous antilymphocyte or antithymocyte globulin (ALG or ATG), and a variety of monoclonal antibodies, cytokines, and anticytokines discussed elsewhere in this issue [1, 2]. Radiation therapy in the form of total body irradiation (TBI) or total lymphoid irradiation (TLI) may be used to suppress immunity and inflammatory processes in RA or SLE [3, 4]. Inadvertent immunosuppression may also follow the use of less extensive radiation [5]. Each immunosuppressive agent has its own specific side effects. In addition immunosuppression *per se* increases the risks of the development of cancer, a complication that is most likely to occur after intense or prolonged therapy [1, 2]. In this report we shall consider the incidence and types of cancers that have occurred in the most extensively studied groups of patients treated with immunosuppressive agents, namely organ transplant recipients. With this background information we shall then discuss the risks of similar or other cancers developing in patients with autoimmune diseases who are given immunosuppressive or cytotoxic therapy.

Cancers in organ transplant recipients

Incidence of post-transplant malignancies

The most common tumours in the general population are carcinomas of the skin, lung, prostate, colo-rectum, female breast, and invasive carcinomas of the uterine cervix [1, 2, 6–8]. A radically different pattern is seen in organ transplant recipients in whom there is a more than three-fold increased incidence of malignancies [9, 10]. Skin and lip cancers are more frequent than in the general population but their incidence varies with the amount of sunshine exposure. In regions with limited exposure, there is a four- to seven-fold increase, but in areas with copious sunshine there is an almost 21-fold increase over the already high incidence seen in the local population [1, 2]. Lip cancers are increased 29-fold in incidence as compared with controls [11]. While *in situ* carcinomas of the uterine cervix show a 14-fold increase over controls [12], there is no increase in the incidence of invasive cancers. Certain neoplasms that are rare in the general population occur relatively frequently in organ transplant recipients. Two epidemiologic studies show that the incidence of non-Hodgkin's lymphomas (NHLs) is 29 to 49-fold above that observed in age-matched controls [9, 10]. There is a 400- to 500-fold increase in the incidence of Kaposi's sarcoma (KS) in renal transplant recipients compared with controls of the same ethnic origin [13]. The incidence of carcinomas of the vulva and anus in renal transplant recipients is increased 100-fold as compared with controls [11]. Hepatobiliary carcinomas show a 38-fold increase in incidence [9, 10]. These incidence figures are borne out by the data of the Cincinnati Transplant Tumour Registry (CTTR) collected up till June 1988. There were 3,670 patients who developed 3,925 types of cancer. Of these 3,492 received kidney, 111 heart, 34 liver, 21 bone marrow, eight pancreas, three combined heart and lung and one lung transplants.

Age and sex of patients

The cancers affected a relatively young group of people whose average age at the time of transplantation was 40 years (range 5 months to 80 years) [1, 2, 6–8]. Forty-eight percent were under 40 years of age at the time of tranplantation. Sixty-five percent

were male and 35% female, in keeping with the 2:1 ratio of male to female patients who undergo renal transplantation.

Time of appearance of tumors

The incidence of cancer increases with the length of follow-up after transplantation [1, 2]. A study of 3,846 Australian renal transplant recipients showed an incidence of 3% at one year, 14% at 5 years and 49% at 14 years [14]. These disturbing figures should be interpreted with caution as most lesions were skin cancers (which are very common in Australia) and the number of long-term survivors was relatively small. Nevertheless, they stress the need to follow transplant patients indefinitely [1, 2, 6–8].

As the length of follow-up of organ transplant recipients has increased it has become evident that certain malignancies appear at fairly distinct intervals after transplantation [1, 2, 6–8]. In contrast with other known oncogenic stimuli in man, which often take 15 to 20 years or more before they cause overt lesions, cancers are diagnosed a relatively short time after transplantation [1, 2, 6–10]. KS is first to appear at an average of 22 (range 2–225.5) months after transplantation [1, 2, 6]. Lymphomas appear at an average of 36 (range 1–196.5) months after transplantation. Carcinomas of the vulva and perineum appear at the longest time after transplantation, at an average of 103 (range 9–241.5) months [1, 2, 7]. All other tumors appear at an average of 65 (range 1–266) months after transplantation.

Types of malignancies

Cancers of the skin and lips

The most common tumors affected the skin and lips [1, 2, 9, 10]. They comprised 1,478 of 3,925 (38%) neoplasms in the CTTR. Their incidence increased with the length of follow-up after transplantation as demonstrated by an Australian study of 3,846 renal transplant recipients of whom 11% had cancers at 5 years, 29% at 10 years and 43% at 14 years [14].

Skin cancers in transplant patients showed some unusual features compared with similar lesions in the general population [1, 2]. Basal cell carcinomas (BCCs) outnumber squamous cell carcinomas (SCCs) in the general population, but the opposite is true in transplant recipients in whom SCCs comprised 51% and BCCs 28%. Another 14% of patients had both types of neoplasm. In the general population these types of skin cancer occur mostly in people in their 60s and 70s but the average age of transplant patients was 30 years younger [1, 2]. In addition, the incidence of multiple skin cancers in this worldwide collection of patients (present in at least 43%) is remarkably high and is similar to that seen only in areas of copious sunlight [1, 2]. Several individuals each had more than 100 skin cancers. Malignant melanomas (MMs) made up 4.7% of skin cancers in this series in contrast with an incidence of 2.7% in the general population of the United States [2]. This finding is consistent with an Australian study showing a five-fold higher incidence of MMs in transplant patients than in age-matched controls [14].

In the general population skin cancers seldom metastasize and cause fatalities. Those that do so are usually MMs. In striking contrast lymph node metastases occurred in 97 of the transplant patients with skin cancers (6.6%). Of these, 82%

resulted from SCCs mostly arising from the skin, rather than from the lips, as occurs in the general population. Even more worrying is that 87 patients (6%) died of their skin cancers. Almost two-thirds of the fatalities were from SCCs, the majority of which had arisen from the the skin rather than from the lips.

Non-Hodgkin's lymphoma

Lymphomas of all types make up 3% to 4% of all neoplasms in the community at large [1, 2], but comprised 534 of 3,925 malignancies (14%) in the CTTR. If we exclude non-melanoma skin cancers and *in situ* carcinomas of the uterine cervix, which are excluded from most cancer statistics, the incidence rises to 19%. The majority (97%) of lymphomas were NHLs whereas Hodgkin's disease (HD) is the most common lymphoma seen in the same age group in the general population [1, 2]. Morphologically most NHLs were classified as immunoblastic sarcomas, reticulum cell sarcomas, microgliomas or large cell lymphomas. Of 137 studied immunologically, 84% arose from B lymphocytes, 15% were T-cell lymphomas, and there was one null cell lymphoma. In one study a spectrum of lesions was described ranging from benign polyclonal B-cell hyperplasias at one end to frankly malignant monoclonal B-cell lymphomas at the other [15]. Whereas extranodal involvement occurs in from 24% to 48% of NHL patients in the community at large [1, 2], it was present in 73% of NHLs in this series. Furthermore, extranodal disease was confined to a single organ in 65% of the transplant patients. The brain was the structure involved most frequently. In the general population about 1% of NHLs affect the brain parenchyma [1, 2], whereas in organ transplant patients 32% involved the central nervous system (CNS), usually the brain. Spinal cord involvement was rare. Brain lesions frequently were multicentric in distribution. Another notable feature was that in 68% of patients with CNS involvement the lesions were confined to the brain, whereas in the general population cerebral lymphomas are frequently associated with lesions in other organs.

Kaposi's sarcoma

KS comprised 175 of 3,925 malignancies (4%) in the CTTR. If we omit non-melanoma skin cancers and *in situ* carcinomas of the uterine cervix, KS then makes up 6.3% of cancers in the CTTR, in comparison with its incidence in the general population in the United States (before the Acquired Immunodeficiency Syndrome [AIDS] epidemic started) where it comprised only 0.02% to 0.07% of all neoplasms [1, 2, 6, 8]. It is striking that the number of KS patients in this series (175) exceeds those with carcinomas of the colo-rectum (141) or breast (127). Apart from individuals with AIDS, who are frequently afflicted by KS, there is probably no other series in which the number of KSs exceeds either of these two common cancers, except possibly in tropical Africa where KS occurs frequently (making up 3% to 9% of all neoplasms) and colonic cancer is rare.

KS affected 123 men and 52 women. The 2:1 male to female ratio is similar to that seen in transplant patients having other cancers, but is far less than the 9:1 to 15:1 ratio seen with KS in the general population [6]. KS was most common in transplant patients who were Jewish, Arabic, black or of Mediterranean ancestry [1, 2, 6]. Sixty-two percent had non-visceral KS confined to the skin, conjunctiva , or oropharyngolaryngeal mucosa and 39% had visceral disease, affecting mainly the gastrointestinal

tract and lungs, but other organs were also affected. The prognosis of patients with visceral involvement was much worse than in those with non-visceral disease. Thirty-six of 44 deaths among the former group were caused by KS, which, in contrast, rarely caused fatalities among patients without visceral involvement.

After treatment complete remissions occurred in 57 of 109 patients (52%) with non-visceral disease. Eighteen of these 57 remissions (32%) occurred when the *only* treatment was a drastic reduction of immunosuppressive therapy [1, 2, 6]. The other 39 remissions followed surgery, radiotherapy or chemotherapy. In patients with visceral disease, 12 of 66 patients (18%) had complete remissions, six of which occurred when reduction of immunosuppression was the only treatment.

Carcinomas of the uterus

Carcinomas of the cervix occurred in 188 of 1,292 women in this series (15%) [1, 2]. *In situ* lesions comprised at least 78% of the cases. It is advisable that all post-adolescent female transplant recipients have pelvic examination and cervical smears on a regular basis [1, 2].

Carcinomas of the vulva and perineum

Carcinomas of the vulva, perineum, scrotum, penis, peri-anal skin or anus occurred in 115 patients, 79 female and 36 male [1, 2, 7]. This 2:1 female to male ratio contrasts with the findings of most other cancers in this series where males outnumbered females by more than 2:1.

The average age of patients in the general population with *in situ* carcinomas of the external genitalia is 40 years. One-third of the patients in the CTTR had *in situ* lesions and were even younger with an average age at the time of diagnosis of 33 years. A more disturbing feature it that transplant patients with invasive lesions were much younger (average age 42 years) than their counterparts in the general population, whose average age is usually between 50 and 70 years. Some transplant patients gave a history of condyloma acuminatum or, less frequently, herpes genitalis suggesting that oncogenic viruses may play an etiologic role in the lesions [1, 2, 7]. Female patients sometimes exhibited a 'field effect' with cancerous involvement not only of the vulva but also the vagina and/or uterine cervix.

Cancers following cyclosporine therapy

With the limited experience gained thus far, the pattern of malignancies observed after treatment with cyclosporine (Cy-A) is somewhat different from that seen with so-called conventional immunosuppressive therapy (CIT), consisting of azathioprine (or cyclophosphamide) and prednisone, sometimes supplemented by ALG or ATG [8].

The average time of appearance of 3,355 neoplasms after CIT was 68 (range 1–266) months, whereas the 570 cancers that followed Cy-A administration occurred much earlier after transplantation, at an average of only 26 (range 1–233.5) months. Several patients at the furthest end of the time spectrum either had been switched from CIT to Cy-A, or had received CIT with a first transplant and Cy-A with a subsequent transplant.

Analysis of the CTTR data shows a disproportionately high incidence of lymphomas (27% *vs* 11%), KS (11% *vs* 3%) and carcinomas of the kidney (5% *vs* 3%) in Cy-A patients compared with CIT patients. In addition there was a lower incidence of skin cancers (23% *vs* 40%), uterine cervical carcinomas (2% *vs* 5%) and carcinomas of the vulva and perineum (< 1% *vs* 3%) in the former group compared with the latter.

One must emphasize that only 14 of the 559 patients (3%) who developed malignancies following Cy-A therapy were treated with this drug only. Other immunosuppressive agents used were mainly prednisone or related compounds (537), azathioprine (301), ALG (153), splenectomy (32), OKT_3 (30) and cyclophosphamide [10]. If for the moment, we exclude patients who received azathioprine or cyclophosphamide and confine ourselves to the 256 tumors that arose in patients who were treated with Cy-A only or with Cy-A plus prednisone the predominant tumors remain as follows: lymphomas (82 patients), skin cancers (53), KS (32), and renal carcinomas (16). With the limited experience gained thus far with Cy-A it is possible that we are seeing mainly the tumors that occur early after transplantation, namely, lymphomas and KS, but with longer follow-up the other malignancies mentioned above will become manifest. As already mentioned skin cancers in particular show a progressive increase in incidence with the length of follow-up. Long-term study of large numbers of patients treated with Cy-A or Cy-A and prednisone will clarify any carcinogenic potential of this agent.

The Cy-A-related tumors showed several differences from those that occurred after CIT. The lymphomas appeared at an average of 14 (range 1–160) months after transplantation in the Cy-A group whereas they appeared after an average of 46 (range 1–196.5) months in CIT patients. The Cy-A-related NHLs more closely resembled those in the general population in that only 59% were extranodal. Small bowel involvement occurred in 42 of 346 NHLs (12%) in the CIT group but in 31 of 145* (21%) of the Cy-A-related NHLs. A striking feature of NHL's in the CIT group was frequent involvement of the CNS which occurred in 134 of 346 patients (39%). Of these, 98 (73%) were confined to the CNS. In contrast, only 22 of 145* (15%) of NHLs in the Cy-A group affected the CNS and of these 11 (50%) involved the CNS only.

A gratifying feature of the NHLs in the Cy-A group is that the disease appeared to respond more readily to treatment than in CIT patients. In 48 of the 145 patients (33%) there were complete remission of the lesions following various treatments, including reduction or cessation of immunosuppressive therapy, surgical excision, administration of acyclovir, radiation therapy and chemotherapy. Twelve of the remissions occurred when the *only* treatment was reduction or cessation of immunosuppressive therapy. The great majority of such remissions occurred in patients whose disease was localized to a single organ.

Cancers in autoimmune diseases

Several factors complicate evaluation of the incidence of cancer in autoimmune diseases treated with immunosuppressive agents. Firstly, malignancies *per se* may

*Of the Cy-A-related lymphomas, 11 were HD or multiple myelomas and are not included with the NHLs.

cause autoimmune, musculoskeletal, connective tissue or other paraneoplastic syndromes [16]. For example, in one study various tumors were complicated by myoneuropathy, myopathy, lupus-like syndromes, secondary gout, polyarthritis, carpal tunnel syndrome, endocrinopathies (Cushing's syndrome; inappropriate secretion of antidiuretic hormone), temporal arteritis, RA and diffuse interstitial fibrosis [16]. Secondly, the more severe autoimmune diseases, especially those complicated by cancer, are likely to be reported in the literature, whereas the straightforward cases go unreported giving a distorted idea of the incidence of cancer in these disorders [17]. Thirdly, certain autoimmune diseases *per se* are complicated by an increased incidence of neoplasms. The tumors usually occur in sites other than the target organ of the autoimmune disease, which itself may be prone to malignancy, as is the stomach in pernicious anemia and the colon in ulcerative colitis. There is some controversy about the risk of cancer in autoimmune disorders. In 1967 Oleinick [18] analyzed the literature on malignancies in patients with SLE or RA and did not find an excessive incidence of lymphoma or leukemia. However, some more recently published reports do show an increased incidence of cancer in these disorders. In a series of 484 patients with SLE, 18 (3.7%) developed cancers ($P<0.0005$) [19]. In addition, Japanese SLE patients had 196 times greater incidence of lymphomas than the general population [20]. A three-fold to 10-fold increased incidence of lymphomas in patients with RA was reported by various workers [10, 21–24] whereas others found an 'unexpectedly low' incidence of neoplasms in these patients [19], or no increase [18], or an unusually low incidence of cancers of the gastro-intestinal tract compared with controls [21]. Seven of 136 patients with Sjögrens Syndrome developed NHLs, an incidence 43.8 times ($P<0.01$) greater than that expected in an age-matched population [25]. In addition, the incidence of Waldenström's macroglobulinemia was increased by a comparable magnitude. The NHLs found in Sjögrens Syndrome show a spectrum of lesions ranging from benign polyclonal lymphoid hyperplasia to malignant lymphomas with B-cell characteristics [26, 27], although occasional T-cell lymphomas have also been described [28]. The incidence of cancer in patients with polymyositis or dermatomyositis was reported to be five to 11 times that expected in the general population [29–31]. However, other studies indicate that these conditions were not associated with a high incidence of malignancies [17, 32]. In one study of 110 patients with bullous pemphigoid there was a highly significant incidence of cancer in 11% [33]. Another eight patients had suspected but unconfirmed internal malignancies. On the other hand other workers found no significant difference in the incidence of malignancies between patients with pemphigoid and age- and sex-matched controls [34]. In a 0–24 year follow-up study of 4,531 Japanese patients with chronic thyroiditis there was no increase in risk for overall malignancies but an increased risk for myelo- and lympho-proliferative neoplasms (13 observed *vs* 4.9 expected $P<0.01$) [35]. The relative risk of malignancy for scleroderma patients is 1.8 times higher than expected, and is due mostly to an increase in lung cancer [36].

An increased incidence of cancer has also been observed in a number of chronic inflammatory disorders and in diseases of obscure etiology. Of 2,544 patients with respiratory sarcoidosis, 48 developed malignancies, whereas 33.8 cases were expected, a statistically significant finding [37]. Lymphomas occurred 11 times and lung cancer three times more frequently than expected. Intestinal lymphangiectasis,

which is characterized by cellular and humoral immune defects, resulting from loss of protein and lymphocytes through dilated intestinal lymphatic channels, was associated with lymphomas in three of 15 patients [38]. In 202 patients with adult celiac disease and idiopathic steatorrhea, 29 developed 31 cancers, 27 of which were either lymphomas or carcinomas of the gastrointestinal tract. The incidence at all sites was significant in men ($P = 10^{-5}$) and in women ($P = 0.032$) [39].

While certain autoimmune diseases are associated with an increased incidence of cancer others such as hyperthyroidism, and Addison's disease are not. In addition patients with allergic disorders have decreased risks for a variety of cancers [40]. Decreased risks of digestive tract cancers also occur in patients with chronic thyroiditis [35] and RA [19]. It is difficult to explain why certain autoimmune disorders are associated with an increased incidence of neoplasms, particularly lymphomas, while others have no increase or even a decreased risk. In some instances the occurrence of cancer and an autoimmune disorder may be pure coincidence. In other instances it is possible that both disorders may have resulted from the same etiologic factor(s). Another possibility, already mentioned, is that certain cancers may cause autoimmune paraneoplastic syndromes. It is also possible that in some autoimmune diseases, in which there is marked B-cell hyperplasia (in response to self-antigen or to infections such as by Epstein-Barr virus [EBV]), T cells, which normally limit the extent of B-cell reactions, are numerically or functionally defective resulting in unrestrained B-cell reactions, which eventually progress from hyperplasia to neoplasia.

Another critical question is whether cancers in patients with autoimmune diseases arose as complications of the underlying defects in immunity or were caused by immunosuppressive or cytotoxic therapy. There are numerous anecdotal reports that include even small series of cancers that occurred in patients with autoimmune diseases given immunosuppressive therapy. In many reports it is not clear if immunosuppressive agents were indeed used, or else it was stated that they were not given. However, the latter statement is misleading as most patients had been treated for prolonged periods with prednisone or other adrenal corticosteroids, which are potent immunosuppressive agents. Another criticism of many reports is that they do not provide data on total numbers of patients or patient-years at risk. As a consequence it is not possible to calculate the relative risk to immunosuppressed nontransplant patients for the development of cancers when compared to a suitable control population.

Conflicting findings regarding the incidence of cancer have been reported following immunosuppressive therapy of the autoimmune and other disorders mentioned above. In some studies immunosuppressive therapy did not appear to increase the incidence of cancer. For example, in a study of 311 patients with RA, 20 developed tumors, of whom 10 had received immunosuppressive therapy and 10 had not [41]. In another study of 126 RA patients treated with cyclophosphamide and other cytotoxic agents the incidence of cancer was no different from control RA patients not given this treatment [42]. However, follow-up was less than 5 years and total cumulative dosage of the drugs is not mentioned. The incidence of cancer in 114 patients with Sjögrens syndrome who received no immunosuppressive therapy was 35.7 times greater than expected, but in 20 patients given such treatment the incidence was increased 100-fold [25]. These data must be interpreted with caution

because of the small number of patients in the treated group. In a report from Norway, seven of 49 KS patients (14%) had received prior immunosuppressive therapy [43]. However, the comparison population of 242 patients, with BCCs of the skin, who were not treated with immunosuppressive therapy, is not a satisfactory control as the patients were not matched for age, sex and underlying diseases, many of which were autoimmune disorders [1, 2]. Study of 290 renal allograft recipients, who developed tumors after transplantation, showed no difference in the incidence of cancer between patients with pre-existing autoimmune diseases compared with those whose renal failure was caused by some other disorder [44]. In contrast, patients with idiopathic cardiomyopathy who were less than age 40 years had a significant excess risk of developing lymphomas after cardiac transplantation [45]. Lymphomas developed in seven of 18 patients with both these characteristics but did not occur in 77 other cardiac transplant recipients.

There are several other studies which provide more convincing evidence of an increased incidence of cancer following immunosuppressive therapy for many of the disorders mentioned above. Fifteen of 1,853 patients (0.85%) with RA treated mainly with chlorambucil or cyclophosphamide, developed acute leukemia [46]. The neoplasm was not observed in patients treated for less than 6 months, or in those who received a total dose of less than 1 g of chlorambucil or 50 g of cyclophosphamide. In addition, four of 35 patients (11.4%) treated with chlorambucil for severe psoriatic arthropathy developed acute leukemia [46]. Acute leukemia also developed in two of more than 150 (1.3%) RA patients given azathioprine together with cyclophosphamide in one, and melphalan in the other [47]. In a study of 81 RA patients treated with cyclophosphamide there was a four-fold increase in the incidence of cancer compared with untreated RA patients and persons in the general population [48]. The major increase was in hematologic and lymphoreticular malignancies which showed a 15-fold increase. The mean total dose of cyclophosphamide in patients with malignancy was 82 (range 23–176) g and the mean duration of therapy was 4.5 (range 1.9–8.1) years. In another series, 1,634 patients were treated for RA, Crohn's disease, ulcerative colitis, chronic glomerulonephritis, and other disorders for at least 3 months using azathioprine (68%), cyclophosphamide (28%) or chlorambucil (4%). They showed an 11-fold increase of NHLs a nine-fold increase of primary liver cancers, five-fold increase of cutaneous SCCs and four-fold increase of bladder cancers [10]. A long-term retrospective case control study of 119 patients with RA treated with cyclophosphamide and 119 matched RA patients not so treated showed an increased incidence of urinary bladder cancer (six treated *vs* no control patients), skin cancer (eight treated *vs* no control patients), and lymphohemopoietic malignancies (five treated *vs* one control patient) [49]. A key factor was the length of follow-up. The mean was more than 11 years, a much longer time period than in some series in which no treatment-related tumors were reported. Another important factor was the total cumulative dose of cyclophosphamide. The greatest risk of bladder cancer occurred in those given a total dose of greater than 85 g. In another study of 54 patients treated with cyclophosphamide for SLE or RA, two cases of bladder cancer were observed, compared with 0.02 expected [50]. Another study involved 154 patients with histologically verified non-alcoholic liver disease who were randomized to azathioprine or prednisone treatment. After a median period of 91 months 33% (13 of 39) patients in the azathioprine group died of a variety of malignancies,

(including two with hepatocellular carcinomas) as compared with 13% (4 of 32) patients ($P = 0.08$) in the prednisone group [51].

Overall the picture that emerges from all these reports is of a pattern of malignancies similar in many respects to that seen in organ transplant recipients. However, the incidence of cancer appears to be less than in transplant patients perhaps because dosage is less and the duration of therapy is shorter. As with organ transplant recipients there is a preponderance of lymphomas, skin cancers, and liver malignancies. An increased incidence of bladder cancers in patients with treated autoimmune diseases may be related to direct toxic effects of cyclophosphamide or its metabolites on the bladder mucosa. An increased incidence of leukemia in these patients may be related to direct damage to the bone marrow by alkylating agents such as cyclophosphamide, chlorambucil, or melphalan which are known to cause this complication in cancer patients given cytotoxic chemotherapy [1, 2].

Malignancies also complicate radiotherapy used for autoimmune diseases. Cancer is a well recognized long-term complication of radiation therapy. In most instances it appears to result from direct damage to DNA, although immunodepression may play some role in patients who receive extensive radiation exposure [52]. The incidence of cancer following radiation therapy for autoimmune diseases is ill-defined. It is well known that patients with ankylosing spondylitis who received therapeutic irradiation have an increased incidence of acute leukemia [53]. There are conflicting reports concerning the carcinogenic hazards of extensive radiotherapy such as TLI used for conditions such as RA or lupus nephritis. Based on experience with TLI given to over 1,000 patients with HD and followed for periods up to 10 years several investigators found no increase in hematologic malignancies, even when TLI was given with chemotherapy [54, 55]. However, other workers found that HD patients had an increased incidence of acute leukemia after radiotherapy [56, 57] and particularly after combined radiotherapy and chemotherapy, leading to concern about the long-term consequences of TLI used to treat autoimmune disorders [4]. Length of follow-up is of great importance. Most RA patients treated with TLI have been followed for a few months only. It is known from experience with HD patients that acute leukemias arise 4–5 years after treatment while NHLs occur after 7–10 years [1, 2, 4, 52]. Furthermore, most cancers that arise after radiotherapy (such as those of thyroid, lung and breast) have an onset 20–25 years after treatment. Of particular concern in patients with autoimmune diseases is that if TLI fails and immunosuppressive cytotoxic drugs are then given the long-term consequences may be similar to those seen after treatment of HD with a 5% risk of leukemia at 5 years and a 15% risk of NHL by 10 years [4].

Possible causes of cancer in immunosuppressed patients

Space constraints permit only brief mention of possible etiologic factors that are discussed in detail elsewhere [1, 2]. The neoplasms probably arise from complex interplay of multiple factors. Severely depressed immunity *per se* may hamper the ability of the body to destroy cancer cells induced by various carcinogens. Chronic antigenic stimulation by self-antigens, or the foreign antigens of transplanted organs, or by repeated infections may overstimulate a partially depressed immune system and lead to NHLs. Alternatively, defective feedback mechanisms may fail to control

the extent of immune reactions and lead to unrestrained lymphoid proliferation and lymphomas. Furthermore, once loss of regulation occurs, the defensive ability of the immune system is weakened and other non-lymphoid tumors may appear.

Activation of oncogenic viruses is a strong possibility. EBV is strongly implicated in causing NHLs; cytomegalovirus in causing KS; certain papilloma viruses in carcinomas of vulva, perineum, anus, uterine cervix, and skin; herpes simplex virus in carcinomas of the lip, vulva, perineum, uterine cervix, and anus; and hepatitis B virus in hepatocellular carcinomas.

Some immunosuppressive and cytotoxic drugs may directly damage DNA and cause cancer. The alkylating agents are strongly suspected as a cause of leukemias and carcinomas of the bladder. Immunosuppressive or cytotoxic drugs may potentiate the effects of other carcinogens, such as sunlight in causing carcinomas of the skin, ionizing radiation in causing leukemias, or herpes simplex virus in causing carcinomas of the lip or uterine cervix. Genetic factors may affect susceptibility to cancer by affecting carcinogen metabolism, regulation of the immune response, level of interferon secretion, response to virus infections, or they may contribute in other ways.

Summary and conclusions

The fact that immunosuppressive therapy is associated with an increased incidence of certain tumors emphasizes the importance of the immune system in host defenses against cancer. Because of this danger we should try to use immunosuppressive therapy as little as possible. However, it is essential for the survival and function of organ transplants. Attempts are being made to modify the present blunderbuss attack on the immune system with more specific methods of control of certain of its components. Eventually, it is hoped to produce states of immune unresponsiveness directed specifically, and only, at the foreign antigens of the allograft. Because of the increased risks of cancer the use of immunosuppressive therapy in autoimmune and other disorders should be restricted to severe cases that fail to respond to other forms of treatment, and then only in controlled trials with long term follow-up of the patients.

We need to study carefully the cancers that occur in immunosuppressed organ transplant recipients and patients with autoimmune diseases to find clues to their etiology. This information may shed light on the causes of similar tumors that occur in the general population, and on the complex role that the immune system plays in the control of malignancy. Hopefully such knowledge may provide immunological methods for the prevention and cure of at least some cancers.

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Some Viral Infections and Related Disorders Associated with Long-term Immunosuppressive Treatments

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From a local series of long-term renal transplant patients and a survey of the literature, some of the viral infections associated with immunosuppressive treatments are discussed in relation to the duration, type, and magnitude of immunosuppression. Special emphasis is put on chronic viral hepatitis, warts and other papova virus infections associated with benign or malignant skin tumors, herpes virus infections and the sequential steps of serum immunoglobulin abnormalities which may culminate in lymphoma or infectious lymphoproliferative syndromes. Finally the distinct features of iatrogenic Kaposi's sarcoma are described in comparison with other forms of this disease. Some of these disorders fit well with the multi-step hypothesis of carcinogenesis.

In most situations the pathogenesis of these complications appears to be multifactorial and the contribution of each immunosuppressive agent is difficult to ascertain, inasmuch as other factors such as initial virological status, allogenic stimulation (blood transfusions, organ transplants, graft-versus-host disease) and the immune disorder of the underlying disease itself are likely to be involved. The most important characteristics of these complications during the initial stages of their progression is their reversibility on withdrawal of the immunosuppressive agents. This justifies careful clinical and immunological monitoring of these patients.

Introduction

The long-term hazards of immunosuppression should be precisely evaluated in order to obtain better knowledge of the risk/benefit ratio of immunosuppressive treatment in chronic immunologically-mediated diseases. Such treatments were initially used in life-threatening situations such as end-stage tumors and organ transplantation. Development of new molecules (e.g. cyclosporine) and progress in

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the design of less toxic therapeutic regimens (e.g. chemotherapy of leukemia) have improved patient survival and led to the demonstration of some long-term side effects. Some are clearly related to the type of immunosuppressive molecule administered. For instance the mutagenic risk associated with alkylating agents was shown to result in a higher incidence of leukemias and cancers in patients suffering from autoimmune diseases treated with these drugs [1–3]. The long-term side effects of corticosteroid treatment are now well documented. They include primarily osteoporosis, aseptic necrosis of the femoral head, diabetes, obesity, cataracts and hypertension. Some of the side effects of cyclosporine treatment have also been described, including chronic interstitial nephritis, hypertension, gingival hypertrophy and cholestatic syndrome. On the other hand the infectious diseases associated with short-term intensive immunosuppression are well known and some may be prevented by appropriate prophylaxis. However, the long-term hazards of immunosuppression are difficult to classify [4]. They may be analyzed in several clinical situations, as, for instance, organ transplantation [5, 6], bone marrow transplantation, tumor chemotherapy and immunosuppression for autoimmune diseases. In such cases, the immunological alterations associated with the underlying disease, the virological status of the patient and the allogenic stimulation (transplantation, blood transfusions) are likely to play a major role in the occurrence of infections or neoplasia. In the present report we shall summarize our clinical experience of viral infections in renal transplant patients with more than 10-year survival and discuss from this clinical model the possible relationship between immunosuppression, viral infection and oncogenesis.

Hepatitis

Liver cirrhosis is presently one of the major causes of mortality in long-term renal transplant patients [7]. Prophylaxis of hepatitis B virus (HBV) infection was performed by systematic determination of HBV markers (HBs antigen) in all blood donors and by administration of hyperimmune anti-HBV gammaglobulins to recipients at risk. In the late 1970s, HBV vaccination became available and helped to reduce the incidence of HBV infection in hemodialyzed and transplant patients. In a recent survey of 51 patients with a renal allograft functioning for more than 10 years, 46 were found to be infected by HBV before or soon after transplantation [8]. Serial determination of HBV replication was performed in these 46 patients by measuring HBs and HBe antigen and antibody, anti-HBc antibody, HBV DNA (Spot test) and HDV markers (HD antigen and anti-HD antibody). The mean duration of follow-up was 14 years. Results are summarized in Table 1. Less than 50% of these patients achieved partial or complete clearance of the virus (HBs antigen and HBV DNA negative, normal liver function tests) while 54% showed evidence of long-term ongoing infection. Twelve liver biopsies were obtained at the end of the survey. Chronic active hepatitis and/or cirrhosis was clearly associated with the presence of HBV DNA. All the patients had conventional immunosuppressive regimen (prednisone and azathioprine) and most received a short course of anti-lymphocyte globulins for 3 weeks after transplantation. Thus, under immunosuppressive treatment some patients may develop cirrhosis of the liver despite the absence of serum HBs antigen, and HBV DNA should be regarded as a

Table 1. *HBV status of 46 initially infected renal transplant patients.*[1] *Mean duration of follow-up: 14 years*

HBsAg	HBV DNA	*n* (%)	CAH[2]	Cirrhosis
+	+	13 (28)	7 } 8/8	6/8
−	+	5 (11)	1 }	
+	−	8 (17)	0/4	0/4
−	−	20 (44)	ND	ND
		46	12	12

[1]Data computed from reference 8.
[2]CAH, chronic active hepatitis.

more reliable indicator of viral replication than other markers. The high incidence of liver cirrhosis shows that HBV infection is definitely a major risk factor of long-term immunosuppression, although it is not presently regarded as a contra-indication for transplantation. Withdrawal of immunosuppressive therapy in HBV DNA positive patients may result in acute liver failure.

Despite recent progress in the prevention of HBV infection, liver alterations still remain a common complication of long-term immunosuppression. Infection by non A-non B viruses are frequent after blood transfusion and even mild immuno-suppression is sufficient to prevent recovery. According to Laquaglia *et al.* [9], more than 70% of patients with non A-non B hepatitis develop chronic liver diseases with a mortality rate three times above that of controls, with the chief cause of death being due to extrahepatic infections. Conversely, graft survival increased among the hepatitis patients. However, the lack of markers does not allow precise assessment of the prevalence of these infections and their contribution to long-term liver failure. Cytomegalovirus chronic hepatitis has not been precisely documented although liver alterations may occur during acute infection. Finally some immunosuppressive agents exert direct or indirect toxic effects on the liver. Sequential measurements of azathioprine plasma levels after a single oral dose demonstrate abnormally high levels in some patients with macrocytosis or macrocytic anemia, leukopenia and liver dysfunction. Reduction of the doses may improve all these alterations. Cyclosporine treatment induces dose-dependent and reversible conjugated serum hyperbilirubinemia with a moderate elevation of serum alanin-amino transferase but only a minimal increase of serum alkaline phosphatase activities [10]. This cholestasis was reported to be associated with an increased incidence of biliary lithiasis [10].

Herpes virus infections

The herpes virus group comprises herpes simplex virus (HSV), varicella-zoster virus (VZV), cytomegalovirus (CMV) and Epstein-Barr virus (EBV). All four viruses share the property of latency with the presence of viral genome within particular cells (neural tissue for HSV and VZV, B lymphocytes for EBV and leukocytes for

CMV). Any seropositive individual harbours latent viruses which are capable of being reactivated under various circumstances including immunosuppression and allogeneic activation (transplant rejection, graft versus host disease).

HSV infections are frequent in transplant recipients. They are generally distributed in the perioral area and characterized by multifocal, extensive, hemorrhagic lesions often associated with painful oral ulcerations with a persistant evolution. Less commonly, anogenital infection caused predominantly by HSV type 2 may occur with large coalescing ulcerated lesions which facilitate superinfection by invasive bacteria. Eczema herpeticum, a disseminated HSV infection restricted to the skin, has been reported in patients whose skin was previously injured. HSV infections are mostly observed during the first months after transplantation whereas in the long term their frequency is similar to that of controls [11–13].

Herpes zoster occurred in 15 to 20% of our renal transplant patients. Unlike HSV infection it may occur in the long term. It is usually localized on the chest, can be gangrenous, hemorrhagic and persistant so that anti-viral treatment with Acyclovir is recommended. However it does not tend to become generalized nor does it leave post-herpetic neuralgia [13–15]. In children who were not previously exposed to VZV, primary varicella infection can be extremely severe under immunosuppression. Prophylaxis with VZV immune globulins should be initiated promptly in case of exposure to VZV.

CMV is the most frequent form of infection identified in transplant patients, being demonstrable in 60–96% of patients in the first year post-transplant [6]. The peak of onset is between 3 and 8 weeks post-transplant although some cases of clinically severe CMV infections were reported as late as 2 years post-transplant.

Primary CMV infections occur in seronegative recipients. The virus is transmitted by the transplant (90%) or by blood transfusions (10%). The peculiar severity of primary CMV infections is due to the high frequency of superinfections (*staphylococcus* septicemia, bacterial or fungal respiratory diseases), to gastro-intestinal or colonic bleeding and to the immunosuppression induced by CMV infection itself. Prophylaxis relies on donor selection according to the CMV antibody status. Administration of CMV hyperimmune globulins to seronegative recipients has been attempted in several transplantation centers but its efficacy is still controversial [16]. Reactivation of CMV infection in seropositive recipients is less often associated with symptomatic illness and is usually less severe than primary infection, although both types of CMV infection can compromise renal allograft function. Approximately 50% of transplanted patients with CMV infection will continue to excrete the virus in their saliva and/or urine 2 to 5 years post-transplant, with 20% continuing thereafter [17]. Clinical effects of such ongoing CMV infections are usually not apparent, although most of these patients have moderate leucopenia and thrombocytopenia despite much lower doses of azathioprine than in the other patients. In rare cases persistent CMV viraemia is demonstrable, often associated with progressive chorioretinitis [18]. Such long-term complication was not observed in our series in which the only ophthalmological complication was corticosteroid-induced cataract which occurred in 27% of the patients [19].

Clinical EBV infection proved to be difficult to evaluate in transplanted patients because of the high prevalence of CMV and the similarities in clinical manifestations of CMV and EBV infections. Mononucleosis-like syndromes with hepatitis and

pneumonia have been attributed to EBV in transplant children [11] whereas in adults, fever, leucopenia atypical lymphocytes and pulmonary infiltrates may occur in the absence of any evidence for CMV [20, 21]. Whatever the clinical manifestations, EBV reactivation is demonstrated by the high rate of viral excretion, in saliva or throat washings of 50–70% of transplant patients and by the rise in antiviral capsid antigen (VCA) and anti-early antigen (EA) antibody titers. The frequency of such alterations parallel the intensity of immunosuppression. Unlike CMV reactivation, EBV replication does not seem to occur more frequently in transplant patients than in other clinical situations associated with immunosuppression. Futhermore it does not appear to be triggered by allogeneic stimulation. EBV can infect a subset of mature B lymphocytes and pre-B cells by interaction with the CR2/EBV receptor. Cell infection is characterized by the expression of EBV-associated nuclear antigen EBNA-1 and 2 and the membrane antigens Lydma. The latter is recognized by specific memory T cells present in EBV seropositive individuals. Such T cells mount a vigorous cytotoxic response against EBV-infected cells. A defect of this control mechanism may lead to the development of lymphoma, in a manner comparable to the *in vitro* continuous growth of lymphoblastoid cell lines obtained by infection of normal B cells by EBV, providing that T cells have been removed or that lymphokine production is prevented by cyclosporine. The role of EBV in the development of lymphomas after transplantation is suggested by the presence of EBV genome and the expression of EBNA antigen in tumor cells [22, 23, 24].

Lymphomas and immunoglobulin abnormalities

Two clinical categories of lymphoma have been reported in transplant patients. The first is observed in young patients who present soon after transplantation with fever, pharyngitis and lymphadenopathy developing into a fatal multi-organ lymphoproliferative disease similar to that seen in boys with the X-linked lymphoproliferative syndrome after EBV infection [23, 24]. A variant of this category was described as Infectious Lymphoproliferative Syndrome in patients with heavy immunosuppression associating antilymphocyte globulins and cyclosporine [25–27]. In two out of nine of these patients, withdrawal of immunosuppressive therapy led to recovery within 2 months. All cases were characterized by oligoclonal or monoclonal serum components and drastically reduced $CD4^+$ T-cell counts. The contribution of EBV could not be demonstrated in these cases. Lymphomas of the second clinical category in transplanted patients present as solid tumours involving the central nervous system, oropharynx, liver, small bowel, or transplanted kidney. Their clinical course is slow but fatal. They are usually polyclonal B-cell lymphomas classified as immunoblastic sarcoma or polymorphic immunocytoma [28].

The presence of monoclonal or oligoclonal immunoglobulin (Ig) serum components in the absence of characterized myeloma or lymphoma is well documented. Such abnormalities may occur especially in the absence of any characterized disease (so called 'idiopathic benign gammapathy') or they may be associated with autoimmune disease (e.g. Sjögren's syndrome), carcinoma or chronic suppurative infections, congenital toxoplasmosis or CMV infection, or after bone marrow transplantation [reviewed in 29]. Ig abnormalities can be demonstrated by isoelectric focusing on thin-layer (0.5 mm) acrylamide gel in a 3.5–9.5 pH gradient [30]. They

Table 2. *Immunoglobulin alterations in renal transplanted patients and controls*[1]

Period	Series	Treatment	*n*	Ig heterogeneity restriction	Oligo or monoclonal components	ILPS[2]
1981–83	Renal transplant	CsA + AZA + CS (± ALG)	60	11 (18)%	16 (27%)	7
1971–83	Renal transplant	AZA + CS (± ALG)	167	31 (19%)	5 (3%)	1
1983–85	Renal transplant	AZA + CS (± ALG)	100	21 (21%)	8 (8%)	2
1983–85	Hemodialyzed	0	21	0	0	0
1983–85	Viral infections	0	18	8 (44%)	0	0
1968–87	Renal transplant (> 10 years)	AZA + CS	41	8 (20%)	0	0

[1]Data computed from references 25–27.
[2]Infectious lymphoproliferative syndrome.

comprise either a restricted heterogeneity or a 'multibanding' aspect. The presence of oligoclonal components is demonstrable by immunofixation [31] or better by electrophoresis on thin layer agarose and immunoblotting as described by Briault *et al.* [Submitted for publication]. As shown in Table 2, Ig heterogeneity restriction was observed in about 20% of transplant patients, including long-term survivors receiving low doses of corticosteroids and azathioprine (Table 2). It was more frequent in viral infections without immunosuppression but did not occur in hemodialyzed patients despite immunodeficiency and T-cell activation [32, 33]. Conversely monoclonal components were more frequent in patients with major immunosuppression associating cyclosporine and antilymphocyte globulins [25–27]. A high incidence of oligoclonal components has been reported in HIV seropositive patients [31]. It is tempting to speculate on a stepwise progression in the alteration of the T-cell control of B-cell proliferation and maturation. The first step would be characterized by Ig heterogeneity restriction which may be regarded as nearly physiological. For instance the antibody response to polysaccharides is usually oligoclonal. The antibody response to murine OKT3 monoclonal antibody was reported to be oligoclonal [34], suggesting that immunosuppression could restrict antibody heterogeneity to a protein antigen, possibly by decreasing the number of functional T_H cells and thereby restricting their repertoire. The second step is characterized by the presence of monoclonal or oligoclonal serum components with multiple clonal B-cell expansion revealed by Ig gene rearrangement analysis, as described in HIV seropositive patients with lymphadenopathy syndrome but without lymphoma [35]. It is still reversible if immunosuppression can be reduced; otherwise it will progress into a truly malignant oligo- or polyclonal tumour (third step), usually associated with rearrangement or translocation of the *c-myc* oncogene [35]. Although this sequence of events presently remains partly speculative, routine monitoring of oligoclonal components in patients under immunosuppressive therapy should be performed. Occurrence of such components may correspond to an

increased risk of lymphoma, and immunosuppression should be reduced in those patients.

Papovaviruses

These viruses comprise two genera, both of which can significantly affect patients under immunosuppressive therapy: papilloma viruses (HPV) and two polyoma viruses, the BK virus (BKV) and the JC virus (JCV).

Human polyoma viruses infect most normal individuals during childhood, apparently without demonstrable clinical illness. After transplantation approximately 40% of patients either excrete one or both of these viruses or manifest a rise in antibody titre [6, 36]. BKV, JVC and the simian virus SV40 are closely related both antigenically and structurally and they are oncogenic outside their normal host [11, 37–40]. SV40 and JVC have been linked to the development of progressive multifocal leucoencephalopathy in monkeys and man, respectively [11, 37]. In transplanted patients the clinical effects of polyoma virus infection may include pancreatic disease, accelerated atherosclerosis and ureteral strictures but they have not yet been clearly delineated [38–40].

HPV infection under immunosuppression has been extensively investigated in transplant patients [12, 13–15, 41, 42]. HPV is responsible for the occurrence of common or flat warts, mucosal papillomas and *condylomata acuminata*. Such lesions occur in up to 50% of renal transplanted patients [12] and the onset is rather late, generally after one year of immunosuppression and sometimes later. The lesions are often multiple, long lasting and quite resistant to therapy. They are localized on the hands and sun-exposed areas; plantar warts and *condylomata acuminata* are relatively rare [12, 42]. The role of sun exposure as a co-factor is clearly established [14]. Forty-one different types of HPV have been defined according to the structure of their genome and their antigenicity. DNA hybridization methodology can now be used for HPV typing and the data in immunocompromized patients demonstrates high qualitative alterations as compared with warts occurring in control groups (Table 3). HPV4, usually restricted to plantar warts, can be found in common flat warts of transplant patients. A recent study of 34 skin lesions in renal transplant patients showed that about a third of single lesions contain more than one type of HPV [Chardonnet *et al.* in preparation]. Some rare HPV types known to be oncogenic may be detected in transplant patients, as, for instance, HPV5 previously found only in patients with *Epidermodysplasia verruciformis*, a rare genetically-determined condition occurring in children with multiple flat warts, defective cell-mediated immunity and high incidence of squamous cell carcinoma [42–44]. Similarly, in one of our renal transplant patients HPV16 was identified in intra-oral papillomas [12]. HPV16 is an oncogenic HPV detected in genital carcinomas, mucous Bowen's disease and bowenoid papulosis.

Squamous cell carcinoma accounts for nearly half of the neoplasms in renal transplant recipients. Its development increases with sun exposure and with the duration of immunosuppression [45–48]. It occurs at a younger age than in the non-immunocompromized population. The average number of skin cancers per patient is two to three. The anatomic distribution is the same as in the general white population, i.e. sun-exposed areas, face, lower lip, neck, forearms and

Table 3. *Human papillomavirus (HPV) infection under immunosuppression*

	HPV DNA		HPV antigen[1]		
	Control	Transplant	Control	Transplant	Prevalence
Common warts	+ [1, 2][2]	+ [1, 2, 16]	+	±	
Plane warts	+ [3]	+ [3, 5, 8]	±	−	~48%
Plantar warts	+ [1]	+ [1]	+	+	
Condylomata acuminata	+ [6, 11]	+ [6, 11, 16]	+	+	
Keratoacanthoma	− [?]	+ [1, 2, 16, 18]	−	−	
Dyskeratosis	−	+ [16, 18]	−	−	~11%
In situ carcinoma (Bowen's disease)	+ [16]	+ [16]	−	−	~5%
Squamous cell carcinoma	−	+ [1, 2, 16, 18]	−	−	

[1]Percent labelled cells. From Rudlinger *et al.* (*B. J. Dermatol.* 1986, **115:** 681–692) and Chardonnet *et al.* (personal data).
[2]HPV types most frequently involved.

hands. Squamous cell carcinoma is often associated with multiple actinic keratoses, widespread warts, keratoacanthomas and Bowen's disease. The agressiveness and development of metastasis are much greater than in the control population [12, 46–49]. The local immune response, as assessed by the size of the cellular infiltrate, is much lower in transplant patients than in patients without immune deficiency [50]. The possible role of HPV in squamous cell carcinoma is suggested by the demonstration of HPV genoma or HPV antigens in tumours in immunosuppressed patients. HPV18 was detected by *in situ* DNA hybridization in five out of 12 squamous cell carcinomas and one or several HPV were present in 75% of these cancers in transplant patients [Chardonnet *et al.* in preparation].

Adenovirus

These DNA viruses are widespread in the normal population in whom primary infection is most often asymptomatic or associated with upper or lower respiratory tract manifestations, conjunctivitis or hemorrhagic cystitis. Some transplanted patients were reported to develop infections due to adenoviruses types 34 and 35 previously not recognized, associated with interstitial pneumonia or hemorrhagic cystitis [6, 11, 51–53]. Since adenoviruses have been shown to be potentially oncogenic in some species, the role of these viruses, particularly the uncommon types, deserve extensive investigation in immunosuppressed patients.

Kaposi's sarcoma

This type of tumor has not so far been associated with any known infectious agent and it is not regarded as a classical opportunistic infection. However, indirect evidence

would suggest the possible contribution of a viral infection as a co-factor in the development of Kaposi's sarcoma (KS). Transplant patients with KS have high titers of anti-CMV antibodies [54] and CMV genoma was identified in the tumor by DNA hybridization as were CMV antigens by immunohistochemical techniques [55]. The occurrence of KS in patients with the acquired immunodeficiency syndrome (AIDS) led to the hypothesis of a possible role of a retrovirus, especially HIV1, in the generation of the neoplasia, but the search for HIV genes in the cells of KS remained negative [56]. More recently the marked similitude of KS lesions with those of avian hemangiomatosis induced by a fowl retrovirus of the avian lymphoid leukosis group [57] has led to the hypothesis that KS might be associated with a still unrecognized transmitted retrovirus [58].

In fact quite different clinical and epidemiological forms of KS can be recognized (Table 4): (1) the classical Mediterranean, (2) the endemic African, (3) the epidemic HIV1-related, and (4) the iatrogenic form of KS, complicating long-standing immunosuppression without HIV infection. The iatrogenic form of KS occurs in patients treated with immunosuppressive drugs. Among the several associated factors needed for KS to develop, of particular importance are: (1) the nature of the underlying disease (renal transplantation, autoimmune diseases, malignancies), (2) the type of immunosuppressive drugs (combination immunosuppressive therapy or single drug) and (3) geographic and genetic factors. In renal transplantation, combined immunosuppressive therapy with azathioprine, corticosteroids, cyclosporine and/or anti-lymphocytic globulins is associated with a risk of development of KS [59]. However the frequency of KS would seem to be higher in patients treated with cyclosporine than in those under conventional therapy [54]. As is the case for the development of lymphoma, this difference reflects greater immunosuppression achieved by this drug than an oncogenic risk associated with this type of molecule. KS has been described in patients suffering from immunologically-mediated diseases and treated with steroids alone. Such diseases included systemic lupus erythematosus, rheumatoid arthritis, polymyositis, temporal arteritis, *polymyalgia rheumatica*, bullous pemphigoid, pemphigus and hemolytic anemia. The role of corticosteroids in the development of KS has been suggested by the temporal relation between institution of therapy and onset of the tumor. Furthermore, in a number of cases KS improved upon withdrawal of therapy. The mean duration of therapy before development was longer in patients given corticosteroids therapy alone as compared to those given combination immunosuppressive therapy (61 months *vs* 16 months, respectively) [60].

In addition to renal transplantation and autoimmune diseases, iatrogenic KS may also occur in malignancies, especially in those of mesenchymal origin (leukemia, lymphoma, thymoma, multiple myeloma). Similarly, KS was described in hemodialyzed patients. all these clinical situations are characterized by some type of immune defect, especially a deficiency of T-cell responses as demonstrated *in vivo* (contact hypersensitivity, delayed-type hypersensitivity) and *in vitro* (alteration of T-cell subsets or T-cell responses). The relationship between KS and immunosuppression is further suggested by the improvement of KS after withdrawal or reduction of immunosuppression [54, 60, 61]. Nonetheless, considering the large number of immunosuppressed patients and the relatively low frequency of iatrogenic KS, it is obvious that non-immunological factors

Table 4. *Comparison of the various forms of Kaposi's sarcoma*

Form	Geographical distribution	Skin lesion distribution	Visceral involvement	Response to therapy		Prevalence
				Classical[1]	α-IFN	
Classical	Mediterranean	Extremities	Rare	+++	?	0.02%
Endemic	African	Diffuse	Frequent	+	?	9%
Epidemic (AIDS)	Same as HIV1 infection	Diffuse	Frequent	0	++	20% (homosexuals)
Iatrogenic	Mediterranean	Extremities	Uncommon	+[2]	?	?

[1]Vincaleucoblastin and/or radiotherapy.
[2]Irradiation only.

are important in the occurrence of this tumor. Ethnic factors seem to be of major importance. In this respect it is noteworthy that iatrogenic KS, like the classical form of KS, occurs preferentially in patients originating from Mediterranean countries and less frequently in patients of other geographical origins.

Conclusions

Most of the viral infections occurring in long-term immunosuppressed patients may play a role in the development of tumors. This hypothesis is supported by sero-epidemiological data, as well as by DNA hybridization with tumor cells in the case of hepatoma (HBV), lymphoma (EBV), warts (HPV) and squamous cell carcinoma (HPV). It still requires further demonstration for CMV, non A-non B virus, BKV, JVC and adenoviruses. It may be postulated that T-cell mediated immunity contributes to the latency of these viral infections, and that the failure of this control in the immunocompromized host facilitates reactivation and chronic viral infection. The frequent occurrence of similar viral infections and tumors in AIDS patients provides further indirect evidence in support of this view. No single immunosuppressive agent can presently be held responsible for such complications. The duration of immunosuppression respresents a definite risk for hepatoma in HBV-infected patients, for lymphoma and especially for tumors associated with HPV. Conversely, profound immunosuppression induced by combined treatment with cyclosporine and anti-lymphocyte globulins should be avoided in view of the risk of early lymphomas or infectious lymphoproliferative syndromes. Systemic monitoring of oligoclonal serum components and $CD4^+$ circulating T cells may provide a clue to the identification of patients at highest risk of lymphoma. Withdrawal or reduction of immunosuppressive treatment may prevent the development of B-cell malignancies in such cases. Similarly, careful clinical monitoring of skin lesions and typing of HPV by DNA hybridization should help to define high-risk patients. Conversely, in the case of HBV or non A-non B infection, most efforts should be devoted to prophylaxis of the primary infection. Indeed the possible positive effects of anti-viral agents in chronic active viral hepatitis are still under investigation and immunosuppressive treatment cannot be withdrawn in view of the risk of massive immune destruction of infected hepatocytes.

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Approach to the Use of Antigen Non-specific Immunosuppression in Systemic Lupus Erythematosus and Other Rheumatic Autoimmune Diseases

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Optimum therapy of systemic lupus as well as the other autoimmune rheumatic diseases should be based upon pathogenetic considerations. We present an hypothesis as well as supporting data that the B-cell repertoires of individuals with both murine and human lupus initially are relatively normal. Even though there is increased antibody production, it is increased proportionally for many specificities. Later, more selective increases lead to selective representation of certain specificities characteristic of that individual.

Therapy during the polyclonal phase is most likely to be effective in bringing about a sustained remission. Such therapy should be directed at preventing or interfering with the polyclonal activation. Later in disease, after particular immune responses become relatively augmented and possibly fixed, it may be necessary also to interfere directly with the specific augmented responses, especially those which are found to be inducing disease.

Whereas SLE is an antibody-mediated disease with decreased DTH, other autoimmune diseases are mediated less by antibody and more by DTH. As a result, therapies for those diseases, especially those directed at the DTH, should be based upon principles other than those for SLE. Moreover, there is a greater likelihood in the other diseases of antigen-specific therapy and even vaccination for prevention.

Introduction

The rheumatic autoimmune diseases have been treated with antigen-non-specific immunomodulatory drugs with some success for many years. The most highly regarded agents have been corticosteroids, and the cytostatic and cytotoxic drugs.

Although such therapy has been life saving and organ preserving in many individuals, there has been an underlying assumption that antigen-non-specific treatments are a stop-gap type approach until more specific and more definitive treatments are developed. It has been assumed that as more becomes known about the autoimmune diseases, antigen-specific or organism-specific approaches to management would obviate the use of the cytotoxic or cytostatic immunosuppressive drugs currently in use.

However, it is possible that for some diseases the antigen-non-specific therapy will still play a useful role in the distant future by bringing a disease 'under control' prior to more directed therapy. For example, a patient with rheumatoid arthritis and very active disease might be treated with non-specific immunomodulatory agents to 'quiet down the disease process' before pinpoint intervention. On the other hand, even in the future, it is possible that there may be no antigen-specific therapy for a disease like systemic lupus erythematosus (SLE). That is not to say that it will not be possible to identify individual pathogenic autoantibodies and devise ways to reduce their quantity specifically. Instead, it is raising the possibility that the disease may not, *in toto*, be the result, in all patients, of a response to a single specific antigen, and therefore, might not be amenable to solely antigen-specific therapy.

In this paper we will try to place current practices of antigen-non-specific immunomodulation into practical and theoretical frameworks. Our hope is that such a presentation will stimulate others to develop better therapeutic regimens for these common and debilitating diseases as well as apply the ideas to other disorders.

A commentary on the pathogenesis of SLE

Patients with SLE produce large amounts of antibodies reactive with epitopes on a variety of nuclear, cytoplasmic, and cell suface antigens. In most individuals with SLE, such reactivity stems from polyclonal antibody production. Despite the discovery of cross-reactive autoantibodies, most patients do not appear to have oligoclonal expansion of a very few highly cross-reactive antibodies. Moreover, despite the occurrence of some highly cross-reactive autoantibodies, these may not preferentially be associated with lupus [1]. That is, lupus may have proportional expansion of cross-reactive antibodies along with proportional expansion of many other antibodies.

The lupus literature contains data suggesting that autoantibody production may be antigen driven and T-cell dependent [2–5]. A good example of such a mechanism for autoantibody production is the anti-Sm response of MRL mice [2]. Spontaneous anti-Sm antibody production is quite limited in young MRL mice, but is markedly augmented in the face of antigen administration and antigen-dependent helper T cells, factors which also act to increase anti-Sm spontaneously as the mice age. The increase in affinity and isotype switch from IgM to IgG of anti-DNA antibodies which are associated with active lupus also support the view of an antigen-directed process. On the other hand, lupus often is characterized by a degree of polyclonal B-cell activation (Table 1) not usually found in antigen-specific immune responses [6]. Patients have a marked increases in numbers of circulating activated and Ig-producing B cells [6]. Moreover, they have a marked increase in numbers of B cells producing antibodies reactive with a panel of haptens to which the patients were

Table 1. *Numbers of Ig-secreting cells (IgG + IgA + IgM) in the peripheral blood are increased markedly in patients with active SLE*

Group	Number	Ig-secreting cells/10^4PBMC
Controls	12	3
Inactive SLE	12	6
Active SLE	10	37

Table 2. *Patients with SLE have a marked increase in numbers of peripheral blood mononuclear cells (PBMC) producing antibody reactive with chemical haptens (PFC by local hemolysis in semi-solid agar) to which the patients had not been exposed purposefully*

		Number of Ig-secreting cells/10^6 PBMC reactive with chemical haptens	
Group	Number	IgM	IgG
Controls	8	31	2
Inactive SLE	10	110	14
Active SLE	9	479	79

not immunized (Table 2) [7, 8]. Such data have suggested a polyclonal activation mechanism for much of the antibody production found in patients with SLE [9–11]. Determining the relative contributions of polyclonal B-cell activation and antigen-driven responses might be important for designing therapeutic approaches to lupus. A limited number of antigen-specific responses might be treated ultimately by interfering with those responses specifically. On the other hand, if polyclonal B-cell activation underlies disease, a more antigen non-specific immunosuppressive strategy could be required.

Recent studies from our laboratory suggest that polyclonal B-cell activation plays a critical and major early role in the heightened autoantibody production state which characterizes murine lupus. Although all of the autoimmune-prone mouse strains have an absolute increase in numbers of B cells producing antoantibodies [12, 13], when corrected for the increase in total numbers of Ig-secreting cells and expressed on a percentage basis, all of the autoimmune-prone strains had relatively normal repertoires [14, 15]. That is, the percentage of the Ig-secreting cells producing antibodies reactive with the panel of self-antigens (DNA, T cells, myosin, actin, transferrin, etc.) or conventional antigens (TNP-KLH, ovalbumin) was the same in autoimmune-prone and non-autoimmune-prone strains (Table 3). Thus, 1% of the Ig-secreting cells of an NZB mouse were producing anti-DNA; similarly, 1% of the B cells of an NZB.*xid* mouse also were producing anti-DNA [14]. However, since NZB mice have thirty-fold more Ig-secreting cells they have a greater total number

Table 3. *Expressed B-cell repertoire (shown as a percentage of the total number of Ig-secreting cells) of autoimmune and non-autoimmune mouse strains in the ELISA-spot assay using several self and foreign antigens*

Strain	T cells	DNA	Actin	Transferrin	Ovalbumin	TNP-KLH
NZB	1.8	1.1	0.5	0.03	0.26	0.6
MRL-*lpr*	0.7	1.5	0.2	0.02	0.03	0.6
BXSB	2.3	1.2	0.8	0.14	0.19	0.5
DBA/2	0.6	1.2	0.4	0.02	0.06	0.4
BALB/c	0.9	1.8	1.0	0.19	0.18	0.5

of anti-DNA-secreting cells (and greater serum anti-DNA). They also have more cells spontaneously producing antibody reactive with ovalbumin and TNP-KLH. The same is true comparing C57BL/6-*lpr*/*lpr* mice with C57BL/6 − +/+ mice. The data suggest, contrary to our *a priori* views (based especially upon serum antibody titers), that the autoimmune-prone strains have B-cell repertoires which are not especially skewed towards autoantibody production—rather they have a much greater total number of autoantibody producing cells with a distribution of reactivity not much different from that of non-autoimmune-prone strains.

In addition to the repertoire studies, considerable evidence has been advanced to suggest that the polyclonal B-cell activation may play an important role in autoantibody production in murine lupus. There are reports of helper factors, reductions in suppressor factors, and effects of T cells (probably via factor production) acting to augment proliferation and/or antibody production [10, 16–20]. These serve to provide insights into the mechanisms by which the polyclonal B-cell activation may be generated. Moreover, some of the data [21, 22] which have been used to support the hypothesis of a primary B-cell abnormality are also compatible with polyclonal B-cell activation resulting from a non-B-cell activity present very early in life.

How does such a formulation account for the anti-Sm response? Recent studies have evaluated anti-Sm along with the other autoantibody responses in the repertoire studies. Anti-Sm was 'an exception which proves the rule.' Approximately 1% of the B cells of 6-month-old MRL-*lpr*/*lpr* mice were producing anti-Sm antibodies. This was similar to the percentage of B cells producing anti-DNA. However, what differed was the percentage of the repertoire devoted to those specificities at younger ages. Two-month-old MRL-*lpr*/*lpr* mice, for example, had 1% of their B cells devoted to anti-DNA whereas only 0.1% were producing anti-Sm. Therefore, between 2 and 6 months of age, MRL-*lpr*/*lpr* mice manifested a ten-fold increase in anti-Sm as a percentage of the repertoire whereas they showed no change in anti-DNA. Since there was an absolute increase in total numbers of Ig-secreting cells between 2 and 6 months of age, the absolute number of anti-DNA secreting cells per mouse rose between 2 and 6 months of age (as did serum anti-DNA); however, the percentage did not. The anti-Sm studies demonstrate that repertoire analyses can detect a substantial change in the B-cell repertoire and, therefore, could have detected a skewing toward autoantibody production in autoimmune-prone strains had there been one. This point was also made by purposefully immunizing mice with

Table 4. *Preferential expansion (day 35 versus day 1) of autoantibody-secreting cells in splenic fragment assays when small numbers (10^6) of NZB B cells are transferred to NZB.*xid *recipients*

B cells producing antibody to	Relative expansion (fold)
DNA	19
T cells	18
Bromelain treated mouse RBC	17
TNP-KLH	2
TNP-KLH after immunization with TNP-Ficoll (to which *xid* mice cannot respond)	52

either TNP-Ficoll or TNP-KLH and demonstrating a substantial skewing of the repertoire towards anti-TNP-producing B cells (15-fold increase after priming and boosting with TNP-KLH). It is recognized, of course, that repertoire studies provide little information about pathogenic versus non-pathogenic antibodies; nevertheless, such studies do allow assessment of a general skewing towards production of autoantibodies in general or towards production of antibodies having reactivity with a particular antigen.

Evidence for antigen-driven responses

Despite the persuasive evidence from the repertoire studies for polyclonal B-cell activation, as well as evidence for polyclonal immune activating factors [10, 16–18], it remains possible that many of the B cells producing antibodies reactive with self-determinants (as well as those producing antibodies with foreign determinants) are being driven by antigen. In other words, it is possible that the B cells are activated by antigen (perhaps in conjunction with helper T cells) and merely expanded by polyclonal B-cell activation. Such a formulation would still hold polyclonal B-cell activation as the critical reason for increased numbers of B cells producing auto-antibodies without abandoning the idea of an autoimmune response.

We have addressed this question by performing transfer studies from unmanipulated autoimmune or non-autoimmune prone strains of mice to unmanipulated congenic *xid* mice. The first such studies involved the transfer of small numbers (1×10^6) of splenic B cells from NZB to NZB.*xid* recipients. The transferred B cells were able to 'take' in the recipients and expand over a 4- to 6-week period [23, 24]. Since the recipient *xid* mice had very few anti-DNA producing cells and produced very little serum anti-DNA, essentially all of the anti-DNA in the recipients came from the donors. We noted that the number of anti-DNA-producing cells increased exponentially over the first 4–6 weeks after transfer of NZB spleen cells to NZB.*xid* recipients. Similarly, B cells producing antibodies reactive with mouse T cells and bromelain-treated mouse RBC increased dramatically (Table 4). In contrast, cells producing antibodies reactive with TNP-KLH increased much more slowly. Immunization of the mice with TNP-Ficoll, an antigen to which *xid* mice do not respond, produced a marked increase in anti-TNP-producing cells (Table 4). Since

Table 5. *It is the NZB internal milieu (present also in the NZB.*xid *mouse) rather than an intrinsic defect of NZB B cells which primarily is responsible for the expansion of autoantibody-producing B cells when small numbers of splenic B cells are transferred to* xid *recipients*

		Serum autoantibody levels in recipients		
B-cell donor	Recipient	Anti-DNA	Anti-T cell	Anti-BrMRBC
DBA/2	DBA/2.*xid*	2[1]	3	3
NZB	DBA/2.*xid*	2	2	2
DBA/2	F_1.*xid*	9	17	10
NZB	F_1.*xid*	11	29	12
DBA/2	NZB.*xid*	45	34	27
NZB	NZB.*xid*	56	58	61

[1]Antibody quantities are presented in arbitrary units using unmanipulated *xid* mice as baseline controls. The antibody quantities in the three different assays have been normalized so that the antibodies reactive with the different antigens can be directly compared.

xid mice do not respond to this antigen, we believe that the increase represented an effect of donor cells responding to the immunization. These data lead us to suggest that the expansion of antibody-producing cells in the recipient is antigen dependent: autoantibody-producing cells expand dramatically because self-antigens are available in the recipient.

Since self-antigens are so readily available and NZB mice can respond so easily to them, why then is the repertoire of NZB mice not skewed toward autoantibody production? We presume that the acute experiments described in Table 4 are just that—acute experiments—and that eventually environmental antigens even out the repertoire by inducing other B cells to produce antibodies of other specificities. That is, eventually enough antigens cross-reactive with such determinants as TNP induce TNP-reactive B cells to proliferate and produce antibody, as occurred when we purposefully immunized mice with TNP-Ficoll. Alternatively (perhaps in addition and playing a critical role in all NZB immunity, see Table 5) factors in the NZB mice might drive B-cell maturation very rapidly so as to overrepresent germ line encoded specificities relative to those induced by immunization, either endogenous or exogenous.

To determine whether or not the transfer experiments were unique to NZB mice, we repeated the experiment using DBA/2 donor B cells and DBA/2.*xid* recipients. The result was an increase in numbers of antibody-producing cells over time; however, the increase occurred much more slowly than when NZB B cells were transferred to NZB.*xid* recipients. We wished to determine whether the increase in NZB B-cell expansion resulted from an intrinsic hyperactivity of NZB B cells or from an excessively stimulatory environment of the NZB mouse which is shared by the NZB.*xid* mouse (it should be noted that the *xid* gene does not alter the T-cell abnormalities of NZB mice [25], it primarily decreases the terminal maturation of the B cells [26]). To address this issue, we transferred either NZB B cells or DBA/2 B

cells to (DBA/2 × NZB) F_1 recipients. The result was equivalent expansion of autoantibody-producing B cells and comparable levels of serum autoantibodies (Table 5). This result, combined with the previous data, pointed to the NZB internal environment (presumably containing excesses of B-cell stimulatory factors) as the explanation for the excess expansion of autoantibody-forming cells in NZB.*xid* recipients. Additional evidence in favor of this possibility came from transfers of NZB or DBA/2 cells into NZB.*xid*, DBA/2.*xid* or F_1.*xid* recipients. The result was greatest expansion of cells in NZB.*xid* recipients regardless of donor and least rapid expansion in DBA/2. *xid* recipients regardless of donor. The data support the view that the autoantibodies of NZB mice result primarily not from an intrinsic B-cell defect, but rather from a very stimulatory internal milieu.

Although this conclusion might appear to be at odds with a prior study employing bone marrow donors and lethally irradiated recipients, which concluded that NZB marrow was responsible for increased IgM [27], in fact it is not at all contradictory. Rather, the two types of studies provide different and mutually complementary information. The transfers of splenic B cells to unmanipulated *xid* recipients measure effects of the recipient internal environment on the donor B cells directly, whereas the marrow reconstitution studies especially assess stem-cell activity which is excessive in NZB mice [28]. Moreover, it is possible that marrow elements contribute to the NZB internal milieu which promotes B-cell hyperactivity.

There does appear to be a small contribution of NZB B cells to the amount of antibody as seen in Table 5. This relatively less important B-cell effect resulted probably from the prior exposure of NZB donor B cells to factors in the NZB mouse prior to transfer [29], but conceivably could derive from a special property of NZB B cells or a subpopulation thereof. Nevertheless, the data in Table 5 emphasize that the NZB internal environment appears to have a quantitatively important effect on expansion of autoantibody producing cells.

A unifying view of autoantibody production

The repertoire studies and the transfer studies together allow us to put together a coherent story regarding the pathogenesis of autoantibody-producing cells in murine lupus. We believe that the B-cell repertoire develops in normal and autoimmune strains on the basis of stimulation by antigen both of germ line encoded immunoglobulins (as receptors for antigen on B cells) and those which have mutated from germ line configuration. A substantial portion of the repertoires of both normal and autoimmune strains are devoted to autoantibody production, approximately 1% to anti-DNA, another 1% to anti-T cell, and 1.5% to anti-bromelain-RBC. The increase in numbers of autoantibody-producing cells in murine lupus results not from a skewing of the repertoire but from an increase in total numbers of Ig-secreting cells of which autoantibody-producing cells are a relatively constant fraction. Therefore, a primary underlying problem in the generalized autoimmunity we call the lupus syndrome is polyclonal B-cell activation. It is upon this fertile soil for humoral hyperimmunity that the autoimmune disease arises. Those B-cell specificities already present in the repertoire, usually expanded on the basis of antigen-stimulation, are the ones most readily able to participate in the polyclonal expansion. In addition, the polyclonal activation may serve to interfere with self-tolerance

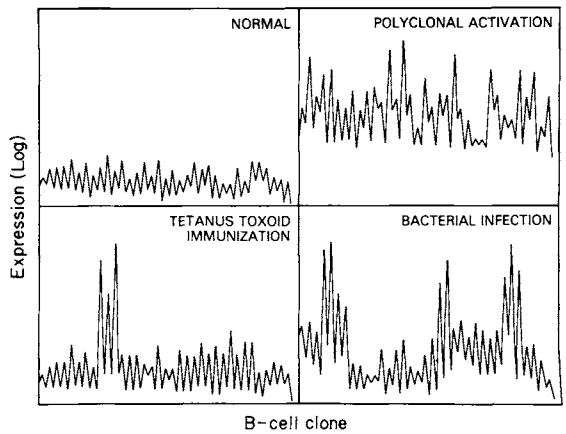

Figure 1. Schematic of different B-cell repertoires. Individual clones of B cells are arrayed along the horizontal axis and the production of immunoglobulin by that clone expressed on the vertical axis on a log scale. However, since there is not enough room for thousands of B-cell clones on the horizontal axis, we have depicted only one clone, the antibody response to a given epitope, and only one epitope for a given antigen. We recognize that the immune repertoire is much more diverse, but it would be difficult to depict that extreme diversity and rely on the reader to extrapolate from these simplified diagrams.

The upper left hand diagram depicts a normal B-cell repertoire. Severe extreme polyclonal B-cell activation in the upper right hand corner is manifested by a marked increase in expression of all of the specificities normally produced. In contrast, immunization with a specific antigen, such as tetanus toxoid, stimulates a minority population of B cells. A somewhat larger population of B cells is stimulated by a bacterial infection; this results in stimulation of a sufficient number of B-cell clones to be manifested as stimulation of some anti-self clones. We believe that this type of stimulation is very important in inducing either the first or subsequent (flares) clinical manifestations of SLE in individuals with an underlying immune abnormality predisposing to SLE.

mechanisms and, thereby, predispose to the ultimate production of pathogenic autoantibodies.

The above omits certain considerations which may be important: (i) The repertoire studies cannot distinguish easily antibodies of different fine specificity, idiotype, charge, etc. (ii) Whereas mice are short-lived, humans are long lived, and disease may be characterized by remissions and exacerbations. (iii) Many pathogenic antibodies may be IgG rather than IgM and the repertoire studies may be weighted in favor of the latter isotype. These will be addressed briefly. It is true that a very small subpopulation of antibodies with unique fine specificity, etc. may be critical to disease and that these would be missed in the repertoire studies. The repertoire studies address aspects of the pathogenesis of hyper-autoimmunity rather than the details of disease induction: we will address disease toward the end of the paper.

Humans with SLE may have a substantial preclinical time for developing autoantibodies, frequently undergo remissions and exacerbations, and nowadays live a long time. Therefore, the formulations from the short-lived mice may only represent the first step in a more complex process. To address the likely human repertoire counterparts at a more theoretical level (though based upon the data that are available, many of which are from our group but unpublished) we put forth Figures 1–3. Figure 1 depicts the B-cell repertoires of people in the basal state, after immunization with tetanus toxoid, after immunization with a bacterium, and after a polyclonal immune activator. The evolution of the B-cell repertoire of a patient with SLE is

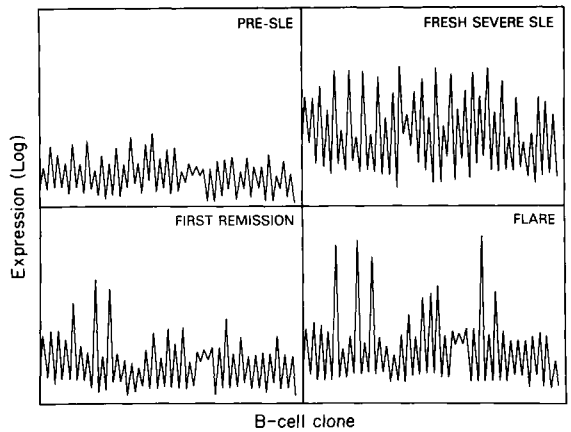

Figure 2. B-cell repertoire in a patient with SLE. This figure is arranged as in Figure 1 with the same proviso with regard to simplification. Prior to the predisposition to SLE, this individual may have a relatively normal repertoire. However, the immune system, perhaps very early in life in some individuals, subsequently becomes activated so that polyclonal B-cell activation results. A mild or moderate degree of polyclonal B-cell activation may precede overt clinical disease for years in some individuals. In others, clinical disease may occur shortly after the induction of polyclonal immunity. In the figure, the polyclonal phase of the disease is emphasized in the upper right hand corner. In this individual, there is sufficient production of antibodies of the proper specificities (? also isotype, charge) to mediate clinical symptomatology. This leads to treatment which induces the first remission (lower left hand corner). However, after reduction in drug dosage, there is a subsequent flare. Although some degree of polyclonal B-cell activation is present, it is not as prominant as in the initial polyclonal phase of the disease (perhaps due to the continued low-level treatment or the lesser strength of the inducer of the flare). However, several clones have begun to dominate the B-cell repertoire and certain specificities will tend to dominate the antibody production in the future, as shown in Figure 3.

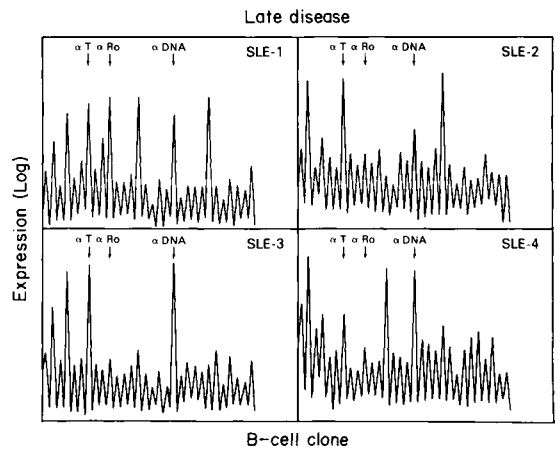

Figure 3. The B-cell repertoires in the latter, fully developed stages of SLE, simplified as in Figure 1. Late in disease, polyclonal B-cell activation may be obvious in some patients; however, in others there is continued disease activity in the absence of hypergammaglobulinemia or other signs of polyclonal activation. This pattern of autoantibody-production without hypergammaglobulinemia frequently is characterized by substantial differences among patients with regard to excessive autoantibody production. That is, some patients will have very high serum titers of anti-DNA and anti-T cell antibodies and very low levels of anti-Ro (SSA). Others may have high anti-Ro and much lower levels of anti-DNA. These differences result from stochastic process in the generation of immunity, genetic factors, hormonal effects, and the environmental antigens to which the particular individuals are exposed. The result is dominance of different specificities in different patients which often results in different manifestations of disease.

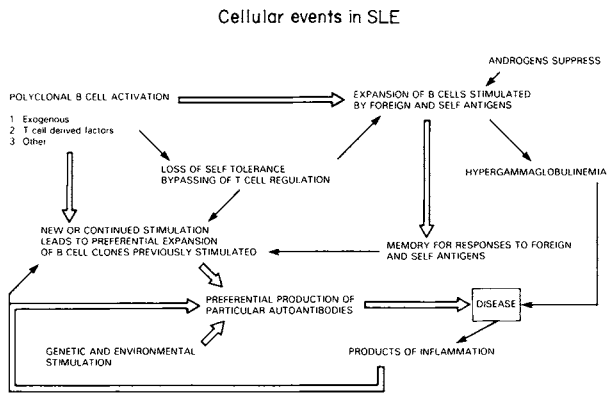

Figure 4. Cellular events in patients with systemic lupus. Shown are many of the factors which lead first to polyclonal B-cell activation and later to expansion of B-cell clones devoted to particular autoantibody responses. Our prejudices are shown by the use of a double (broad) arrow for pathways we believe are especially important. This schematic allows one to visualize the dynamic processes which underlie Figures 1–3.

shown in Figure 2. An initial polyclonal B-cell activation becomes relatively skewed towards one or another set of B-cell clones by virtue of a combination of genetic predisposition, antigenic stimulation, and random processes. By the time disease is clinically obvious, people with SLE may differ substantially as shown in Figure 3. The processes by which such a state may occur are outlined in Figure 4.

It is also worth noting that a switch from inactive to active SLE is associated with both an increase in total numbers of Ig-secreting cells [6] and also a switch from IgM to IgG antibody production [8, 11, 30–32]. The switch to IgG antibodies substitutes a long-lived isotype for a much shorter-lived one. Therefore, even without any change in total numbers of Ig-secreting cells, there would be a substantial increase in serum autoantibody titers. This effect of isotype switch must be kept in mind not only with regard to pathogenesis but also in designing therapy.

Undiscussed aspects of SLE pathogenesis

In the interests of brevity we have omitted discussion of aspects of SLE which may be critical to pathogenesis. These include (i) rapid generation of antibody-producing cells very early in life with consequent impaired tolerance induction and excess ability to mount pathogenic anti-self responses, (ii) transplacental transmission of antibodies which induce antoantibodies in children, (iii) idiotype regulation [which also may be important for (i) and (ii)], and (iv) inability to manifest suppressor function adequate to prevent or subsequently to down-regulate excess B-cell activity (we touch on this later).

Approaches to therapy of SLE based upon pathogenetic considerations

The above discussion provides a good framework for analysis of therapy. At the stage of polyclonal B-cell activation, the dictated therapy would appear to be removal of

the polyclonal B-cell activator(s) or counteracting the effect of the polyclonal stimulation. Thus, if an exogenous polyclonal activator were the culprit (as may occur in certain forms of so-called drug-induced lupus), removal would be expected to improve markedly or even ameliorate the process. If an endogenous activator is operative, identification might allow a specific counter. For example, excess production of a cytokine might be treated by agents which reduce the secretion, binding to target, or effect of that cytokine. Such therapy would be antigen-non-specific, but still might be quite directed and physiologically specific. However, if a state of polyclonal B-cell activation is determined, but the cause remains elusive, more general antigen-non-specific immunosuppression may be necessary. In some individuals it might be possible to measure various likely components of the pathways to polyclonal B-cell activation and still interfere with a particularly active pathway. In others, the only course available may be more generalized immunosuppression—interfering with final common pathways of B-cell activation and/or differentiation.

Interfering with the disease process at the stage of polyclonal B-cell activation may prevent emergence of dominant B-cell responses to particular autoantigens. Vigorous treatment at this earlier stage may produce a much higher frequency of 'cures' or 'sustained remissions' than either later intervention or more modest early treatment. We have observed that cytotoxic drug therapy of patients presenting wth lupus nephritis and the greatest degree of polyclonal B-cell activation (and highest anti-DNA) has led to the highest probability of a sustained remission, even though such patients often had the most severe multi-organ systemic illness. In other words, among patients with SLE and renal disease, early therapy of patients with the highest anti-DNA led to the best responses. A series of similar observations have been made in murine lupus where it has been possible to prevent disease emergence by treatment with any of a great variety of agents during the period of polyclonal B-cell activation [e.g. ages 4–6 months in (NZB × NZW) F_1 mice] but much more difficult to treat after that [33].

This discussion does, however, remind us that murine lupus is a disease in which the information for abnormalities in the lymphocytes are present in the bone marrow stem cells [34]. Patients with SLE also manifest excessive bone-marrow activity, especially when the disease is active [11]. As a result, therapy which kills marrow cells may have an ameliorating effect by virtue of the non-specific reduction in numbers of mature cells generated. This effect of killing some of the marrow cells may be temporarily effective in reducing the load of disease-producing cells and ameliorating disease, but may not as reliably bring about long-term remission after the stage of primarily polyclonal B-cell activation gives way to the more antigen-specific phase of the autoantibody response.

When there is emergence of a limited number of specificities dominating the immune repertoire of a given patient, it may be possible to direct therapy in an antigen-specific manner. This might include approaches to the antigen-specific response or to a limited part of that response which was found to be pathogenic, e.g. by idiotype intervention. However, even in this situation, it may be necessary to include some form of antigen non-specific therapy to bring the disease under better control or shift the immune system away from a very activated state so as to allow the specific therapy to be employed effectively. However, if several specificities or several idiotypes are involved, it still may be necessary to focus on more antigen non-specific

Table 6. *Preservation of renal function by immunosuppressive drug therapy of patients with lupus nephritis*

Therapy	Time for 25% of group to reach renal failure
1. Prednisone-H	60 months
2. Azathioprine + prednisone-L	40 months
3. Cyclophosphamide (PO) + prednisone-L	> 160 months
4. Cyclophosphamide (PO) + prednisone-L + azathioprine	> 160 months
5. Bolus cyclophosphamide (IV) + prednisone-L	> 160 months

H, high dose prednisone; L, low dose prednisone (0.5 mg/kg/day which was tapered rapidly to about 0.25 mg/kg every other day or less); PO, oral; IV, intravenous, typically 0.5 to 1.0 G/m^2 every 3 months after an initial 3-monthly doses.

approaches to therapy which can act at a final common pathway of B-cell activation and/or effect.

Current approaches to immunosuppression in SLE

A number of cytostatic and cytotoxic drug regimens have been tried in patients with SLE over the past 4 decades. These have included such drugs as 6-mercaptopurine, 6-thioguanine, azathioprine, chlorambucil, nitrogen mustard, cyclophosphamide, methotrexate, and cyclosporine as well as corticosteroids [35, 36]. In addition, lymphoid ablative procedures have been used: thoracic duct drainage, leukapheresis, splenectomy, thymectomy, and total nodal irradiation [35]. All of these therapies have been designed to reduce the immune–inflammatory state characteristic of the patient with active lupus. Many patients receiving these treatments have had improvement in the lupus syndrome. The best documented has been the benefit to patients with lupus nephritis of therapy with such drugs as azathioprine and especially cyclophosphamide (Table 6) [37, 38]. However, all of the above non-specific treatments have been associated with undesirable effects in a substantial percentage of patients. In general, the more effective the drug regimen the greater the toxicities of therapy.

It appears that a problem in the antigen-non-specific therapy of SLE is not the lack of possible treatments, but, rather, the lack of minimally toxic treatments. Very high doses of corticosteroids are effective in the great majority of patients with SLE but are too toxic to be administered for long periods of time. If one could give 2 grams of methylprednisolone daily (the way one gives 2 grams of acetylsalicylic acid daily—6 aspirin/d) with minimal toxicity in only a small minority of patients, much less attention would have to be given to the problems of patients with rheumatic autoimmune diseases. The same would hold if 2 grams of cyclophosphamide were non-toxic. Alas, not only are these doses lethal, but even much lower doses have unacceptable side effects. In fact, the treatments currently available do not allow us to

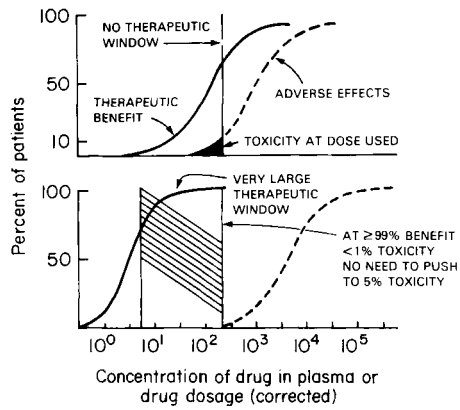

Figure 5. The therapeutic window in rheumatology. The concentration of drug in plasma or corrected drug dosage is shown in arbitrary units on the horizontal axis. The vertical axis represents the percentage of treated patients experiencing benefit (solid line) or toxicity (dashed line) at each drug dose. If our arbitrary goal is to benefit at least 75% of patients while inducing toxicity in less than 5%, the drug used to generate the upper two curves is totally without a therapeutic window: approximately 10% of patients experience unacceptable toxicity before 75% are benefitted. This is the type of therapeutic window available for drugs used in the treatment of moderately severe to severe autoimmune rheumatic diseases. In contrast, the lower two curves allow a good response to be achieved in 99% of patients before as many as 1% experience toxicity. This type of curve is available for the treatment of many bacterial infections with antibiotics, but is not available for such drugs as cyclophosphamide or prednisone in autoimmunity. The challenge for therapeutics of autoimmune diseases is to develop interventions with therapeutic windows resembling that shown in the lower half of the figure.

design a therapy for SLE which will benefit a substantial majority of patients and induce toxicity in a small minority.

This problem is demonstrated in Figure 5. Whereas the therapeutic window for many of the drugs we use in clinical medicine is quite large, and for others adequate, in the autoimmune–rheumatic diseases there is essentially no therapeutic window. That is, there is no dose range where the great majority of patients are treated effectively while very few suffer toxicity. Therefore, the challenge before us is the development of treatments which are at least as effective as those available today but which are much less toxic. Fortunately, modern molecular biology holds out the promise of therapeutic cytokines, cytokine analogues (which could bind to a receptor but prevent triggering), suppressor molecules, Class II MHC fragments, tailored human monoclonal antibodies to cell surface receptors, and a whole host of other molecules which might be used favorably to modulate the immune system.

A theoretical note on immunity and its modulation

In view of the possible new agents that soon may be available to our therapeutic armamentaria, we would like to close with an abbreviated and somewhat simplified theoretical discussion of immunity in relation to therapy which may serve as a springboard for future therapeutic considerations. This view is a restatement of the long observed phenomena of immunological tolerance and immune deviation. It is well known that different doses of antigen lead to different degrees of antibody production to that antigen [39]. A very low dose of antigen may be insufficient to engage the immune system. A little more antigen leads to 'low zone' tolerance. (The tolerance is functionally effective even if mediated by antigen–antibody complexes,

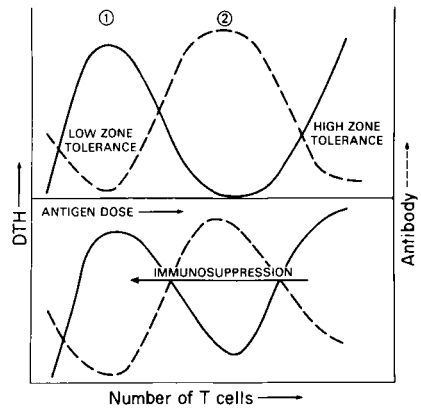

Figure 6. A theoretical view of the relationship between delayed type hypersensitivity (DTH), antibody production (Antibody), and either antigen dose (upper panel) or numbers of antigen-specific T cells (lower panel) based especially on references 39 and 40. At very low numbers of T cells, antibody production is low (analogous to low zone tolerance) but DTH becomes expressed. As antibody production increases with increased numbers of T cells (comparable in some ways to increasing antigen dose) DTH is suppressed. At high zone tolerance, antibody production is again suppressed. However, immunosuppressive drug treatment at this high T-cell number could shift the curve to the left so that there is now actually increased antibody production. Further immunosuppression might lead to low antibody but substantial DTH. Cells participating in DTH may actually suppress antibody production (illustrated by the 1 in a circle in the upper panel). Symmetrically, during maximum antibody production there may be skewing away from DTH (2 in a circle).

receptor blockade or suppressor cells or their factors.) A greater antigen dose induces a good antibody response but even higher doses lead to 'high zone tolerance.' These phenomena are shown in Figure 6. Also shown in this Figure is the idea that the more T cells the more help for antibody until one achieves suppression. Finally the Figure also depicts the concept that often there is a tendency towards a reciprocal relationship between the degree of antibody production and the degree of delayed type hypersensitivity (DTH) [40]. This relationship is well known to students of SLE because the active phase of disease is characterized by markedly impaired DTH in the face of enormous increases in antibody production [36]. Moreover, immunosuppressive drug treatment of the antibody production leads to a paradoxical increase in DTH.

This oversimplified view of immunity allows certains rough predictions regarding immune interventions. For antibody-mediated diseases, an estimate that there is an optimal antigen dose and/or degree of T-cell help suggests that reduction in antigen dose or in T-cell help will be beneficial. However, if the doses are supra-optimal, a modest degree of immunosuppression might actually worsen the condition by bringing the doses into the optimal range for antibody production. It is possible that this phenomenon is operative in the clinical situation in which some patients with active SLE treated with low doses of corticosteroids may actually worsen.

If low doses of helper T cells or antigen are present and suppressor cells of antibody production are operative, increasing the antigen dose (e.g. increasing exposure of the immune system to a self-antigen through inflammation or infection) might allow increased autoantibody production. Patients with SLE and mice with lupus have suppressor functions inadequate to control the B-cell hyperactivity

[41, 42] which may be important in allowing persistent autoantibody production. A manipulation which impairs the degree of suppression also might allow increased autoantibody production. Such a manipulation might be low doses of cyclophosphamide which could actually worsen disease.

In addition, intermittent administration of substantial doses of agents which kill rapidly dividing cells, such as stimulated B cells, would be expected to reduce markedly antibody production and benefit patients with antibody-mediated diseases. Therefore, intermittent therapy with cyclophosphamide or methotrexate would be expected to be effective. Moreover, these drugs provide a second benefit by limiting marrow stem-cell activity which is excessive in SLE and which appears to contribute to disease [34].

In constrast to the antibody-mediated diseases, those disorders mediated primarily by DTH would be expected—at an oversimplified level—to have a totally out-of-phase response to changes in antigen or helper T-cell dose. In other words, at optimal doses for antibody production, DTH would be limited; a moderate amount of immunosuppression might lead to increased DTH and worsening disease. Boluses of cell cycle specific drugs (e.g. cyclophosphamide or methotrexate) would be expected to be less effective for DTH than daily immunosuppressive therapy. Cell cycle non-specific agents would be much more effective here than for antibody-mediated diseases.

To the extent that we can learn the cellular basis for disease in a given patient, the better we could tailor current therapies or employ new ones. However, many of the patients we categorize as having a given disease may actually have a similar syndrome but different diseases with different mechanisms of action. Among patients with rheumatoid arthritis, some may have primarily DTH, some antibody-mediated disease with bystander destruction, and others various combinations as is seen in the animal models of RA [43]. Therefore, the best current treatment for one patient might be quite the opposite of that for the next. The same argument would hold for other rheumatic autoimmune diseases: a patient with polymyositis mediated primarily by cell-mediated cytotoxicity could respond very differently from a patient with prominent antibody-mediated muscle destruction.

However, as mentioned above, there is every hope that antigen-specific therapy will be available soon for the non-SLE diseases. The progress in RA research has been rapid and fruitful. The evidence implicating Epstein-Barr virus (EBV) as at least a co-factor in RA has been impressive [44, 45]. Moreover, even if specific therapy cannot be developed rapidly, a vaccine against EBV would undoubtedly markedly reduce the amount of autoimmunity humans experience [44].

If antibody and DTH tend to vary inversely, why are the same immunosuppressive drugs used for both antibody- and DTH-mediated diseases? It is largely because (i) the current immunosuppressive therapies are so diverse in action (with effects against both antibody-mediated and cell-mediated mechanisms), (ii) diseases are mediated by more than one immune mechanism, and (iii) such very large doses of drug are given (so as to drive both DTH and antibody way to the left of Figure 6) that the same drugs appear to be useful against such deverse disorders. The problem, of course, is that the drugs are so very toxic at high doses. The challenge now is to use the information available to analyze disease mechanisms in individual patients and devise much less toxic and yet substantially beneficial interventions. We hope that

these antigen non-specific therapies will be temporary, but recognize that for SLE they may be with us for a very long time.

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Immunointervention in Skin Disorders

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The original definition of an autoimmune disease propounded by Witebsky *et al.* in 1975 was (a) demonstration of a circulating antibody (b) recognition of a specific antigen, (c) production of antibodies against antigen in experimental animals, (d) production of pathological changes in corresponding tissues in sensitized experimental animals. Since that latter two involve animals and therefore species differences, the criteria have to be modified. In addition, with particular reference to skin disease, other criteria have been suggested. These are (a) production of lesions by passive transfer with antibodies or immuno-competent cells, (b) production of the lesions in organ culture experiments with serum from the patient and (c) *in vivo* demonstration of reaction between antigen and antibody.

The criteria imply that autoimmune disease is mediated in a specific pattern, i.e. with specific antibodies against a specific antigen. The criteria do not take into account other mechanisms such as abnormal T- and B-cell function or abnormal antigen processing with no measurable antibody production. Such mechanisms may well operate in diseases such as lupus erythematosus, scleroderma and dermatomyosites. In addition there is now accumulating evidence that some skin disorders, e.g. psoriasis and lichen planus may also have similar mechanisms operating in the production of the disease state. In addition, the concept of localized autoimmunity has to be considered where antibody is only produced at the site of damage as a result of cross-reactivity between an external agent and a tissue component. The implications of these suggestions are that skin disorders which in the past have been labelled as of unknown aetiology now appear to have an immune basis with autoimmunity playing an important role.

The classification of autoimmune skin disease now has to be altered to take into account current and emerging concepts of autoimmunity. It would appear reasonable to consider skin disorders with a probable immunological basis in the following groups, (a) those with a definable circulating autoantibody, (b) those disorders in which there is accumulating evidence for a local autoimmune reaction, (c) disorders which may possibly prove subsequently to have an autoimmune basis.

Disorders with circulating autoantibodies

Pemphigus and pemphigoid are the classical autoimmune skin disorders. The pemphigus antibody was first described by Beutner and Jordon in 1964 [2] and the pemphigoid antibody was described in 1965 [3]. The pemphigus antibody was originally thought to act against the 'intercellular cement substance' of the epidermal cells but now has been shown to be directed against the plasma membrane of these cells [4]. The pemphigoid antibody localizes to the basement membrane region and on immunoelectron microscopy has been detected in the lamina lucida [5], and the antigen is synthesized by the epidermal cell [6]. There is good evidence that the pemphigus antibody is of primary pathogenic significance [7] but the evidence for the pemphigoid antibodies is not as convincing, but this may be due to antibody concentration. As with all circulating autoantibodies, there is always the question as to whether they are primary or secondary and it is possible that the primary ones are bound to the tissues and it is the secondary ones which circulate.

Other skin disorders with cirulating autoantibodies, which may well have a pathogenic role, are linear IgA disease, cicatricial pemphigoid and dermatitis herpetiformis. Systemic lupus erythematosus is discussed separately (see pages 575–592). Linear IgA disease is so called because the IgA antibody is deposited in the skin along the line of the basement membrane in a linear homogenous pattern, and has been recognised as a separate entity, distinct from dermatitis herpetiformis [8]. Linear IgA disease is seen both in children and adults but there are clinical and immunological differences. In children there is a high incidence of clinical remission and circulating antibodies [9] whereas in adults there is a low incidence of spontaneous remission, and of circulating antibodies [8]. The localization of the antibody on immunoelectron microscopy has been found to vary in linear IgA disease. In some studies it has been reported in the lamina lucida [10] whilst others have reported it below the basal lamina [11], and in a third study it was found below the basement membrane in some patients, and in the lamina lucida in others [12]. It is therefore possible that there are two separate antigens in linear IgA disease.

Cicatricial pemphigoid is a disorder which predominantly affects the mucous membranes and leads to scarring unlike the other bullous disorders. A circulating antibody has been found which localizes to the lamina lucida region of the basement membrane of the skin, oral mucosa and conjunctiva [13]. Herpes gestationis occurs in pregnant or post partum patients with the antibody again localizing to the lamina lucida region of the basement membrane of both skin and amnion [14].

Finally, dermatitis herpetiformis, which is due to gluten sensitivity, and has an associated gluten-sensitive enteropathy identical to coeliac disease has the anti-reticulin antibody in approximately 20% of patients [15]. The anti-reticulin antibody may well be as a result of cross-reactivity between gliadin and reticulin. Gliadin binds to reticulin in a lectin-like manner [16] and may render the reticulin immunogenic, thus initiating an autoimmune process.

Possible autoimmune disorders without circulating autoantibodies

Vitiligo and alopecia areata

Both diseases are considered to have an autoimmune basis because of the increased incidence of organ-specific circulating autoantibodies and disorders associated with

these antibodies, e.g. thyroid disease and pernicious anaemia. However as yet no antibodies have been found to melanocytes invitiligo or the hair follicle in alopecia areata.

Psoriasis

There is increasing evidence that psoriasis is an immunologically mediated disorder [17]. There is recruitment of T-helper cells, and their subsequent activation in the developing and established psoriatic lesions and resolution is associated with an influx and activation of T-suppressor cells into the epidermis. The known classical trigger in psoriasis is a streptococcal infection and it has recently been shown that there is cross reactivity between monoclonal antibodies to streptococcal antigens and keratinocytes [18]. Thus it is possible that an external agent may initiate a local autoimmune state mediated by T cells.

Eczema and lichen planus

There is also accumulating evidence that both eczema and lichen planus are immunological disorders. Auto-sensitisation i.e. spread of eczema from one part of the skin to another, particularly with hypostatic eczema or contact eczema from nickel to distant sites was described over 60 years ago. At present it seems more likely that the phenomenon of autosensitization in eczema is due to cell-mediated immunity rather than an antibody.

Lichen planus is a skin disorder of unknown aetiology characterized by a T-cell infiltrate but has no identifiable circulating autoantibody.

Neoplasms

The concept that malignant disease may develop due to a breakdown in normal immunological mechanisms is well recognised. The future management of these disorders may well be some form of immune intervention. T-cell lymphomas and basal cell carcinomas have already been found to improve with immunotherapy.

Treatments

It is obvious that the more that is known of the underlying pathogenetic mechanisms of immunologically-mediated disorders the more precise can be the treatment. However, at present we are still trying to determine what triggers the autoimmune state and at what stage we may block the process before the clinical end-stage of the disease process appears. The mechanism of action of many of our current therapeutic agents is unknown but new drugs are now becoming available with known actions which will make treatment, particularly of skin disease, less empirical.

Corticosteroids

These drugs have revolutionized the management of skin disorders over the last 40 years. Systemic steroids form the basis of treatment of pemphigus and pemphigoid.

Unfortunately, very high doses, i.e. prednisolone 120 mg daily, are required to induce remissions in pemphigus and their side effects are a very common feature in the treatment of this disease. The site or sites of action of steroids in pemphigus and pemphigoid are not known. It is possible that antibody production is decreased, or that the principle site of action is local on antigen–antibody response, or on the subsequent production and/or action of the inflammatory mediators. In support of a central action, steroids induce a remission and thus enable the dose to be reduced after a few weeks, and in addition, topical steroids have very little effect in pemphigus compared to other skin diseases.

However, in pemphigoid and herpes gestationis the diseases respond to lower doses of steroids. If the same basic mechanism of a breakdown in normal homeostatic factors controlling autoimmunity occurs in both pemphigus and pemphigoid, it is surprising that differing doses of the drug are required to achieve the same result, i.e. suppression of the clinical lesions. This observation would argue for more than one site of action of corticosteroids in this group of diseases.

It is interesting that in the other autoimmune disorders, such as linear IgA disease and cicatricial pemphigoid, systemic steroids do not have the same efficacy as in pemphigus, pemphigoid and herpes gestationis. Thus a central action in controlling antibody production could possibly be different in these diseases compared to pemphigus and pemphigoid. Alternatively, a local action of steroids combined with a central one is effective in pemphigus and pemphigoid, but not in linear IgA disease and cicatricial pemphigoid. This infers that the local pathogenetic mechanisms are different in cicatricial pemphigoid and linear IgA disease compared to pemphigus and pemphigoid.

Topical corticosteroids are highly effective drugs in eczema and lichen planus, and have a reasonably good clinical effect in psoriasis (systemic steroids are also effective in these diseases, but not usually necessary). The clinical response of these disorders is proportional to the strength of topical steroids. However, it would have to be admitted that how topical corticosteroids exert their beneficial effect is not known, although in psoriasis it has been shown that T cells are cleared from the skin prior to the clinical response [19] and it is therefore possible that this action on T cells is at least one of the sites of action of topical steroids.

Ultraviolet light

Ultraviolet light (UVL) has been known to be beneficial for both eczema and psoriasis for many years. It has also been one of several treatments used in alopecia areata. UVL has been shown to impair lymphocyte function *in vitro* [20] and *in vivo* contact hypersensitivity responses [21] UVL has also been shown to decrease surface markers, including DR expression, on antigen presenting cells [22]. Thus UVL is likely to suppress skin disorders which are dependent on the functioning of antigens presenting and T-lymphocyte cells in the skin. Indeed eczema, psoriasis and lichen planus all benefit from irradiation with UVL. More recently the effect of UVL on skin disease has been increased by using UVA (long wave UVL 320–400 nm) with psoralens, and this combined treatment has been termed PUVA. PUVA therapy has been found to be far more effective than UVL alone in clearing psoriasis, eczema and alopecia areata. As with topical steroids it has been shown in psoriasis that T cells are

significantly decreased in the skin before clinical improvement with PUVA [23]. Because of its effect on T lymphocytes, PUVA therapy has been used to treat T-cell cutaneous lymphoma with excellent results. Why T lymphocytes are so sensitive to UVL irradiation compared to the epidermal cells, and how it interferes with the function of T and the antigen presenting cells is not known. PUVA is currently one of the best immunosuppressive treatments available for T-cell mediated skin disease. Despite its action on the 'central' immune system, UVL irradiation does not appear to benefit autoimmune skin disease which is not associated with a T-cell infiltrate in the skin.

Methotrexate

Methotrexate was first used for the treatment of psoriasis in 1958. It was introduced following a chance observation by Gubner in 1951 [24]. Gubner was using aminopterine to treat patients with rheumatoid arthritis as he thought aminopterine was an anti-inflammatory drug. One of the patients had psoriasis and the latter responded far better than the arthritis. Aminopterine then became a standard treatment for psoriasis, and amethopterine (Methotrexate) was introduced in 1958 because it had a lower incidence of side effects. It was thought for many years that the action of methotrexate in psoriasis was by inhibiting mitosis. However, it was subsequently shown that in psoriasis treated with methotrexate, histological improvement occurs before there is a fall in mitosis, implying the primary site of action is on some other pathogenetic factor. It is only since the observation that T cells probably play a part in the pathogenesis of psoriasis, that it can be appreciated that methotrexate may well be exerting its anti-psoriatic effect by its immunosuppressive properties.

Methotrexate has been used in pemphigus with variable results, and it is likely that any beneficial effect is due to a central immuno-suppressive effect on antibody production.

Azathioprine

This drug has been used for many years in a variety of skin disorders and it is likely that it exerts its beneficial effect by immunosuppression. It has been used extensively in pemphigus and pemphigoid in an attempt to reduce the steroid requirement to induce remission in those disorders. Its site of action is likely to be a central one, possibly by interfering with antibody production. Azathioprine has also been used with good effect in patients with severe eczema, particularly in severe forms of photosensitive eczema. In this instance the beneficial mechanism of action is less clear. It may be a local and central effect on T-cell function.

Cyclosporine

The first skin disease to be shown to be improved with cyclosporine was psoriasis in 1979. The observation came about in exactly the same way as with aminopterine some 30 years previously. Cyclosporine was being used to treat patients with rheumatoid arthritis and four of them had psoriasis which cleared [25]. However, this observation was not persued. It was only following the reports that psoriasis was

likely to be a T-cell mediated disease [26] and therefore cyclosporine should be effective, that trials with it were undertaken in psoriasis. There are now studies [27, 28] showing that cyclosporine is indeed effective in clearing psoriasis and fortunately at a relatively low dose. The above reports showing cyclosporine can clear psoriasis at doses ranging from 3–5 mg/kg/day. Cyclosporine appears to have an immunosuppressive action which is more specific than other immunosuppressive drugs such as steroids, methotrexate and azathioprine. Therefore not only has cyclosporine proved to be a valuable drug in the treatment of psoriasis it has also been helpful in defining the immunopathology of the disease.

Cyclosporine is now being used in the treatment of other immunologically mediated skin diseases. It has been used with variable results in pemphigus and pemphigoid [29] with good results in eczema [30] and alopecia areata [31]. The mechanism of action of cyclosporine is thought to be by inhibiting IL-2 production from activated T helper lymphocytes. Whether it may have other additional actions in clearing skin diseases remains to be seen.

Sulphones and sulphonamides

Sulphapyridine has been used to control the rash of dermatitis herpetiformis since 1940, and dapsone since 1950. Sulphamethoxypyridazine is also equally effective. These drugs act very quickly in that they control the itch within 48 h and clear the rash within a week. However the relapse is equally rapid when the drugs are stopped. In addition to controlling the rash of dermatitis herpetiformis the same drugs are effective in linear IgA disease. They also have a beneficial effect in cicatricial pemphigoid, particularly on the oral and genital lesions, but to a lesser extent on ocular involvement. There are also reports of a beneficial effect in some patients with pemphigus [32]. The speed of clearance and relapse would seem to imply that these drugs act on a late stage in the disease process. It is possible they act on either the production of inflammatory mediators or block their action in the skin. They appear to have no effect on T- or B-cell function, as the enteropathy in dermatitis herpetiformis which is T-cell mediated [33] is not affected by dapsone or the sulphonamides.

Plasmaphoresis

This therapy has been employed for severe pemphigus, usually those not responding to drugs, with good effect in some patients [34]. However, it is doubtful whether its use could be justified in other skin disorders. Plasmaphoresis probably exerts its beneficial effect by the removal of the circulating autoantibodies and immune complexes.

Interferons

Interferons are produced by leucocytes, fibroblasts and T-cells and have the ability to inhibit cell growth. There are reports of the effectiveness of alpha interferon in basal cell carcinomata [35] and viral warts [36]. Gamma interferon has been used in psoriasis but the results have been disappointing [37]. It appears likely that

interferons will prove to be more effective in neoplasms than in the inflammatory dermatoses characterised by activated T-cell infiltrates such as psoriasis. In the latter there is unlikely to be a lack of production of interferons. It is possible that it is the failure to respond to these substances which is important in the aetiopathology [38].

Diet

The disease par excellence which responds to diet manipulation is dermatitis herpetiformis. This skin disorder has been shown to be due to gluten and removal of this from the diet clears the rash, and re-introduction produces a recurrence [39]. The precise mechanism by which gluten produces the rash is not known, but as it takes on average 2 years for complete clearance it could be argued that it is unlikely that gluten would remain in the skin for that length of time. This is therefore support for the observation than an autoimmune state is induced by gluten and it is the anti-reticulin antibody which subsequently is responsible for skin pathology.

Dietary control of other skin disorders is less impressive, particularly in atopic eczema where claims have been made. The problem in atopic eczema would appear to be a defect in dealing with antigens (such as food ones) and it is probably not one particular antigen which is responsible for the rash.

Conclusion

It could be argued that a large proportion of the current skin disorders seen in the clinic probably have an immunological basis and are treated by immunointervention. This would certainly be true for two of the commonest one, i.e. eczema and psoriasis. The two commonest viral infections, warts and recurrent herpes simplex may well in the future be treated by immunointervention with interferons. Neoplastic disorders may also be treated with interferons or other immunological modulators in the near future. Monoclonal antibodies targeted against tumour cells, although not used as yet in dermatology may well have a part to play in the treatment of melanomas or T-cell lymphomas. If autoimmunity is a breakdown in the normal defence system, its greater understanding will lead to more appropriate treatments.

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Therapeutic Immunosuppression in Type I (Insulin-dependent) Diabetes

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Introduction

The autoimmune hypothesis of the origin of Type I (insulin-dependent) diabetes mellitus (IDDM) is based on numerous data obtained both from human and animal models (BB rat and NOD mouse). The immunological aggression mainly involves T lymphocyte-mediated immunity. It results in the selective destruction of insulin-secreting cells (beta-cells) in the pancreatic islets of Langerhans, following an intense lymphocytic infiltration (insulitis) [1–3].

First open trials of immunosuppression

The pathogenic role of T cells in Type I diabetes made it logical to attempt to prevent the course of the disease by the induction of T-cell selective immunosuppression. After the pioneering report of Like and colleagues showing prevention of Type I diabetes in the BB rat by anti-lymphocyte serum, [4] several immunosuppressive therapies were investigated with variable efficacies (Table 1). An effect was only obtained when the drug was given before disease onset. This could be simply explained by the rapid and complete destruction of beta cells which has occurred at the time of overt diabetes. Ultimately, it was shown both in BB rat and NOD mice that cyclosporine A (Cy-A) could very efficiently prevent the disease at non-toxic doses [5–7]. Cyclosporine is of major interest as a non-cytotoxic drug,

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Table 1. *Trials of immunosuppression in Type I diabetes*

Animals (BB rat, NOD mouse)	Human
Anti-lymphocyte serum (ALS)	
Neonatal tolerance (blood transfusion bone marrow graft)	ALS—Azathioprine—steroids
Neonatal thymectomy	Corticosteroids alone
Anti-lymphocyte subset MoAb[1]	Plasmapheresis
Anti-MHC[2] Class II MoAb	Azathioprine
Total lymphoid irradiation	CBL—1 MoAb
Corticosteroids	Cyclosporine
Cyclosporine	
Cyclosporine—steroids—ALS	
Anti-IL-2-receptor MoAb + cyclosporine	
L3T4 (anti-helper lymphocyte) MoAb	

[1]MoAb, monoclonal antibody.
[2]MHC, major histocompatibility complex.

acting in a reversible fashion by inhibiting interleukin-2 production [8], and consequently all T-cell functions without toxicity for other blood cells. A cure of overt diabetes in BB rat was obtained and the effect persisted after stopping immunosuppression, especially when the combination of Cy-A and anti-IL-2 receptor monoclonal antibody was used [9].

Immunoprevention treatment can be initiated in animal models of diabetes before onset of beta cell destruction but this is not feasible in man. One may assume however that a sufficient beta cell mass is still present in human diabetics at the onset of their disease to induce a significant clinical effect (remission of insulin dependency) if immunosuppressive treatment is started very rapidly after the appearance of clinical symptoms and before too much of the beta cell mass has been destroyed.

Various immunosuppressive agents have been used, often in a preliminary fashion in recent-onset human Type I diabetes (Table 1). Steroids, azathioprine and anti-lymphocyte serum were used alone or in association but no clearly interpretable results were obtained in these trials. Much more convincing results were obtained using Cy-A (Sandimmune, Sandoz). Cyclosporine is undoubtedly, with anti-T lymphocyte monoclonal antibodies, the most powerful T-cell immunosuppressive agent presently available. This consideration, together with the wide clinical experience gained in its use in organ transplantation [10] prompted two groups of investigators to use it in diabetes [11, 12]. These pilot studies performed in France and in Canada suggested that Cy-A could indeed induce remission of insulin-dependency in patients with diabetes of recent onset. The effect was particularly clear-cut when immunosuppressive treatment was started within 6 weeks of the start of insulin therapy.

These open trials did no more, however, than suggest the effect, because of the frequency of spontaneous remissions ('honeymoon' phenomenon) [13, 14] commonly seen at the clinical onset of Type I diabetes. Somewhat paradoxically, insulin requirement (and more generally dosage adaptation) are not fully objective criteria,

Table 2. *Cyclosporine/IDDM: results of placebo-controlled trials*

	Rates of complete remission (%)		
	6 months	9 months	12 months
CDF study ($n = 122$)			
Cy-A	25.4	22.4	17.5
(Cy-A blood > 300 ng/ml)[1]	(37.5)	(37.0)	(32.3)
Placebo	18.6	5.8	0
Canadian-European study ($n = 188$)			
Cy-A	38.7	—	24.2
(Cy-A, symptoms < 6 weeks)	(55.3)	—	(31.6)
Placebo	19.1	—	9.8

[1]Whole blood trough cyclosporine level—RIA determination—Non-specific polyclonal serum (cyclo-kit, Sandoz).

since an investigator may be inclined to reduce insulin dosage in a more aggressive fashion than one would do otherwise in the absence of immunosuppressive treatment. These two considerations prompted the undertaking of double-blind placebo-controlled trials which appeared necessary to provide definite conclusions with regard to the immunopreventive effect of Cy-A in Type I diabetes.

Placebo-controlled studies with cyclosporine

Two placebo-controlled double-blind trials were set up. The French multicenter trial [15] (CDF study: Cyclosporine-diabetes-France) included 122 patients, aged 15–40 years. Cyclosporine was initially given at a dose of 7.5 mg/kg/d increased to 10 mg/kg/d after 3 months in the absence of remission and systematically decreased after 6 months to 5 mg/kg/d. In the Canadian European Study [16] patients received 10 mg/kg/d Cy-A for 12 months. In both studies Cy-A was reduced, when high Cy-A blood concentration or nephrotoxicity occurred, and stopped when it failed after 3 or 6 months.

The two studies gave very similar results. The rates of complete remission at 6 months were 37.5% in the CDF study in the subgroup of patients who had received sufficient immunosuppression (whole blood Cy-A concentration ≥ 300 ng/ml trough level measured by the polyclonal RIA kit) and 38.7% in the Canadian-European study. At 12 months these rates were 32.3% and 24.2% respectively. At the opposite end, the rates of remission in the placebo group were respectively 18.6 and 19.1% at 6 months and 0% and 9.8% at 12 months. These values are significantly lower than those observed in the Cy-A groups (Table 2). The French study confirmed the importance of adequate immunosuppression since the remission rate was lower in patients who had a low blood Cy-A concentration (< 300 ng/ml) as defined by the experience gained in other conditions (renal transplantation).

The Canadian-European study showed that the short duration of the disease at the start of immunosuppression was a predictive factor of response to Cy-A: the remission rate reached 55.3% in the subset of patients who had less than 6 weeks of symptoms.

Mechanisms of remissions

Patients enrolled in the French study were submitted to extensive metabolic (beta cell function) and immunologic (anti-beta cell autoimmunity) investigations in order to provide some insight in the mechanisms of Cy-A-associated remissions.

Insulin secretion capacity

In most patients, the clinical course paralleled the evolution of insulin secretion capacity measured by plasma C peptide secretion following glucagon injection [17]. This evolution can be separated in three phases. Firstly, C peptide secretion was initially very low (1.3 ng/ml as a mean, compared to 6.6 ng/ml in healthy controls), and partially recovered at 3 months both in Cy-A treated and in placebo receiving patients. One may assume that such spontaneous improvement reflected the reversal of a functional inhibition of insulin-secreting cells rather than their replication. This early beta cell function recovery is probably due to the correction of the initial profound metabolic disorders afforded by intensive exogenous insulin therapy. The correction of metabolic disorders also explains the disappearance of initial peripheral insulin resistance. One may hypothesize that it is the conjunction of these two phenomena (increased beta cell function and decreased insulin resistance) which explains the occurrence of clinical remissions seen in recent onset diabetics, whether or not they are treated with Cy-A. One should note at this point that Cy-A administration does not enhance spontaneous recovery of insulin secretion above that noted in absence of Cy-A.

However, continuous Cy-A therapy allows the persistence of insulin secretion capacity which has reappeared after the correction of metabolic disorders. In sharp contrast, non-Cy-A treated diabetics present a continuous decline of their stimulated C peptide secretion, which becomes significantly lower than in the Cy-A group after 6 months. When Cy-A treatment is interrupted the C peptide levels decline. Some patients with poor C peptide levels, who had transient remission, relapsed in the absence of an abrupt drop in C peptide levels. This latter point suggests that changes in sensitivity to insulin can also contribute to the occurrence of remissions and relapses.

Lastly, one should note that the patients with the best C peptide levels (measured at 3–6 months, after the reversal of the initial functional inhibition) had the highest remission rate and that the most prolonged remissions fitted consistently with the aforementioned relationship between a short duration of symptoms (and an expected well preserved residual beta cell mass) and an improved remission rate.

Anti-beta cell immunity

The study of the humoral and cellular immune status of Cy-A-treated patients has provided interesting information on the immunological mechanisms by which Cy-A preserved the residual beta cell mass.

Cyclosporine had only minimal effects on the production of anti-insulin and anti-islet cell antibodies whether directed to islet cytoplasmic antigens as detected by immunofluorescence (ICA) or membrane antigens as evaluated in a cytotoxicity assay, even in patients undergoing remission [18]. A slow decrease of ICA titers was

observed in Cy-A treated patients over a one-year follow-up period but this decrease was also seen in placebo-treated patients and was not statistically significant between the two groups at any time of observation. The occurrence of a relapse of insulin dependency was never accompanied by a reappearance or increase in ICA titer either in patients receiving Cy-A or placebo.

Cell-mediated immunity directed against beta cells was studied in parallel to humoral immunity [19]. Anti-beta cell cellular immunity was assessed by an *in vitro* test based on inhibition of insulin release from cultured rat islet cells by patient's mononuclear cells. This T lymphocyte-mediated suppressive effect on beta cells was abrogated in Cy-A-treated patients within one month of treatment, and was not detected in the 12 months of follow-up. Conversely the inhibitory lymphocytes persisted unchanged in placebo-treated patients during the 12 months of follow-up. No correlation was observed between the clinical course of diabetes (remission or failure) and evolution of anti-beta cell cellular immunity. Finally, these immunological studies point to the major role of cell-mediated immunity in the pathogenesis of human Type I diabetes. Abnormalities of cell-mediated immunity are completely reversed by Cy-A while anti-islet cell antibody production persists. Cyclosporine-induced reversal of anti-beta cell specific cellular immunity was observed in every patient including those who did not show clinical remission of diabetes. Taken together with data on C peptide secretion, these results suggest that an increased remission rate will not be achieved by increasing immunosuppression but rather by improving the residual beta cell mass.

Placebo-controlled studies clearly demonstrated that a potent immunosuppressive agent can slow down or stop the evolution of IDDM when started at the time of clinical onset. This result has major theoretic importance by confirming the autoimmune origin of the disease in man. It also represents the first clear demonstration of the efficacy of an immunosuppressant on the clinical course of human Type I diabetes.

Duration of remissions

Insulin dependency

Trials show that maintenance of remissions requires the continuation of Cy-A treatment. In the French study where Cy-A was interrupted, insulin needs increased progressively from 0.30 to 0.58 u/kg/d over one year. At the same time the residual insulin secretion as a mean (measured by the serum C peptide concentration), declined, reaching the levels observed in patients not treated with Cy-A, but who had similar duration of diabetes.

Cy-A blood concentration required for maintenance of remissions is lower than that required for induction of remissions. A trough level of 200 ng Cy-A per ml whole blood (polyclonal RIA kit) was associated with remissions persisting for 2 years or more. However, 50% of subjects with Cy-A concentrations persistently below this level (including patients who stopped Cy-A although in remission after 6 months) had relapsed at 12–15 months and 80–90% at 2 years. The problem of relapse is complicated by the possible interference of immunological events independent of decreased levels of immunosuppression, such as a burst of autoimmunity or appearance of Cy-A-resistant immune effectors (Table 3).

Table 3. *Putative causes of relapses of insulin-dependency*

Immunological	Metabolic
Insufficient immunosuppression	Insufficient initial residual beta cell mass
Development of cyclosporine-resistant immune effectors	Increased insulin needs (infections, growth, etc.)
Burst of autoimmunity	Insulin-resistance of peripheral tissues due to chronic low insulin level
	Progressive islet sclerosis (?)

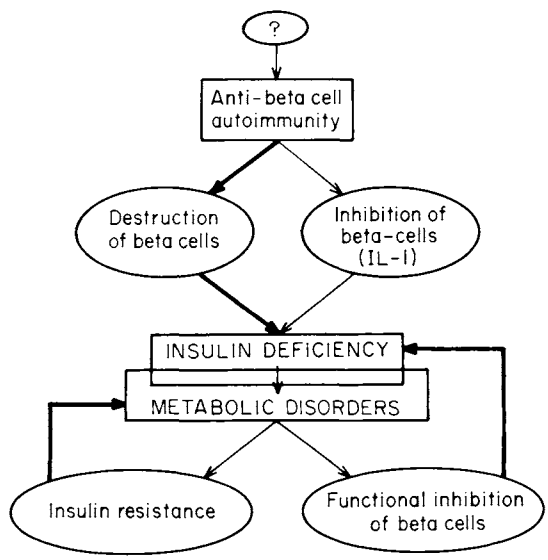

Figure 1. Interactions of immunologic and metabolic factors resulting in the insulin deficiency.

Residual beta cell mass and insulin sensitivity

The course of plasma C peptide concentration in patients who relapsed after initial remission suggests that both insulin secretion and insulin sensitivity may contribute to the relapse. In fact we did not observe an abrupt drop of insulin secretion at the time of relapse. Moreover C peptide levels measured at the time of relapse were not significantly lower than the best C peptide levels previously observed in each subject, at a time of remission. It should be realized however that these patients persistently had an insulin-secretion lower than subjects with continuing remissions: as a mean, 2.0 ng/ml versus 2.9 ng/ml respectively (glucagon-stimulated C peptide). Consequently, relapse may be due to a progressive decrease in insulin-sensitivity following chronic insulinopenia rather than to an acute flare-up of islet damage. These data throw light on the complex interactions taking place between the immunological and metabolic factors resulting in the onset of remission of insulin-dependency (Figure 1).

It must be stressed that in most cases the remaining beta-cell mass is far from normal. Even in patients where it is compatible with the absence of exogenous insulin

Table 4. *Cyclosporine specific side-effects (CDF study)*

	Cyclosporine, % ($n=58$)	Placebo, % ($n=56$)
Acute nephrotoxicity (creatinine increase >50% over basal level)	47	4
Hypertrichosis		
moderate	38	9
severe	7	0
Gum hyperplasia	29	16
Paresthesias	6	9
Hypertension requiring treatment	3	0
Abdominal discomfort	16	4
Haemoglobin (g/dl)	12.7±0.2	14.1±0.2
Potassium (mM)	4.8±0.05	4.5±0.06

therapy in basal conditions, residual beta cell mass does not allow patients to fullfil increased insulin needs, e.g. oral glucose overload, infection, etc. This limitation explains the occasional occurrence of transient insulin requirements during remissions (Table 3).

Side effects

We have already mentioned that stopping Cy-A treatment exposes the risk of relapse of insulin requirement. The need for prolonged immunosuppressive treatment prompts a very careful assessment of Cy-A chronic toxicity with the hope of avoiding, or limiting, this toxicity. Nephrotoxicity is certainly the major current concern [20]. In the CDF study a moderate increase in plasma creatinine (rise by 50% over basal value) was observed in 47% of patients treated with Cy-A, as opposed to 4% in those receiving placebo. This 'acute nephrotoxicity', was correlated with Cy-A blood concentration. It was reversible in all cases when Cy-A dosage was decreased. A relationship clearly appeared between the severity of acute nephrotoxicity as detected by increase in plasma creatinine and the ultimate appearance of renal histological lesions, especially interstitial fibrosis (chronic nephrotoxicity). No relationship was noted between histological renal abnormalities and duration of treatment or accumulated Cy-A dosage [21]. Taken together, these data suggest that prevention of acute nephrotoxicity can prevent chronic nephrotoxicity. The risk of chronic nephrotoxicity becomes minimal when maintaining residual Cy-A blood concentration around 300 ng/ml (polyclonal radioimmunoassay) and decreasing Cy-A dosage as soon as plasma creatinine increases by more than 30% over basal value.

Other Cy-A side effects (Table 4) included hypertension, reversible after interruption of Cy-A, hypertrichosis, gum hyperplasia, paresthesiae, abdominal discomfort, mild anemia or an occasional and moderate hyperkaliemia. All side effects were reversible after decreasing the dosage or stopping Cy-A.

Table 5. *Side effects of cyclosporine limited to immunosuppression*

	Number of affected patients		
	Cyclosporine		Placebo
Infections[1]			
Benign	37/61	ns[2]	26/54
Severe	0	—	0
Lymphoma			
Clinical symptoms	0/650	—	0/150
Restriction of immunoglobulin heterogeneity (IEF)[3]	0/59	—	0/38

[1]CDF study.
[2]Not a significant difference.
[3]IEF: Electrofocusing of serum, CDF study.

Cyclosporine treatment exposes to two classical side effects of immunosuppresion: infection and Epstein-Barr virus associated B lymphomas. In fact, the risk of lymphoma is low (less than 0.1%) when moderate Cy-A doses are used as in autoimmune diseases, particularly when Cy-A is used alone. To date, no lymphoma was observed among more than 650 diabetics treated with Cy-A for 6 months to 4 years. Moreover no abnormalities of circulating immunoglobulins, namely a restriction of heterogeneity which could be an early sign of B lymphocyte proliferation, were detected in 59 Cy-A treated diabetics serially studied for up to 2 years (Table 5). Similarly no severe or opportunistic infection occurred. The frequency of intercurrent infections (upper respiratory tract, varicella, etc.) did not differ over 12 months from the placebo group in the CDF study. All infections had a benign course.

New trials

Controlled studies have outlined the two main requirements regulating Cy-A-induced remissions of insulin-dependency in Type I diabetes: (1) adequate immunosuppression (as assessed with blood Cy-A concentrations); (2) sufficient residual beta cell mass which means early diagnosis. Such early diagnosis implies that patients selected for Cy-A have shown a short duration of symptoms and a modest weight loss. The study of remissions in large patient groups revealed no obvious influences of sex, age, presence or titer of autoantibodies to insulin or to islet cell antigens. The study of HLA phenotype did not show any clear-cut difference between HLA-DR3 and DR4 patients. The frequency of remissions was lower in non-DR3 and non-DR4 subjects, but these patients represent less than 10% of Type I diabetics.

New (open) trials have been undertaken to explore other directions that may improve or extend the clinical application of Cy-A in Type I diabetes, both in children and in adults.

Children

Cyclosporine was given to 40 children with Type I diabetes (Saint-Vincent de Paul Hospital, Paris) in strictly controlled conditions in order to ascertain a safe use of the

drug [22]. Cyclosporine was initially given at a dosage of 7.5 mg/kg/d and then adjusted in order to maintain Cy-A whole blood trough level between 200 and 300 ng/ml (non-specific radioimmunoassay) and to avoid plasma creatinine increase exceeding 30% above baseline.

At 4 months, 27 patients had interrupted insulin therapy. Seventy-five percent of these early remitters were off insulin at 12 months with a good glycemic control. Several major (and statistically significant) differences were observed between the 27 remitters and the 13 non-remitters including prediagnostic duration of symptoms (27 *vs* 48 days), intensity of weight loss (3% *vs* 10% body weight), initial HbA1c (10.7 *vs* 13.2%) and frequency of ketoacidosis (11.0 *vs* 61.5%). Of all these closely related parameters, weight loss was the most reliable predictor of remission. The initial C peptide response to intravenous glucagon (1.50 *vs* 0.50 ng/ml) as a measure of the endogenous insulin secretion was another important independent predictive factor at variance with adult diabetics.

The usually brisk onset of symptoms in children (nycturia) could explain the shorter duration of symptoms and subsequently the higher remission rate than that observed in adults.

Adults

New trials were set up and are currently running in adults with the aim of improving the treatment: treatment of patients with the highest probability for a remission (short duration of symptoms), evaluation of drug combinations to obtain remissions with a lower dose of Cy-A, reinduction after a relapse of insulin dependency (resume treatment) [17]. An absolute prerequisite shared by all these new trials is of course to use therapeutic schedules avoiding chronic Cy-A kidney toxicity, a goal which can now be easily achieved (see above). At the present rate of patient enrolment it may be difficult to attain and maintain remission rates exceeding 50% for more than 12 months, even if a large proportion of them are still in remission over 2 years.

Evaluation of the risk/benefit ratio

Improvements in insulin therapy and blood glucose monitoring over the last 20 years have not solved the problems associated with acute and chronic constraints and complications of Type I diabetes, which is still the origin of common major morbidity and mortality. Two main problems remain: (1) The constraints of daily insulin injections with their intrinsic complications (both local and systemic, hypoglycemia) and the need for meticulous monitoring to ensure precise metabolic control, critical to its long-term effectiveness; (2) Major degenerative complications that persist despite intensive insulin therapy. IDDM represent more than 25% of indications for chronic hemodialysis [23]. It is the main cause of blindness in adults, and it causes severe cardiovascular disorders (hypertension, coronaritis).

Cy-A-induced immunosuppression results in remission of insulin-dependency and a partial restoration of insulin secretion. The maintenance of endogenous insulin secretion provides several marked advantages for the patient. First, metabolic control (as assessed by blood glucose and glycosylated hemoglobin levels) is better in remitters than in non-Cy-A-treated patients receiving conventional insulin treatment, without insulin-induced hypoglycemias. Second, one may assume that the

persistence of endogenous insulin secretion with improved porto-peripheral gradients in insulin concentration, affords better protection from occurrence of degenerative complications. One should emphasize however that before extending this new treatment of diabetes from therapeutic trials to routine therapy one should improve the remission rate and the duration of remission. These two goals are the objective of future experimental and clinical research in the field.

Perspectives

Improvement of the residual beta-cell mass

It has appeared difficult so far to induce remissions in more than 50% of adults whatever the immunosuppressive regimen used. As mentioned above, no parameter predictive of remission has emerged clearly, except early treatment after short duration of symptoms, minimal weight loss and absence of keto-acidosis. All parameters that presumably assess the residual beta cell mass at the clinical onset of the disease are unreliable because insulin deficiency at that time may be due altogether to immune (T-cell mediated) and metabolic factors (hyperglycemia and/or insulinopenia) which induce a functional degradation of beta cells. Some of these factors are reversible others are irreversible.

To be fully efficacious Cy-A therapy should probably be started within 2 or 3 days after the beginning of insulin therapy. Ideally, one would like to start Cy-A earlier, before insulin therapy is required, as soon as hyperglycemia is detected. This is practicable with children, when the family is adequately informed of the first symptoms of the disease. One could even envisage starting Cy-A earlier before detectable hyperglycemia and extensive beta cell damage has occurred. Genetic, immunologic and metabolic screening programs aiming at the early detection (preglycosuric) of patients at risk of diabetes are in progress in several countries both in adults and children [24, 25].

Another positive direction would be to induce the regeneration of beta cells by using beta cell growth factors. Platelet-derived growth factor or insulin-like growth factor 1 have demonstrated some effect on the replication of fetal rat islets of Langerhans *in vitro* [26], but no factor regenerating the islets in adult humans is currently available.

Maintenance therapy

To attain long-lasting remission after stopping Cy-A immunosuppression remains a major goal for the patient. The two placebo-controlled randomized trials, Canadian-European and French, show that maintenance of Cy-A treatment (eventually at reduced dosage) is required to maintain remission. So far, no alternative therapy to Cy-A has proved to be efficient at inducing remissions in randomized trials, but active research of new immunosuppressive methods should be pursued. The clinical use of Cy-A may be improved by a combination of drugs to prevent its nephrotoxicity or by decreasing Cy-A therapy and administering other immunosuppressants, which is currently done with Cy-A in organ transplantation.

Corticosteroids are successfully used in autoimmune diseases other than diabetes. Their deleterious effect on glucose metabolism limits their use in diabetes [17].

However, a wide spectrum of other possibilities has to be tested, including bromocriptine, azathioprine, monoclonal antibodies directed against either T lymphocytes, Class II MHC antigen or cytokines or cytokine receptor. Eventually one may even entertain the idea of inducing beta cell specific tolerance (idiotypic manipulation, T-cell vaccination, induction of autoantigen specific suppression . . .). However, all these methods, which present various advantages in terms of efficacy and toxicity, will need sufficient initial beta cell mass outlined above.

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The Clinical Use of Immunosuppressants in the Treatment of Putative Autoimmune Intra-ocular Diseases

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Uveitis refers to a large number of intra-ocular inflammatory disorders with both endogenous and exogenous causes, the latter including such disorders as toxoplasmosis and cytomegalovirus retinitis. A large number of blinding disorders are of putative autoimmune origin, requiring effective immunosuppression. High dose steroids have been used to treat several endogenous conditions. Alkylating agents appear anecdotally to be more effective than anti-metabolites. However, the concern of long-term and short-term side effects, as well as the question as to whether vision is being preserved in these patients has made this approach somewhat less attractive. Cyclosporine has been utilized in the treatment of several sight-threatening disorders. Approaches have concentrated on therapeutic regimens that will give the least in secondary effects, while other studies are presently evaluating the usefulness of cyclosporine G. Several conditions, such as Behçet's disease and the intermediate and posterior uveitic entities that lead to macular edema appear to respond well. It is imperative to evaluate the patient for other causes of poor vision that may not respond to this type of therapy.

Any intra-ocular inflammatory disease is referred to as uveitis. This term, an old one, does not in any way suggest an etiology. The cause of the disease can be due to exogenous factors such as toxoplasmosis or cytomegalovirus, or it may result from endogenous factors, some of which are thought to be attributed to autoimmunity. The underlying clinical feature for all of these conditions is the presence of inflammatory cells and an increase in the proteinaceous content of the eye. These changes

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can be observed and quantified by the ophthalmologist [1]. Since a long list of underlying disorders can manifest itself in the eye, the anatomic site of the inflammation is noted. Therefore, the uveitis will be referred to as being either an anterior, intermediate or posterior uveitis.

The term anterior uveitis indicates that the major centre of inflammatory activity is located in the front portion of the eye. Though this may be a phenomenon localized to the eye, it can be associated with several systemic conditions, such as ankylosing spondylitis and sarcoid. Intermediate uveitis includes those disorders that affect the mid-portion of the eye. These inflammatory conditions can be a manifestation of such systemic disorders as sarcoidosis, or can be localized exclusively to the eye, such as pars planitis. Posterior uveitides can present in a multitude of ways. This includes those that are usually unilaterally active and focal, such as toxoplasmosis. Others can present as a diffuse retinitis, with large areas of the retina destroyed by the process, such as in cytomegalovirus retinitis associated with AIDS.

One of the major group of entities that are known to cause severe sight-threatening intermediate and posterior uveitis are those that are of putative autoimmune origin. Though extensive attempts have been made to identify a causative agent for such disorders as Behçet's disease, the Vogt-Koyanagi-Harada syndrome, and pars planitis, none have been definitively linked to these diseases. Alterations in various arms of the immune system have been reported [1].

The significant role of the immune system and genetic restriction in this cluster of diseases can be underscored by citing two examples. The first is the strong association with specific HLA antigens. One of the first HLA-associated diseases was that of ankylosing spondylitis and HLA-B27 [2]. A common component to this disease is that of anterior uveitis. Anterior uveitis in Caucasians not associated with ankylosing spondylitis is also associated with the same antigen [3]. Some disorders capable of causing severe alterations to the posterior portion of the globe have been associated with other antigens. Behçet's disease is associated with HLA-B51 in the Japanese [4], while HLA-DR53 (previously called MT-3) has been associated with the Vogt-Koyanagi-Harada syndrome in the same population [4]. In Caucasians, several studies have now established the association between HLA-A29 and birdshot retinochoroidopathy [5, 6], with a relative risk of about 50. This purely ocular disorder has many characteristics of the animal models for uveitis (see below), and most of these patients demonstrate immunologic evidence of sensitization to retinal elements [5], similar to what is seen in animals with experimentally induced uveoretinitis.

The isolation, purification and evaluation of retinal antigens capable of inducing an intra-ocular inflammatory response have revolutionized our ability to evaluate these disorders. The retinal S-antigen (S-Ag) was the first of these antigens to be described, by Wacker and associates [7], and by Faure and colleagues [8]. More recently, the inter-photoreceptor binding protein (IRBP) has also been studied [9]. Immunization with either of these glycoproteins at a site far from the eye will lead to a bilateral uveitis in both lower mammals as well as in non-human primates [10, 11]. The disease induced begins as a retinitis, with a marked perivasculitis, and an initial attack of the photoreceptor region of the retina. Experiments have pointed clearly to the predominant role of the T cell in this model, and certainly by extension in human uveitis as well. T-cell lines will efficiently transfer the disease to naïve hosts [12]. More recently, several uveitogenic regions of the S-antigen have been identified [13].

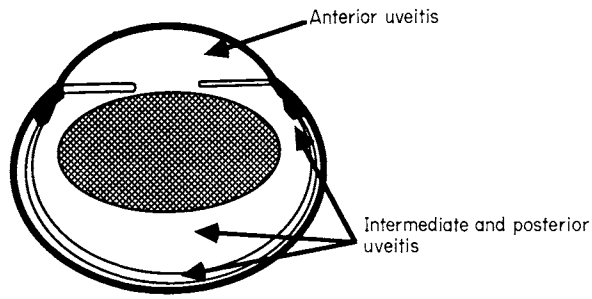

Figure 1. Commonly used therapeutic modalities for endogenous uveitis. Anterior uveitis: topical and peri-ocular steroid, mydriatics. Intermediate and posterior uveitis: peri-ocular and systemic steroid, cytotoxic agents (alkylating and anti-metabolites), cyclosporine; other: virectomy, plasmapharesis colchicine.

Since we felt that the S-antigen model was T-cell mediated, and had a resemblance to the disorders seen in humans, we have used this laboratory-induced disease to evaluate various therapeutic modalities. One agent that proved particularly effective in altering the clinical picture was that of cyclosporine [14]. When this unique anti-T cell drug, whose major mode of action appears to be through the inhibition of IL-2 release during the T-cell activation and recruitment cascade, was given to animals at the time of retinal S-Ag immunization, the disease could be inhibited from being expressed. A similar finding was noted when the cyclosporine therapy was begun even one week after immunization, though higher dosages needed to be used. Of interest is cyclosporine's poor penetration through the cornea, thereby not permitting high concentrations to appear intra-ocularly. Therefore, topical administration of the agent did not yield protection from EAU, but this protection could be achieved if the drug was given intra-camerally [15].

Therapeutic approaches to uveitis

Several approaches to therapy for uveitis exist (Figure 1). Certainly, if an infectious agent can be identified, then the appropriate anti-viral, bacterial, fungal, or parasitic agent is indicated. However, in a large number of patients, the cause of the disease seems to be due to an internal dysregulation of the immune system, with no organism found in the eye. It is certainly conceivable (and probable) that exogenous factors may have initiated the immune response, but that its propagation is subsequently mediated by several factors, including autoimmunity. In these patients, immunosuppressive approaches to therapy are those that are followed.

Corticosteroids

In cases of endogenous uveitis involving the anterior and intermediate segments of the eye, either topical or periocular steroids can be considered as the initial therapies of choice. If given on an hourly basis for treating the acute inflammatory attack, topical steroids can be quite effective. Peri-ocular steroid injections can have a particularly beneficial therapeutic effect in those cases which are especially severe, only unilateral, and where cystoid macular edema is a confounding problem. Some of the local effects of corticosteroids include the re-activation of corneal herpes simplex infections, cataracts, an increase in intra-ocular pressure, proptosis of the globe, scarification of the extra-ocular muscles, and perforation of the globe.

In the many cases of intermediate and posterior endogenous uveitis, where the disease is severe enough to threaten sight in both eyes, systemic corticosteroids would be the approach to consider as a first line therapeutic modality. One important exception to this approach is discussed below (Behçet's disease). In a large proportion of these patients, extended therapy with not inconsequential dosages of corticosteroids are needed to control the disease. For sight-threatening disorders, it is not unusual to begin therapy with 1–2 mg/kg/d of prednisone. A quick taper of the drug will often lead to a recurrence of the disease. Therefore the patient must be very slowly tapered, and then kept at a maintenance dose of prednisone that can be relatively high, from 25–40 mg of prednisone/d. Optimally one certainly tries to continue tapering the medication, but frequently there is a 'breakthrough' at lower dosages. Further, every-other-day therapy has not been especially helpful as a therapeutic schedule, even after the disease has come under good control. The possibility of secondary effects of corticosteroids, not only those seen acutely, but those seen after chronic use, must be explained to the patient [1].

Cytotoxic agents

In some uveitis patients, corticosteroid therapy yields only a partial therapeutic response, or though the therapeutic effect has been realized, the secondary effects of the agent preclude continued therapy at the dosage required. In these cases, cytotoxic agents, particularly alkylating agents, have been suggested as a second line of drugs. The International Uveitis Study Group, in the early 1980s, had suggested the use of cytotoxic agents, particularly for the ocular complications of Behçet's disease, where it was clinically evident that long-term corticosteroid therapy was not effective, and in cases of sympathetic ophthalmia where initial systemic steroid therapy was not thought to be helping clinically [1]. In addition, it could be used in cases of severe endogenous uveitis where steroids were inducing too much in the way of secondary problems, and that there was a reasonable clinical expectation that sight could be restored. It must be emphasized that this therapeutic approach must never be used when an infectious agent is thought to be the cause of this disorder, as in cases of toxoplasmosis and viral retinitis. Though no randomized study demonstrating the efficacy of cytotoxic agents in the treatment of uveitis has been performed, there had been reports lauding the efficacy of these drugs in uveitis, most often in the treatment of Behçet's disease [16]. However, a more recent evaluation of these agents' effects has dimmed this point of view. Tabbara has looked at the long-term visual outcome of Behçet's patients and did not find that alkylating agent therapy had a striking effect on the ocular disease [17]. A randomized study recently performed in Israel [18], would support these observations, and suggests that these agents are probably not the most ideal for Behçet's disease (see below). Further, the concern of long-term side effects, such as sterility, chromosomal abnormalities and neoplasm make these agents less attractive.

Cyclosporine

Once cyclosporine was demonstrated to have a beneficial effect on the animal model for uveitis, the agent was used for endogenous uveitis patients with sight-threatening

bilateral disease, who had received either high-dose systemic corticosteroid therapy or cytotoxic agents, but for whom they were discontinued because of a poor therapeutic response or side effects. It was noted that patients with various types of endogenous intermediate and posterior uveitis had beneficial therapeutic responses when cyclosporine was used as the sole immunosuppressive agent [19]. It was of particular note that patients with Behçet's disease had a positive clinical response, with a rapid decrease in their inflammatory disease and as well a marked decrease or total disappearance of their explosive retino-vascular inflammatory episodes, which frequently lead to visual handicap. Two randomized double masked studies have now confirmed these observations [18, 21]. In one, the cyclosporine was compared to cytotoxic agents and in the other to colchicine, which is given to prevent recurrences.

The use of a relatively high initial dose of 10 mg/kg/d of cyclosporine permitted us to use the drug as the sole agent in the treatment of the uveitis, but it also lead to renal alterations. In a study of 17 uveitis patients, renal biopsies were obtained from them and compared to age-matched controls [22]. The results, which showed renal alterations now recognized as being due to cyclosporine, were noted. On the basis of more recent studies which suggest that renal alterations can be markedly avoided, recommendations for the treatment of uveitis with cyclosporine were produced and published [18]. The recommendations would start the dosage at 5 mg/kg/d combined with low-dose prednisone (or equivalent corticosteroid). During the course of therapy, the dosage of the drug should not exceed 7.5 mg/kg/d, and that only for a relatively short period of time. It is hoped that with this schedule the impressive clinical efficacy seen at the higher dosages can in the main be achieved, but that the renal toxicity will be markedly reduced or abrogated.

Other therapeutic approaches

Immunoenhancing agents such as levamisole, have in the past been suggested as being useful in the treatment of endogenous uveitis. It is now clear that patients with these entities do not have a deficient immune response but rather one that is inappropriately responding. Therefore these agents do not have a role in the treatment of these diseases. Additionally, prostaglandin inhibitors may have a partial benefit in the treatment of mild anterior uveitis, but will not be helpful in treating severe intraocular inflammatory conditions. Non-steroidal anti-inflammatory agents should be avoided when using cyclosporine since the renal toxicity will be markedly enhanced. Colchicine has been used with at best moderate success in treating the recurring explosive ocular episodes of Behçet's disease.

Two approaches not involving medication deserve note. Plasmapharesis has been suggested as helping the acute episodes of Behçet's disease [23]. The problem with this approach is the need for continued anti-immunosuppressive therapy, and the difficulty in continuing this approach on a chronic basis. Vitrectomy, the removal of the vitreous from the eye has been suggested as helping in the course of the inflammatory disease [24]. In our hands, though the eye may look better when examined, the number of recurrent attacks did not decrease, nor did the major cause of decrease in vision in intermediate and posterior uveitis, macular edema, improve.

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A Theoretical Framework for Self-tolerance and its Relevance to Therapy of Autoimmune Disease

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Therapeutic intervention in autoimmune diseases should be based on a knowledge of how the normal immune system maintains unresponsiveness to 'self' and how this state of unresponsiveness may be broken. We have proposed that 'self' from the viewpoint of T cells may represent only a small fraction of the peptides that are available in the body. These would be the peptides that successfully access MHC molecules on a limited number of antigen presenting cells. As the number of self peptides is far greater than that of useful MHC molecules, then the set that are privileged to access MHC on presenting cells will compete or buffer out the others. In other words the peptides which are immunologically visible establish tolerance to themselves whilst ensuring that many others remain cryptic. On this model, organ-specific autoimmunity is not a breakdown of tolerance but rather a failure to keep certain peptides from associating with MHC molecules on cells involved in antigen presentation. This could be at either the inductive side of the response or on the target side if mimicry by foreign antigens has primed the effector arm of the immune response.

Monoclonal antibodies (MoAbs) have proved to be useful immunosuppressive agents. MoAbs to certain T-cell adhesion molecules may also permit tolerance to occur to antigens administered simultaneously with them. The possibility of establishing tolerance to exposed peptides in autoimmunity is discussed. We propose that T cells whatever their stage of maturation can be tolerized as long as they see antigen in the absence of helpful stimuli from other cells.

Introduction

T-lymphocytes are central to all immune responses and are therefore prime targets for therapy at control of immune function. Somehow T-lymphocytes happen to be unresponsive to self antigens although this unresponsive state is lost in autoimmune disease. The natural unresponsive state exists for probably two main reasons. First, certain proteins are likely to be hidden or 'cryptic' and therefore unavailable to

induce and sustain autoimmunity. From a T-cell's viewpoint any protein whose processed fragments never make it to an MHC-associated presentation will be 'cryptic'. This could be for reasons of concentration, accessibility to specialized antigen presenting cells (APC), or indeed to competition with other peptides for access to MHC molecules in APC. Second, unresponsiveness can arise from acquired immunological tolerance. Such tolerance could only occur if the relevant peptides were visible i.e. were associated with MHC on cell-types that could 'present' for tolerance.

To understand autoimmunity fully and to plan proper intervention strategies, an appreciation of processes maintaining unresponsiveness in T lymphocytes is required. In this article we offer an hypothesis of how unresponsiveness occurs and from this model suggest how therapeutic intervention may be directed to reverse autoimmune disease.

The secretary model

We have in the past proposed a model of self-tolerance [1] in which we argue that the number of self-peptides to which T cells are tolerant need only be a relatively small number. We termed this model 'The Secretary Hypothesis' because its theme was that MHC molecules act as busy secretaries preoccupied with choosing and presenting very few clients (the peptides) to T-cell receptors. In as much as there are many more clients than secretaries there is inevitably competition for presentation. Certain peptides, by virtue of concentration or access to processing pathways involving MHC molecules, would be favoured before others and would therefore be 'visible'. Below a certain critical threshold all these others would be cryptic. If the favoured peptides were truly an abundant species then there would be a large safety margin to prevent autoimmunity, which could otherwise follow an increase in the level of a particular 'cryptic' peptide. In other words the 'visible' peptides would buffer out the 'cryptic' ones and essentially keep them cryptic. The developing T-cell pool need only be tolerant of the 'visible' peptides to maintain unresponsiveness to self.

The majority of visible peptides would include the processed products of abundant molecules both extra- and intracellular in origin: for example, gamma globulins, albumin, complement components, haemoglobin, a range of intracellular enzymes and receptors and even MHC molecules themselves; whereas a range of other tissue-specific peptides might be considered 'cryptic'. For such peptides ever to be 'visible' they would need to become associatd with MHC in the correct cell (any APC) at a level sufficient to induce a recognition response. That recognition response could of course result in either tolerance or immunity. (How that choice is made we will discuss later.) Obviously the terms 'visible' and 'cryptic' are operational. There may be peptides associated with MHC that cannot induce a response, but these could still be targets for effector systems induced in other ways. For example, a potential target cell such as the beta-cell of the pancreas, may not be able to release enough antigen to be processed by APCs, but what it carries could be sufficient for recognition by primed effector cells. This differential sensitivity of the induction and effector arms may in certain circumstances lead to breakdown of non-responsiveness by molecular mimicry. Here a foreign peptide (in conjunction with host MHC) may induce a response leaving host effector systems to detect and attack the low levels of

a cross-reacting 'cryptic' self peptide + MHC. If this response were sufficient to evoke damage than inflammation and subsequently a cascade process might expose a range of other cryptic peptides that would perpetuate damage and thus sustain a chronic autoimmune disease. There may be many peptides sitting in the clefts of self-MHC at levels that fail to induce responses but that can be targets for them in the rare circumstances of mimicry. When the foreign antigen disappears by clearance or otherwise then these same peptides may not access APC in sufficient quantities to perpetuate the response which would then fade away.

By chance certain favoured self-peptides may be polymorphic. Predictably these peptides would behave as minor transplantation antigens. Those MHC-binding peptides that are not polymorphic would similarly serve a secretarial/buffering role in allotransplantation by competing out other cryptic peptides and lowering the effective concentration of 'minors' that would be available to sensitize.

The essence of this model is that access of peptides to T-cell receptors (for tolerance or immunity) is competitive. Let us for the present stay with immunity. In this setting the obvious question is how can an external antigen evoke an immune response, if it is having to compete with this wealth of self-peptides? We would argue that indeed this is the case—that many foreign antigens may fail to immunize. It is a common experience for the experimenter to fail to immunize to a simple protein unless it is presented appropriately or in an adjuvant. The response to infectious agents is however an impressive one; and we would argue that infectious agents are privileged for access to the secretary (MHC) in this sea of peptides. This privilege relates to a number of special properties. For example, each microbe will possess multiple repeats of any given protein from which multiple peptide fragments will be generated. Viruses will have special means of achieving intracellular entry and privileged processing pathways. Similarly for a range of bacteria natural sugar receptors or preexisting antibodies can facilitate entry and degradation in cells of the macrophage/monocyte lineage on the pathway to presentation. In brief, we see no problem of generating a protective immune response in this setting of competing self-peptides.

We proposed this model prior to the resolution of the X-ray crystallographic structure of the MHC [2]. The structure reveals a cleft where peptides may sit. It is for this binding site that competition could occur.

In the setting of autoimmunity we would argue that we are considering a situation where cryptic antigens have become visible. In other words it may not be a question of tolerance being broken (because true tolerance never existed to these peptides); but rather that particular peptide fragments successfully competed to associate with sufficient MHC Class II or Class I molecules on APC capable of initiating a response or on target cells capable of being recognized by a response induced through mimicry. Where the resulting inflammatory reaction does not become self-limiting then autoimmune processes will continue.

On this scheme intervention would aim to: (1) eliminate the inciting antigen by drugs in the case of infectious mimicry or by reducing local inflammation in the case of cryptic 'revelations'; (2) establish a state of tolerance to these revealed cryptic antigens. That tolerance need not be long lived, but simply long enough to cover the period in which destructive tissue damage can be resolved and relevant cryptic peptides encouraged back into their holes.

Monoclonal antibodies for immunosuppression and tolerance

There have been many publications demonstrating the benefit of monoclonal antibodies in the treatment of autoimmunity in rodent models. Perhaps the most widely documented are those using CD4 MoAbs. As $CD4^+$ T cells play such a central role in both cell mediated and humoral responses they constitute an obvious target. Furthermore the CD4 molecule itself seems to participate in the processes of receptor occupancy by antigen and perhaps in signalling. Therapy with CD4 MoAbs could, therefore, both deplete as well as block function of $CD4^+$ T cells.

Clearly CD4 MoAbs can be very immunosuppressive, and this may contribute substantially to the control of inflammation, and as a consequence interrupt the cascade of processes that expose the cryptic antigens and perpetuate autoimmunity. It is likely that this in itself can explain much of the available data. However, in the last few years our own laboratory [3–6] and that of Wofsy and Seaman [7, 8] have determined that CD4 MoAb therapy may in certain circumstances lead to the induction of a tolerant state to antigens administered around the same time. We shall recall a few salient features from our own published data to establish a theoretical framework for how monoclonal antibodies might be used to permit therapeutic tolerance to endogenous antigens.

The key findings using human immunoglobulin as the 'tolerogen' were [5, 6] that: (1) the 'tolerogen' could be administered for up to a week after the end of MoAb therapy and still produce tolerance; (2) there was no requirement for CD8 cells for the generation of tolerance; nor was there any evidence of transferable suppression; (3) tolerance could be maintained indefinitely if antigen were administered every 5 weeks or so, without a need for further CD4 therapy. This phenomenon we have called 'reinforcement'; (4) there was no need for depletion of $CD4^+$ cells as low concentrations of CD4 antibody pairs or even F(ab')2 fragments were sufficient to permit tolerance to occur; (5) at the cellular level tolerance was maintained at the level of T cells but not of B cells. In other words there was evidence for functional deletion or paralysis of HGG-specific $CD4^+$ cells; (6) this was not uniquely a property of CD4 MoAbs but could also be reproduced by a non-depleting MoAb to LFA-1 (CD11a). Similarly, for CD8 in a system of tolerance to minor histocompatibility antigens, we have shown [Shixin Qin *et al.* in preparation] that a comparable tolerant state can be induced in $CD8^+$ cells using F(ab')2 fragments of a CD8 MoAb. The conclusion is that peripheral T cells, both $CD4^+$ and $CD8^+$, can be turned off (tolerised) if presented with antigen in circumstances where the CD4, CD8 and LFA-1 surface molecules have been peturbed with appropriate MoAbs.

On the basis of this information we propose a model of how tolerance may arise in normal development and how monoclonal antibodies may be used to simulate this process in the adult. We make two basic assumptions. The first requires that T-cell-receptor occupancy and the signal provided by recognition of peptide-MHC is equivalent for both tolerance and immunity. The second assumption is that the critical decision (ON or OFF) is decided by secondary signals in a Bretsher–Cohn [9] manner. We argue that these costimulatory signals arise through multiple cell interactions, each contributing some level of help which ultimately reaches a threshold which pushes the system to ON. By inference a T cell which has adequately bound antigen but is isolated from collaborating partners cannot receive costimulatory signals and defaults to OFF. In other words, the choice ON or OFF is dictated by the

frequencies of antigen-reactive T cells and the chance that these can be brought together into a collaborative unit. For a potent antigen with many foreign epitopes the chance would be relatively high. However, any situation that would isolate T-cells from each other in space or time would predispose to tolerance. By this token we would explain the ease of tolerance induction in the neonatal mouse, the irradiated animal, the animal depleted of lymphocytes by thoracic duct drainage or by administration of anti-lymphocyte globulin. Normal self tolerance would arise by what we would call the 'Lemming effect'. Simply put, the thymus is a relatively helpless environment. No sooner does a T cell express an anti-self receptor than it comes to the antigen-cliff and jumps over or deletes. There is a perpetual loss of self-reactive cells one by one. Therefore no helpful cells can accumulate to provide costimulator activity to the incoming self-reactive set, and of course these newcomers die. The situation would be different in a peripheral lymph-node following exposure to a foreign antigen. Mature T cells are continuously recirculating from blood to lymph. Antigens with multiple epitopes are likely to recruit specific T cells successfully from the recirculating pool in sufficient numbers to generate an active collaborative unit wherein all the necessary requirements for triggering and growth are met; and thus tolerance is prevented. Bystander effects would also interfere with tolerance induction to other antigens in this environment.

The model explains tolerance through MoAb therapy in the following way (Figure 1). Where depletion has occurred then the number of residual antigen-specific T cells may be too small to form a collaborative unit and so tolerance would occur. If antigen persisted then some part would be available to tolerize new T cells as they formed and this process of tolerogenesis would continue until antigen levels dropped to below a threshold value. Present levels of depletion would probably still leave too many T cells thus precluding tolerance for many antigens. However CD4 MoAbs would still blockade any cells that had not been depleted. Once antibody therapy is stopped then cells would gradually emerge from blockade. This staggered exit from blockade would mean a staggered contact with antigen. The competent T cell would find itself isolated and helpless and as a result tolerance-susceptible. Tolerance would again be a consequence of a low frequency of available T cells. If however the antigen possessed too many foreign epitopes then the chance of adequately controlling exit from blockade in a tolerance-effective manner may be poor and so a positive immune response would ensue.

The essence of the argument is that T cells could only become tolerant if receptor occupancy occurred and this would require CD4 participation. In other words, the initial confrontation between antigen and T cells is symmetrical; the critical decision (ON or OFF) being determined by the number of T cells or T cells and other collaborative cells that could be brought together. Whether one deals with depletion or blockade the outcome reflects the frequencies of helpful cells available at the time of antigen confrontation.

With time, antigen levels would drop and so would the levels of the depleting and blocking antibodies. Gradually, antigen reactive T cells would return by *de novo* formation. If antigen were to be reintroduced to the system at a time when only a small number of antigen-specific T cells had developed, then inevitably these would simply fall off the antigen-cliff and become tolerant as they would be lacking the necessary help. This would explain the reinforcement data without a need to entertain

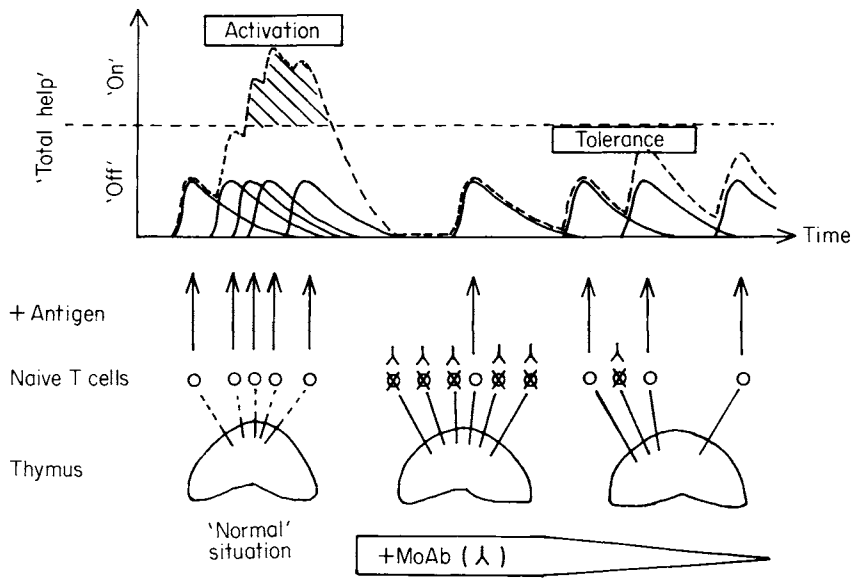

Figure 1. A model for how T cells make the ON or OFF choice following exposure to antigen. The basis of the hypothesis is that T cells depend upon collaborative encounters with other T cells or other helpful cells for an ON response to occur. Lack of helpful signals leaves a T cell isolated in a way that results in the antigen signal (peptide + MHC) being OFF, i.e. in the functional deletion of that cell.

If we move along the figure from left to right then the key points are: (1) In the normal adult animal challenged with antigen, many T cells are brought into critical sites of induction in lymphoid tissues. The direct cellular interactions with adhesion molecules and growth factors result in a cumulative ON effect where all participants survive and may be activated. (2) Following administration of CD4, CD8 or LFA-1 antibodies cells bearing these markers are either eliminated (i.e. reduced in numbers substantially) or blocked. The remaining cells and those gradually coming out of blockade (as monoclonal antibody levels decline) will now contact antigen in relative isolation. The ability of any lymphocyte to be helpful or be receptive to help will only last for a limited period, and failure to form a collaborative unit will result in OFF and possibly death of that cell. If not death then at least functional inactivation.

On this model tolerance of T cells to any antigen would require a temporally controlled functional isolation of the T-cell population so that all antigen-specific T cells would eventually see antigen in helpless conditions. Experimental failures to induce tolerance to many antigens with CD4 MoAbs may reflect simply that such helpless conditions were not achieved for all lymphocytes.

ideas of suppression etc. The same ideas would be applicable to tolerance following LFA-1 therapy and following therapy with IL-2 receptor antibodies. In the latter case, one source of help would be compromised. The possibilities are that interference with other growth-factor receptors and adhesion molecules would also facilitate the process of isolating a T cell from help, and thus enable tolerance to be induced.

Future prospects

The basis of the above hypotheses is that a T cell is tolerizable if it can be isolated from other helpful cells and yet exposed to antigen on appropriate APCs. The ease with which such isolation may be achieved will of course depend on the circumstances. For example tolerizing memory populations may be harder than tolerizing virgin cells both on the basis of real frequencies and the effective chance of interactions based on the expression (up or down regulated) of adhesion molecules and

growth factor receptors. However, a component of interference at all levels of interaction by a synergistic battery of reagents may be a way of achieving tolerance for memory cells comparable to that elicited by single agent therapy with virgin cells. For man, a controlled debulking of lymphocytes coupled with more subtle use of non-depleting blocking MoAbs as well as other tolerance therapies may perhaps be the way to create the operational isolation to favour tolerance whilst simultaneously limiting the phlogistic consequences of T-cell activation.

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Clinical Use of OKT3: The Role of Cytokine Release and Xenosensitization

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Introduction

The clinical use of immunosuppressive anti-T cell murine monoclonal antibodies (MoAbs) has so far mostly been confined to transplantation programs. An indicative although non-exhaustive list of the different membrane molecules targeted by MoAbs used as immunosuppressors in transplantation is shown in Table 1 [1–13].

The MoAb that has been the most widely used in this setting is OKT3 (anti-CD3). It was introduced into clinical practice in 1980 and has been marketed since 1986. OKT3 has been shown by different authors to be a very potent immunosuppressor able effectively to treat and also prevent acute allograft rejection.

OKT3 thus is a good example of a MoAb that in many transplantation centers is progressively replacing conventional serotherapy with polyclonal anti-lymphocyte sera. Although over the past few years very convincing data have been accumulated showing the effectiveness of some anti-T cell MoAbs in the treatment or prevention of both spontaneous and induced autoimmune diseases [14–17], very few attempts have been made to extend these results to clinical practice (details in Table 2) [18–22].

One major reason for this paradox is that the *in vivo* administration of MoAbs may have side effects that not only interfere with the therapeutic effectiveness of the

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Table 1. *Xenogeneic anti-T cell antibodies used as immunosuppressive agents in clinical transplantation*

Kidney	OKT3 (CD3)	[1–5]
	WT32 (CD3)	[6]
	T12 (CD6)	[7]
	BCL1 (blast cells)	[8]
	33B 3.1 (CD25)	[9]
	BMA 031 (Ti)	[L. Chatenoud *et al.* in preparation]
Heart, liver	OKT3 (CD3)	[10]
Bone marrow	OKT3 (CD3)	[11–12]
	anti-LFA_1	[13]
	33B 3.1 (CD25)	[D. Maraninchi *et al.* personal communication]

Table 2. *Xenogeneic anti-T cell monoclonal antibodies used as immunosuppressors in autoimmune diseases*

Rheumatoid arthritis	Leu 3 (CD4)	[18]
	RFT2 (CD7)	[19]
Multiple sclerosis	Anti-T12 (CD6)	[20]
	Anti-T4 (CD4)	[21]
	Anti-T11 (CD2)	[21]
	OKT3 (CD3)	[22]
Autoimmune diabetes	OKT3 (CD3)	[Our personal unpublished data]

product but also, if an inadequate protocol is applied, may be harmful to the patient. Our aim is to analyze the pathophysiological basis of these side effects using our experience with OKT3 in renal allograft recipients, in an effort to delineate better strategies to preclude such manifestations. Schematically these side effects may be separated into three major categories related to: (1) The specificity of the MoAbs used; once binding has occurred, the target molecule to which the MoAb is directed may trigger a series of consequences closely related to its physiological role. This is the case for the clinical syndrome following the first two or three anti-CD3 MoAb injections induced, at least in part, by a transient state of T-cell activation provoked by the *in vivo* triggering of the T3 molecular complex. (2) The xenogeneic nature of the MoAbs used; this factor is responsible for the sensitization observed, especially when the MoAbs are administered without associated immunosuppressors. (3) The degree of immunosuppression induced by the anti-T cell murine MoAb. In practice, if we consider again the case of OKT3, the different protocols undertaken clearly show that, in order to avoid the occurrence of severe viral infections, this MoAb should not be used for periods longer than 2 to 3 consecutive weeks.

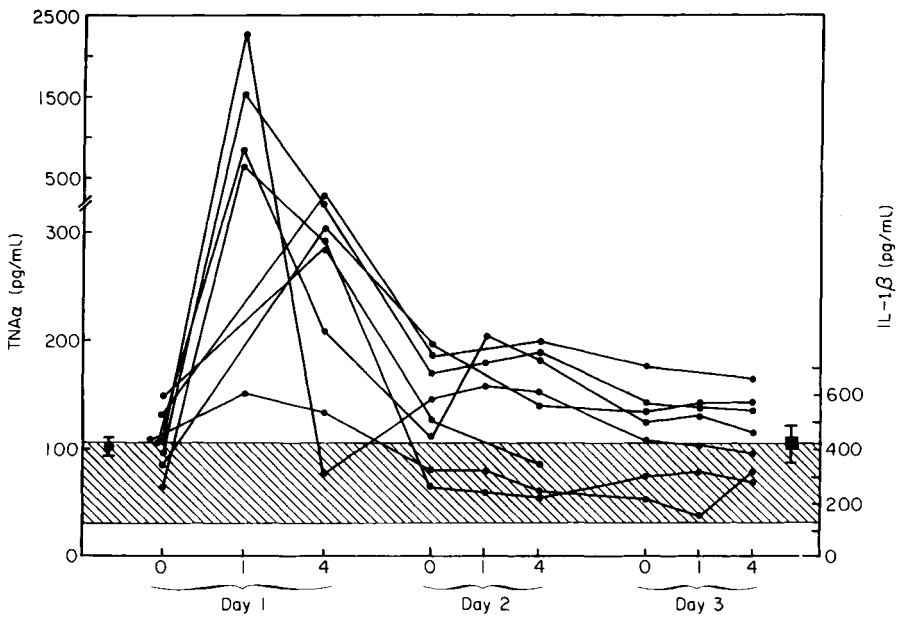

Figure 1. Serum TNFα (curves) and IL-1β concentrations (hatched area) in eight OKT3-treated renal allograft recipients. Samples were tested before and at 1 and 4 h following the first, second and final OKT3 injections. ●, mean ± SEM TNFα serum concentration in patients before treatment (105.37 ± 8.88 pg/ml); ■, mean ± SEM IL-1β serum concentration in patients before treatment (262.5 ± 19.55 pg/ml). In all patients, IL-1β serum concentrations remained within the normal range, shown in the figure by the hatched area (in normal controls serum IL-1β concentrations: 336.50 ± 17.31 pg/ml).

We will focus on the first two points since they represent important pitfalls specifically related to the use of anti-T cell murine MoAbs. Over-immunosuppression (leading to an increased incidence of infections or tumors) may in fact be considered a general risk of all types of chronic immunosuppressive regimens.

Transient activation induced by OKT3

It has been a common experience of all centers using OKT3 that the first injections of MoAb invariably induce a 'flu-like' syndrome including, in variable proportions depending on the patient, chills, headache, pyrexia, vomiting, diarrhea, tachycardia, respiratory distress, hypotension and arthragia [1, 2, 4]. Meningismus is seldom observed; pulmonary edema was related in all cases to patient fluid overload [1]. A more severe reaction than that observed in allograft recipients was seen in the few patients treated so far with OKT3, who presented an autoimmune disease (either multiple sclerosis [22] or autoimmune diabetes [our personal unpublished data]).

This reaction is spontaneously reversible and is not life-threatening although the association of the symptoms listed above may become highly disabling for the patient. It has been confirmed that this reaction is not related to hypersensitivity and, since its symptoms are highly reminiscent of the clinical side effects induced by the *in vivo* injection both in humans and animals of different recombinant cytokine molecules [23–26], it has been attributed to an acute release of cell mediators. However, it has been impossible to test this hypothesis until very recently, when the availability of specific radioimmunological assays (RIA) allowed us to detect these various molecules namely IL-1β, IL-2, TNFα, IFNα and IFNγ in biological fluids.

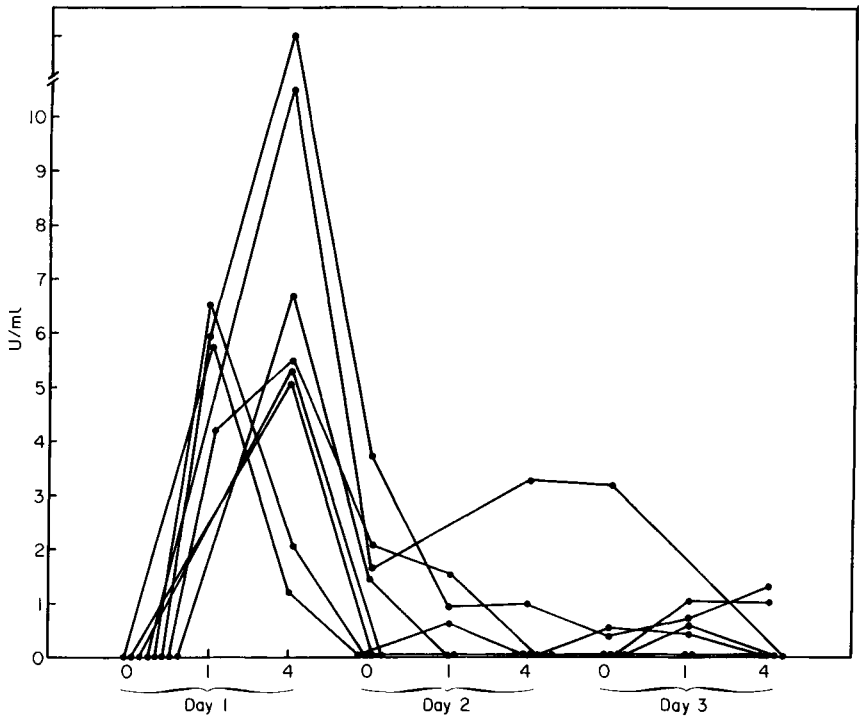

Figure 2. Serum IFNγ serum concentrations in the same patients and with the same kinetics described in Figure 1.

Table 3.

	Ig anti-OKT3			Ig anti-OKT3	
	day 10	1–3 months		day 13	1–3 months
PUI[1]	–	–	QUO[2]	+	+
TRO[1]	–	–	TIA[2]	+	–
CHA[1]	–	–	NAC[2]	+	+
BOU[1]	–	+	XAT[2]	+	+
COU[1]	–	+	HAD[2]	+	+
BER[1]	–	–	GUI[2]	+	+

[1]These patients received OKT3 5 mg/d, i.v., day 0–10; cyclosporine A 7.5 mg/d starting at day 8 of OKT3 treatment.

[2]These patients received OKT3 5 mg/d, i.v. only.

We analyzed by means of RIA sera collected from eight renal allograft recipients for the presence of these various cytokines. Seven of these patients were treated prophylactically (that is, to prevent rejection) with OKT3 (5 mg/d, i.v. bolus injection) for 20 to 30 consecutive days in association with low dose steroids (0.25 mg/kg/d) and full dose azathioprine (3 mg/kg/d). In all seven patients, a 1 g bolus injection of methylprednisolone was administered 1–3 h prior to the first

OKT3 injection. The last patient received a haploidentical intrafamilial graft and thus corticosteroids were avoided. Sera were collected prior to the first OKT3 injection and then samples were drawn at 1, 4 and 14 h following the first, second and third injections. The RIAs used were all soluble phase immunoprecipitation tests that have already been described in detail elsewhere [27–29].

In all patients but one a sharp increase in the circulating levels of TNFα was observed as early as 1 h following the first OKT3 injection (Figure 1). Very significant increases in serum levels of circulating IFNγ were also found but, at variance with TNFα, peak values were reached at 4 h following the first injection (Figure 2). Importantly, following OKT3 treatment, IL-2 serum levels were significantly increased only in the patient who did not receive the methylprednisolone bolus prior to the OKT3 injection (i.e. serum IL-2 was undetectable before treatment: at 4 h following the first OKT3 injection 8.1 U/ml were found). In all the other patients IL-2 serum levels remained within the normal range throughout the study period. An interesting dissociation was observed since no IL-1β (Figure 1) and no IFNα could be detected at any time following OKT3 injection.

Just as the clinical syndrome that is spontaneously reversible, this massive release of cytokines into the circulation was self-limited. In all patients tested, pretreatment levels were reached at 14–17 h following the first OKT3 injection, and neither the second nor the third injections induced any further modification. Moreover the highest cytokine levels were recorded in the patient in whom steroids were avoided; this was in fact the patient that presented the most severe clinical reaction. Currently, the corticosteroid bolus is administered prior to OKT3 to decrease the intensity of the clinical reaction.

The mechanisms that can explain this massive although transient cytokine release are related to T-cell opsonization (and depletion) and T-cell activation. T-cell opsonization implies subsequent trapping by the reticuloendothelial system and T-cell lysis, a phenomenom that could easily explain the TNFα release by activated macrophages. However it is worth mentioning that other opsonizing and/or depleting anti-T cell MoAbs have been used both in humans and in experimental models (anti-CD2, anti-CD4, anti-CD6 and anti-CD8) [14, 30, 31] that, however, do not provoke the same clinical syndrome induced by anti-CD3. Thus one cannot ignore the possibility that at least part of this TNFα release is mediated by T cells. Recent data in the literature have in fact confirmed that pure human T lymphocytes may indeed produce significant amounts of both TNFα message and protein when appropriately stimulated, especially by anti-CD3 [32, 33].

In addition, it is evident that OKT3 induces a T-cell activation that explains the presence of circulating IFNγ and in some patients of IL-2. This activation clearly represents the *in vivo* counterpart of the extensively described mitogenic capacity of OKT3 on human lymphocytes. In fact it was recently shown that a pure monocyte activation, like the one observed by injecting *Escherichia Coli* endotoxin into normal human volunteers, promotes only TNFα release into the bloodstream without any evidence for the presence of associated T-cell derived lymphokines [34].

The fact that patients with autoimmune disease exhibit a stronger reaction than allograft recipients may well be related to the fact that the latter always present an immune deficit at the time of transplantation, thus being less sensitive to the anti-CD3 activation effect.

Finally it is worth mentioning that the same reaction is observed in mice treated with the hamster MoAb 145-2C11 that recognizes the murine CD3 molecule [35]. Using this murine model we are trying to determine different strategies to circumvent this important side effect, among which is the use of either polyclonal or monoclonal antibodies that specifically block *in vivo* the cytokine activities previously described.

Anti-monoclonal antibody sensitization

Despite the fact that MoAbs are administered at much lower dosages than traditional anti-lymphocyte sera and that they represent a homogeneous source of deaggregated immunoglobulins, usually administered by a route that is not highly immunogenic (i.e. intravenously) sensitization of the recipient occurs. This sensitization has been regularly observed not only in humans but also in mice, rats and monkeys especially when MoAbs are injected alone, that is, in the absence of associated conventional immunosuppressors [1–3, 5, 31, 36]. However, it should be underlined that with the MoAbs used so far no hypersensitivity reactions leading to clinically evident serum sickness have been observed. Thus the major consequence of this xenosensitization is the complete abrogation of the MoAb therapeutic effectiveness related to its accelerated clearance [5, 37]. At present only one exception has been described, namely the rat MoAb anti-L3T4 that when administered at high dosages has been shown to be tolerogenic [38].

Anti-OKT3 antibodies present isotypic heterogeneity; both IgM and IgG are produced and only anti-OKT3 antibodies to the latter are able to exert a clearcut neutralizing effect on the immunosuppressive capacity of OKT3, an observation probably linked to the lower affinity of IgM anti-OKT3 immunoglobulins compared to IgG [5]. Concerning their fine specificity, essentially two types of anti-OKT3 antibodies have been detected and purified. The first includes anti-isotypic antibodies that react with all mouse IgG2 (OKT3 is an IgG2a) but do not recognize either mouse IgG1, IgG3 or IgM or OKT3 F(ab')2 fragments and do not interfere with OKT3 binding to T cells [5, 39]. Secondly, anti-idiotypic antibodies are also found that react with determinants unique to the variable position (Fab or F(ab')2 fragments) [5]. These latter anti-idiotypic antibodies are oligoclonal and may very effectively block the binding of the MoAb to its target antigen [5, 40]. This particular immunogenicity pattern displayed by OKT3 has been extended to other anti-T cell MoAbs showing different specificities [31, 36]. Thus, similar data have been obtained in monkeys treated with different anti-CD4 and anti-CD8 antibodies [36].

There are now several means available to overcome such deleterious anti-MoAb sensitization. They basically involve variations in the treatment protocol or structural manipulation of the injected MoAb.

Among possible variations in the treatment protocol one finds the association of OKT3 to conventional immunosuppressive agents [4–5]. An associative treatment including low dose corticosteroids and azathioprine has proved to be highly effective in preventing and/or delaying the onset of sensitization [4–5]. Such a strategy, which is at present followed by various centers, allowed effective OKT3 treatment for 20 consecutive days in 75 to 85% of patients.

Cyclosporine is also effective in preventing the anti-MoAb response. This is well illustrated in Table 3 where the immunization pattern is compared between two

series of patients; the first includes six renal allograft recipients that received OKT3 (5 mg/d, i.v.) as the only immunosuppressive treatment for 13 consecutive days; the second includes six patients presenting recent onset Type I diabetes that received OKT3 (5 mg/d, i.v.) for 10 consecutive days and in whom replacement therapy with cyclosporine A was started at 8 days of OKT3 treatment. The results show that the antibody response is very significantly diminished by such cyclosporine A treatment.

Another type of protocol that may be applied, given the restricted specificity and the oligoclonality of the anti-MoAb anti-idiotypic response, is the sequential use of MoAbs with the same specificity but expressing different idiotypes [41]. Indeed, M. Jonker *et al.* were able, with such a strategy, to achieve sustained *in vivo* immunosuppression in a rhesus monkey model by using consecutively different anti-CD4 MoAbs [42].

Structural manipulation to 'humanize' MoAb molecules has been successful with the chimeric form of the CAMPATH 1 rat immunoglobulin [43]. In addition human MoAbs of the IgG isotype and exhibiting high affinity may now be obtained by using heteromyelomas as fusion partners [P. Lake, personal communication].

However, clinical trials are necessary to determine whether these new types of MoAbs will allow circumventing anti-idiotypic sensitization.

The results obtained by Benjamin *et al.* [31] argue against this hypothesis. These authors showed that it was impossible to tolerize mice against the idiotypic determinants of rat MoAb injected *in vivo* when they are directed against host tissue antigens.

Immunotoxins also represent good alternatives and are discussed in detail elsewhere in this volume.

Conclusions

Several characteristics of anti-T cell MoAbs make them very useful potential tools in clinical autoimmune diseases, as complements and/or alternatives to conventional drugs, notably cyclosporine A, steroids, azathioprine and cyclophosphamide. Firstly, they are potent immunosuppressors and their effectiveness in experimental autoimmune models is now well established. Secondly, they can be associated with other immunosuppressors, the more so since these therapeutic associations, when adequately conducted, may prevent xenosensitization.

However the disadvantages are that prolonged treatment cannot be performed without exposing the patient to the risk of over-immunosuppression. MoAbs may thus represent good candidates for treatment of autoimmune patients at a very precise stage of the disease, that is preferentially at exacerbation of the immunological injury. Thus it seems obvious that future progress in this field will rely less on the characterization of new monoclonal antibodies, since we already have very sophisticated ones, than on the definition of new means to monitor disease activity in autoimmune patients.

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The Multichain Interleukin-2-Receptor: A Target for Immunotherapy of Patients with Adult T-cell Leukemia, Autoimmune Disorders and Individuals Receiving Allografts

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Antigen-induced activation of resting T cells induces the synthesis of interleukin-2 (IL-2), as well as the expression of specific cell surface high-affinity receptors for this lymphokine. There are at least two forms of the cellular receptors for IL-2, one with a very high affinity and the other with a lower affinity. We have identified two IL-2-binding peptides, a 55-kD peptide reactive with the anti-Tac monoclonal antibody and a 75-kD non-Tac IL-2-binding peptide. Cell lines bearing either the p55 (Tac) or the p75 peptide alone manifested low- to intermediate-affinity IL-2 binding, whereas cell lines bearing both peptides manifested high- and low-affinity receptors. Fusion of cell membranes from low-affinity IL-2-binding cells bearing the Tac peptide alone with membranes from a cell line bearing the p75 peptide alone generated hybrid membranes bearing high-affinity receptors. We propose a multichain model for the high-affinity IL-2-receptor in which both the p55 Tac and the p75 IL-2-binding peptides are associated in a receptor complex. An additional 90-100-kD peptide may also participate in the multi-subunit high-affinity form of the IL-2-receptor. The p75 peptide is the receptor for IL-2 on large granular lymphocytes and is sufficient for the IL-2 activation of these cells. In contrast to resting T cells, the activated T cells in certain neoplasias of mononuclear cells, select autoimmune disorders and in organ allograft rejections express the Tac antigen. Specifically, human T-cell lymphotrophic virus Type I (HTLV-I)-associated adult T-cell leukemia cells constitutively express large numbers of IL-2 Tac receptors. A 42-kD tat protein encoded predominantly by the pX region of HTLV-I may play a role in directly or indirectly increasing the transcription of the 55-kD Tac IL-2-receptor gene. To exploit the fact that IL-2-receptors are present on abnormally activated T cells but not on normal resting cells, clinical trials have been initiated involving patients with neoplastic or autoimmune disorders as well as those receiving organ allografts. These patients are being treated with unmodified anti-Tac, with anti-Tac conjugated to truncated *Pseudomonas* toxin, with isotopic (^{212}Bi and ^{90}Y) chelates of anti-Tac and with recombinant 'humanized' anti-Tac.

Introduction

T cells mediate important regulatory functions, such as help or suppression, as well as effector functions, like the cytotoxic destruction of antigen-bearing cells and the production of soluble products termed lymphokines. Failure of T cells to function normally may lead to immunodeficiency or neoplastic and/or autoimmune disease. Successful T-cell-mediated immune responses require that the T cells change from a resting to an activated state.

The activation of T cells requires two set of signals from cell surface receptors to the nucleus. The first signal is initiated when appropriately processed and presented foreign antigen interacts with the 90-kD polymorphic heterodimeric T-cell surface receptor for the specific antigen. Following the interaction of antigen presented in the context of products of the major histocompatibility locus and interleukin-1 or interleukin-6 with the antigen receptor, T cells synthesize IL-2 [1, 2]. To exert its biological effect, IL-2 must interact with specific high-affinity membrane receptors. Resting T cells do not express high-affinity IL-2-receptors, but receptors are rapidly expressed on T cells after activation with an antigen or mitogen [3, 4]. Thus, the growth factor IL-2 and the high-affinity form of its receptor are absent in resting T cells, but after activation both proteins are expressed. Although the interaction of appropriately presented antigen with its specific receptor confers specificity for a given immune response, the interaction of IL-2 with high-affinity IL-2-receptors determines its magnitude and duration.

Robb [3], utilizing purified, biosynthetically labeled IL-2, demonstrated specific, saturable, high-affinity binding sites on IL-2-dependent T-cell lines as well as mitogen- and alloantigen-activated T cells. Further progress in the analysis of the structure, function and expression of the human IL-2-receptor was greatly facilitated by our production of an IgG_{2a} mouse monoclonal antibody (termed anti-Tac) that was shown to recognize the human IL-2-receptor [5–7]. We have utilized the anti-Tac monoclonal antibody and radiolabeled IL-2 in cross-linking studies to (1) define multiple IL-2-binding peptides that participate in the human receptor for IL-2; (2) molecularly clone cDNAs for the 55-kD peptide of the human IL-2-receptor; (3) define the cellular distribution of IL-2-receptors; (4) determine the immunoregulatory effects that require the interaction of IL-2 with its receptor; (5) analyze disorders of IL-2-receptor expression on leukemic cells, as well as those participating in autoimmune disorders; and (6) develop protocols for the IL-2 receptor-directed therapy of patients with IL-2 receptor-expressing adult T-cell leukemia, patients receiving organ allografts and individuals with autoimmune disease.

Chemical characterization of the multichain IL-2 receptor

The IL-2-binding receptor peptide identified by the anti-Tac monoclonal on phytohemagglutinin (PHA)-activated normal lymphocytes is a 55-kD glycoprotein [6, 7] that is sulfated [8] and phosphorylated on a serine residue [9].

There were a series of unresolved questions concerning the IL-2-receptor that were difficult to answer when only the 55-kD Tac peptide was considered. These questions concerned (1) the structural explanation for the great difference in affinity

between high- and low-affinity receptors; (2) the mechanism used to transduce receptor signals to the nucleus in light of the short cytoplasmic tail of 13 amino acids (see below); and (3) the non-proliferative responses of Tac^- cells (e.g. large granular lymphocytes [LGL] that are precursors of natural killer [NK] and lymphokine-activated killer [LAK] cells) to IL-2. Furthermore, IL-2 has been shown to upregulate the expression of Tac mRNA in multiple cell types, including some that initially do not express the Tac peptide. Finally, certain cell lines (e.g. MLA-144) are Tac^- yet manifest IL-2 binding sites.

We [10] and others [11] have resolved most of these questions by the definition of a new non-Tac IL-2-binding peptide with a molecular weight of 68,000–76,000 (p75). Using cross-linking methodology, we demonstrated the p75 peptide on MLA-144, a gibbon ape T-cell line that does not express the Tac antigen but manifests relatively low-affinity ($K_d = 14$ nM) IL-2-binding sites. The p75 peptide was also identified in additional to the Tac peptide (p55) on all cell populations expressing both high- and low-affinity receptors. We proposed a multichain model for the high-affinity IL-2-receptor in which an independently existing Tac or p75 peptide would represent low- and intermediate-affinity receptors, respectively, whereas high-affinity receptors would be expressed when both peptides are expressed and associated in a receptor complex [10]. To test this working hypothesis, a variety of T-cell lines were examined for IL-2 binding and were subjected to IL-2 cross-linking studies to determine if there was a correlation between the affinity of IL-2 binding and the IL-2-binding peptides expressed. In these studies, cell lines bearing either the p55 Tac or the p75 peptide alone manifested low- and intermediate-affinity IL-2 binding, respectively, whereas a cell line bearing both peptides manifested both high- and low-affinity receptors. Furthermore, fusion of cell membranes from a low-affinity IL-2-binding cell line bearing the Tac peptide alone (MT-1) with membranes from a cell line bearing the p75 peptide alone (MLA-144) generated hybrid membranes bearing high-affinity receptors [12]. These studies support the proposed multichain model for the high-affinity IL-2-receptor.

There is evidence suggesting a more complex subunit structure that involves peptides in addition to the p55 and p75 IL-2-binding peptides. Two monoclonal antibodies, OKT27 and OKT27b, were produced that react with distinct epitopes of a 95-kD peptide. The OKT27b antibody inconsistently coprecipitated the 55-kD Tac peptide as well as the 95-kD peptide [13]. A flow cytometric energy transfer technique was used to demonstrate a close non-random proximity between p55 Tac and the 95-kD T27 peptides [13]. Furthermore, fluorescence photobleaching recovery measurements suggest that the Tac and T27 peptides interact physically *in situ* in HUT-102 membranes [14]. In independent chemical cross-linking studies with radiolabeled IL-2, Herrmann [15] and Saragovi [16] presented evidence for a 90-100-kD IL-2-binding peptide in mice associated with the 55- and 75-kD chains of the high-affinity form of the IL-2-receptor on mouse T-cell blasts, CTLL-16 cells and sublines of EL-4 transfected with the gene encoding the p55 peptide. This 90-100-kD peptide was not precipitated by an anti-p55-specific antibody. Taken together, these studies suggest that three IL-2-binding chains (p55, p75 and p95) are associated in the multi-subunit high-affinity IL-2-receptor.

The three-dimensional structure of the 133 amino acid lymphokine IL-2 has been defined [17]. These studies, taken in conjunction with studies using site-specific

mutagenesis of IL-2 and monoclonal antibodies directed toward defined regions of IL-2 in neutralization and binding assays [18–20], have aided in the analysis of the structure-function relationships of human IL-2. Furthermore, they have led to the identification of the amino acid residues required for binding to the different IL-2-receptor peptides and for biological activity. IL-2 has an α-helical tertiary structure involving six α helices that suggests certain portions of the molecule form a structural scaffold that underlies the receptor binding facet of the molecule [17]. A short helical segment (helix A, amino acid residues 11–19) and the associated amino acid 20 is required for biological activity and appears to be involved in binding to the p75 IL-2-binding peptide. The second helix on the structural scaffold helix is an extended loop involving residues 33–56 that form a helix interrupted in the middle by Pro^{47}. These two segments are referred to as B and B′. This segment appears to be required for binding to the p55 Tac peptide. An additional α helix E (amino acids 107–113) is also positioned on the binding plane and could theoretically bind the proposed 90-100-kD IL-2-binding peptide. However, no extensive studies of this region of IL-2 have been made. Finally, the carboxy-terminal residues 121–133 and two of the three cysterine residues (58 and 105) are required for full biological activity and binding [18].

Molecular cloning of cDNAs for the human 55-kD Tac IL-2-receptor peptide

Three laboratories [21–23] have succeeded in cloning cDNAs for the p55 Tac IL-2-receptor peptide. The deduced amino acid sequence of the IL-2-receptor indicates that this peptide is composed of 251 amino acids, as well as a 21 amino acid signal peptide. The receptor contains two potential N-linked glycosylation sites and multiple possible O-linked carbohydrated sites. Finally, there is a single hydrophobic membrane region of 19 amino acids and a very short (13 amino acid) cytoplasmic domain. Potential phosphate acceptor sites (serine and threonine, but not tyrosine) are present within the intracytoplasmic domain. However, the cytoplasmic domain of the IL-2-receptor peptide identified by anti-Tac appears to be too small for enzymatic function. This receptor differs from other known growth factor receptors that have large intracytoplasmic domains with tyrosine kinase activity. Thus, the p75 peptide, the p90–100 peptide or other peptides associated with the Tac peptide may play a critical role in the transduction of the IL-2 signal to the nucleus.

Distribution of IL-2-receptors

The majority of resting T cells, B cells or monocytes in the circulation do not display the 55-kD peptide of the IL-2-receptor. Specifically, less than 5% of freshly isolated, unstimulated human peripheral blood T lymphocytes react with the anti-Tac monoclonal antibody. The majority of T lymphocytes, however, can be induced to express IL-2-receptors by interaction with lectins, monoclonal antibodies to the T-cell antigen receptor complex or by alloantigen stimulation.

In addition, the Tac peptide has also been demonstrated on activated B cells [24]. Such Tac-positive B cells manifested both high- and and low-affinity IL-2-receptors at a ratio of 1:10, comparable to that observed with IL-2-dependent T-cells lines and activated T lymphocytes. The size of the IL-2-receptors on Tac^+, cloned, normal

B cells was comparable (53–57 kD) to that of receptors on PHA-stimulated T lymphoblasts [24]. Finally, Tac^+ B cells transcribed 1,500 to 3,400 base mRNAs for the IL-2-receptor. Thus, certain malignant as well as activated normal B cells display the Tac antigen and manifest high-affinity receptors for IL-2.

IL-2-receptors identified with the anti-Tac monoclonal antibody have been detected on activated cells of the monocyte-macrophage series, including cultured monocytes, Kupffer cells of the liver, cultured lung macrophages, Langerhan's cells of the skin and Reed-Sternberg cells in Hodgkin's disease [25, 26].

Rubin [27] demonstrated that activated normal peripheral blood mononuclear cells and certain lines of T- and B-cell origin release a soluble form of the IL-2-receptors into the culture medium. Using an enzyme-linked immunosorbent assay with two monoclonal antibodies that recognize distinct epitopes on the human IL-2 Tac receptor, they showed that normal individuals have measurable amounts of IL-2-receptors in their plasma and that certain lymphoreticular malignancies, autoimmune disorders and allograft reactions are associated with elevated plasma levels of this receptor. The release of soluble IL-2-receptors appears to be a consequence of cellular activation of various cell types that may play a role in the regulation of the immune response. Furthermore, the analysis of plasma levels of IL-2-receptors appears to provide a very valuable new approach to the analysis of both normal and disease-associated lymphocyte activation *in vivo*.

The p75 IL-2-binding peptide is expressed along with the 55-kD Tac peptide on activated T and B lymphocytes. Furthermore, it is expressed on certain circulating cells that do not express the Tac antigen. It has been known that Tac-non-expressing LGL can be stimulated by IL-2 to enhanced NK activity and to generate the cytotoxic LAK cells that can lyse NK-resistant tumor targets [28, 29]. Using cross-linking methodology with radiolabeled IL-2, we demonstrated that normal LGL and leukemic LGL from all individuals tested expressed that p75 IL-2-binding peptide but did not express the Tac peptide [30].

Lymphocyte functions that are regulated by the interaction of IL-2 with its receptor

The anti-Tac monoclonal antibody has been used to define those lymphocyte functions that require an interaction of IL-2 with the 55-kD inducible receptor on activated T and B lymphocytes. The addition of anti-Tac to cultures of human peripheral blood mononuclear cells inhibited a variety of immune reactions. Anti-Tac profoundly inhibited the proliferation of T lymphocytes stimulated by soluble antigens and by cell surface antigens (autologous and allogeneic mixed lymphocyte reactions). Upon activation, human T cells acquire other surface structures that in large measure are growth factor receptors that are not easy to detect during their resting stage [31, 32]. The addition of anti-Tac at the initiation of T-cell cultures stimulated by mitogens, antigens or the T3 antibody inhibited the expression of these late-appearing activation proteins examined, the insulin and transferrin receptors and the Ia proteins [31, 32]. Anti-Tac was also shown to inhibit a series of T-cell functions, including the generation of both cytotoxic and suppressor T lymphocytes in allogeneic cell cultures, but did not inhibit their action once generated. In contrast to the action of T cells, anti-Tac did not inhibit the IL-2-induced activation of LGL into effective NK and LAK cells. As noted above, LGL express the p75 but not the

55-kD Tac peptide. Furthermore, upregulation of the expression of Tac mRNA and Tac peptide by IL-2 has been demonstrated for a number of cell types (e.g. LGL, B cells and resting T cells), including some that initially express few if any Tac molecules [24, 33, 34]. The addition of IL-2 to such Tac^- cells, including LGL leukemia cells, augmented transcription of the Tac gene and induced and expression of the Tac peptide [30]. Neither the IL-2-induced activity of LGL nor the upregulation of Tac gene expression was inhibitied by the addition of anti-Tac. These results strongly suggest that the p75 peptide is responsible for IL-2-induced activation of LGL and that the p75 peptide can mediate an IL-2 signal with co-expression of the Tac peptide. Thus, the p75 peptide may play an important role in the IL-2-mediated immune response not only by participating with the Tac peptide in the formation of the high-affinity receptor complex on T cells but also by contributing to the initial triggering of LGL activation so that these cells become efficient NK and LAK cells.

Disorders of IL-2 expression in malignant and autoimmune diseases

Normal resting T cells, B cells and monocytes do not express the Tac peptide of the IL-2-receptor. In contrast, this receptor is expressed by a proportion of the abnormal cells in certain forms of lymphoid neoplasia, select autoimmune diseases and in individuals rejecting allografts. That is, a proportion of the abnormal cells in these diseases express the Tac antigen on their surface. Furthermore, the serum concentration of the soluble form of the Tac peptide is elevated. In terms of the neoplasias, certain T-cell, B-cell, monocytic and even granulocytic leukemias express the Tac antigen. Specifically, virtually all of the abnormal cells of patients with HTLV-I ATL express the Tac antigen. Similarly, a proportion of patients with cutaneous T-cell lymphomas, including the Sézary syndrome and mycosis fungoides, express the Tac peptide. Furthermore, the malignant B cells of virtually all patients with hairy cell leukemia and a proportion of patients with large and mixed cell diffuse lymphomas are Tac^+. The Tac antigen is also expressed on the Reed–Sternberg cells of patients with Hodgkin's disease and on the malignant cells of patients with true histiocytic lymphoma. Finally, a proportion of the leukemic cells of patients with chronic and acute myelogenous leukemia are Tac^+. In addition to these Tac-expressing leukemias and lymphomas, there are certain leukemias (e.g. acute lymphoblastic leukemia and LGL leukemia) that do not express the Tac peptide but do express the p75 peptide of the IL-2-receptor.

Autoimmune diseases may also be associated with disorders of Tac-antigen expression. A proportion of the mononuclear cells in the involved tissues express the Tac antigen, and the serum concentration of the soluble form of the Tac peptide is elevated. Such evidence for T-cell activation and disorders of Tac-antigen expression are present in patients with rheumatoid arthritis, systemic lupus erythematosus, subsets of patients with aplastic anemia and individuals with HTLV-I-associated tropical spastic paraparesis. Disorders of IL-2-receptor expression have also been demonstrated in animal models of these diseases, including adjuvant arthritis of rats, the rodent models of Type I diabetes (NOD mouse and BB rat), experimental allergic encephalomyelitis (EAE) of mice and in rodent models of systemic lupus erythematosus.

We have focused our studies of disorders of IL-2-receptor expression on a distinct from of mature T-cell leukemia that was defined by Takasuki [35] and termed adult T-cell leukemia (ATL). ATL is a malignant proliferation of mature T cells that have a tendency to infiltrate the skin, lungs and liver. HTLV-I has been shown to be the primary etiologic agent in ATL [36]. All the populations of leukemic cells we have examined from patients with HTLV-I-associated ATL expressed the Tac antigen [37]. An analysis of HTLV-I and its protein products suggests a potential mechanism for this association between HTLV-I and IL-2-receptor expression. The complete sequence of HTLV-I has been determined by Seiki [38]. In addition to the presence of typical long terminal repeats (LTRs) (e.g. *gag*, *pol* and *env* genes), retroviral gene sequences common to other forms of retroviruses, HTLV-I and -II were shown to contain an additional genomic region between *env* and the 3′ LTR referred to as pX that encodes at least three peptides of 21, 27 and 40–42 kD. Sodroski [39] demonstrated that one of these, a 42-kD protein they termed the tat protein, is essential for viral replication. The tat protein acts on a 21-bp enhancer-like repeat within the LTR of HTLV-I, stimulating transcription [40, 41]. This tat protein also appears to play a central role in directly or indirectly increasing the transcription of host genes such as the IL-2 and especially the IL-2 Tac receptor genes involved in T-cell activation and HTLV-I-mediated T-cell leukemogenesis [42–44].

The IL-2-receptor as a target for therapy in patients with Tac-expressing leukemia, patients with autoimmune disorders and individuals receiving organ allografts

The observation that T cells in patients with ATL, select autoimmune diseases and individuals rejecting allografts express IL-2-receptors identified by the anti-Tac monoclonal antibody, whereas normal resting cells and their precursors do not, provides the scientific basis for therapeutic trials using agents to eliminate Tac-receptor-expressing leukemic cells or activated T cells involved in other disease states. Patients treated with anti-Tac should retain their Tac^- mature normal T cells and their precursors that express the full repertoire of antigen receptors for T-cell immune responses. The agents that we have used in human or animal models include (1) unmodified anti-Tac monoclonal; (2) toxin conjugates of anti-Tac (e.g. A chain of ricin toxin, *Pseudomonas* exotoxin [PE] and truncated PE [PE40]); (3) IL-2 truncated toxin fusion proteins (e.g. IL-2 PE40); (4) α- and β-emitting isotopic (e.g. ^{212}Bi and ^{90}Y) chelates of anti-Tac; and (5) hybrid 'humanized' anti-Tac with mouse light and heavy chain variable or hypervariable regions joined to the human constant κ light chain and IgG_1 or IgG_3 heavy chain regions.

We have initiated a clinical trial to evaluate the efficacy of intravenously administered anti-Tac monoclonal antibody in the treatment of patients with ATL [45, 46]. None of the nine patients treated suffered any untoward reactions, and only one, a patient with anti-Tac-induced clinical remission, produced antibodies to the mouse immunoglobulin or to the idiotype of the anti-Tac monoclonal. Three of the patients had a temporary mixed, partial or complete remission following anti-Tac therapy.

These therapeutic studies have been extended by examining the efficacy of toxins coupled to anti-Tac selectively to inhibit protein synthesis and viability of Tac^+ ATL lines. The addition of anti-Tac antibody coupled to PE inhibited protein

systhesis by Tac-expressing HUT-102-B2 cells, but not that by the acute T-cell line MOLT 4, which does not express the Tac antigen [47].

The initial PE-anti-Tac conjugate was hepatotoxic when administered to patients with ATL. Funcational analysis of deletion mutants of the PE structural gene has shown that domain I of the 68-kD PE molecule is responsible for cell recognition; domain II for translocation of the toxin across membranes; and domain III for ADP-ribosylation of elongation factor 2, the step actually responsible for cell death [48]. A PE molecule from which domain I has been deleted (PE40) has full ADP-ribosylating activity but extremely low self-killing activity when used alone because of the loss of the cell-recognition domain. The PE40 was produced in *Escherichia coli*, purified and conjugated to anti-Tac. The anti-Tac PE conjugates inhibited the protein synthesis of Tac-expressing T-cell lines but not of Tac-nonexpressing lines.

IL-2-PE40, a chimeric protein composed of human IL-2 genetically fused to the amino-terminal of the modified form of PE40 was constructed to provide an alternative (lymphokine-mediated) method of delivering PE40 to the surface of IL-2-receptor Tac^+ cells [48, 49]. The IL-2-PE40, a cytotoxic protein, was produced by fusing a cDNA encoding human IL-2 gene to the 5′ end of a modified PE40 gene that lacks sequences encoding the cell-recognition domain [48, 49]. The addition of IL-2-PE40 led to the inhibition of protein synthesis by the toxin moiety of IL-2-PE40 when added to human cell lines expressing either the p55, p75 or both IL-2-receptor subunits. The receptor internalization was much more efficient when high-affinity receptors composed of both units were present. IL-2-PE40 is a powerful reagent for studying IL-2-receptor interactions and for analyzing pathways of human immune response and its regulation. This chimeric protein toxin is being evaluated as an agent for IL-2-receptor-directed therapy in rodents with different forms of autoimmune disease, as well as in primates receiving allografts.

The action of toxin conjugates of monoclonal antibodies depends on their ability to be internalized by the cell and released into the cytoplasm. Anti-Tac bound to IL-2-receptors on leukemic cells is internalized slowly into coated pits and then endosomic vesicles. Furthermore, the toxin conjugates do not pass easily from endosonic vesicles to the cytosol, as required for their action on elongation factor 2. To circumvent these limitations, alternative cytotoxic reagents were developed that could be conjugated to anti-Tac and that were effective when bound to the surface of Tac-expressing cells. In one case, it was shown that ^{212}Bi, an α-emitting radionuclide conjugated to anti-Tac by use of a bifunctional chelate, was well suited for this role [50]. Activity levels of 0.5 μCi or the equivalent of 12 rad/ml of a radiation targeted by ^{212}Bi-labeled anti-Tac eliminated greater than 98% of the proliferative capacity of the HUT-102 cells, with only a modest effect on IL-2-receptor-negative lines. This specific cytotoxicity was blocked by excess unlabeled anti-Tac but not by human IgG.

In parallel studies, the β-emitting ^{90}Y was chelated to anti-Tac using the chelate 1(2)-methyl-4-(P-isothiocyanatobenzyl) diethylenetriamine-pentaacetic acid. The addition of the chelate did not alter the capacity of the monoclonal antibody to bind to its antigenic target nor did it alter its rate of metabolism or pattern of distribution. There was no elution of the radiolabeled yttrium from the monoclonal antibody chelate. As noted below, Rhesus monkeys receiving an allograft of a cynomolgus monkey heart showed a marked prolongation of xenograft survival following the

administration of ^{90}Y-labeled anti-Tac. Thus, ^{212}Bi-labeled anti-Tac and ^{90}Y-labeled anti-Tac are potentially effective and specific immunocytotoxic agents for the elimination of Tac-expressing cells.

In addition to its use in the therapy of patients with ATL, antibodies to the IL-2-receptors are being evaluated as potential therapeutic agents to eliminate IL-2-receptor-expressing T cells in other clinical states, including certain autoimmune disorders and in protocols involving organ allografts. The rationale for the use of anti-Tac in patients with the disease aplastic anemia is derived from the work of Zoumbos *et al.* [51], who have demonstrated that select patients with aplastic anemia have increased numbers of circulating Tac^+ cells. In this group of patients, the Tac^+ but not Tac^- T cells were shown to inhibit hematopoiesis when cocultured with normal bone marrow cells. Furthermore, we have demonstrated that anti-Tac inhibits the generation of activated suppressor cells [52]. Studies have been initiated to define the value of anti-Tac in the therapy of patients with aplastic anemia.

IL-2-receptor-directed therapy has also been used in rodent models of autoimmunity. Anti-murine IL-2-receptor antibodies were shown to suppress murine diabetic insulitis, lupus nephritis, EAE and adjuvant arthritis [53, 54].

Monoclonal antibodies that recognized the IL-2-receptor have been used to inhibit organ allograft rejection. Anti-Tac was shown to inhibit the proliferation of T cells to foreign histocompatibility antigens expressed on the donor organ and to prevent the generation of cytotoxic T cells in allogeneic cell cocultures [55, 56]. Furthermore, in studies by Kirkman [57], the survival of renal and cardiac allografts was prolonged in rodent recipients treated with an anti-IL-2-receptor monoclonal antibody. In parallel studies, the administration of anti-Tac for the initial 10 days after transplantation prolonged the survival of renal allografts in cynomolgus monkeys [58]. Unmodified anti-Tac did not lead to a prolongation of graft survival in heterotopic cardiac xenografts in which Rhesus monkeys received cardiac xenografts from cynomolgus donors. In contrast, animals receiving ^{90}Y-labeled anti-Tac showed a prolongation of graft survival from a value in untreated animals of 6–8 days to a mean graft survival of 33 days in animals receiving a dose of radioactivity that had acceptable toxicity. In the light of these encouraging results, human recipients of cadaver donor renal allografts are receiving different anti-IL-2-receptor monoclonal antibodies as adjunctive immunotherapy [59–61]. Antibody treatment has been well tolerated, and 50 of 53 recipients treated retain a functioning allograft. Thus, the development of monoclonal antibodies and toxin-lymphokine conjugates, directed toward the IL-2-receptors expressed on leukemia and lymphoma cells, on autoreactive T cells of certain patients with autoimmune disorders and on host T cells responding to foreign histocompatibility antigens of organ allografts may lead to the development of rational, novel therapeutic approaches for these clinical conditions.

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Anti-IL-2-R Monoclonal Antibody in Allograft Recipients

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A rat IgG2a monoclonal antibody (33B3.1) directed at the human Tac antigen and interacting with the high affinity binding site of IL-2 on its receptor was tested for treatment of kidney allograft recipients. This antibody is efficient in preventing graft rejection in the weeks following transplantation. Its activity compares to polyclonal rabbit anti-thymocyte globulin but it has fewer side effects. The dose of 33B3.1 given is critical for its preventive effect. A majority of the recipients developed IgM and/or IgG against rat Ig. Rejection episodes occurring during 33B3.1 therapy were associated with an early rise in host anti-33B3.1 antibodies and a drop in their monoclonal antibody (MoAb) circulating levels. When 33B3.1 was given to treat ongoing rejection, it had only an inconsistent and/or delayed effect. Various factors are discussed, such as circulating free 33B3.1 levels, host anti-33B3.1 immune response and the presence of inhibiting concentrations of soluble Tac chain, which can interplay with the protective effect of the anti-interleukin-2-receptor monoclonal antibody.

The use of bioreagents which interfere only with determinants present on host activated lymphocytes may represent a new step toward selective immunosuppression in allograft recipients.

Interaction of interleukin-2 (IL-2) with its high affinity receptor (IL-2-R) is required to sustain the expansion of alloimmune T lymphocyte clones *in vitro*. When this interaction is inhibited *in vivo* by antibodies which interfere with the IL-2-binding site on high affinity IL-2-receptors, a prolongation of allograft survival is observed [1–3]. Anti-IL-2-R antibodies which do not inhibit this interaction are inefficient [4, 5]. In a pilot study, we tested such a blocking antibody (33B3.1) [6], a

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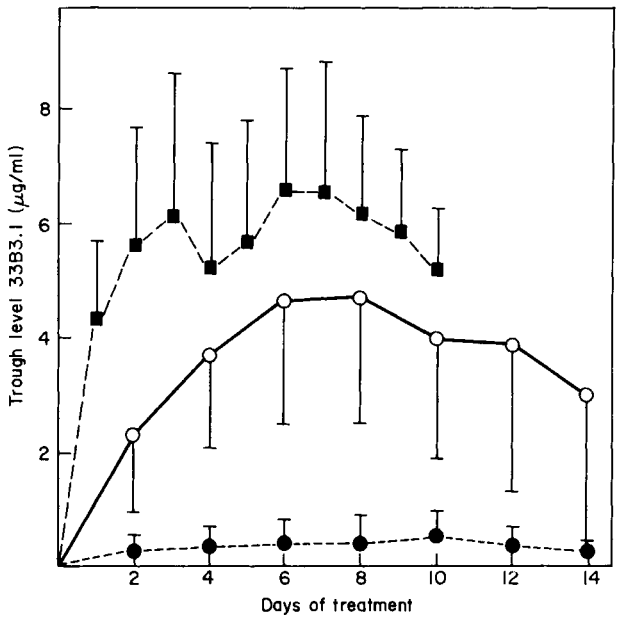

Figure 1. Shows trough level of 33B3.1 according to the different protocols used:in prophylactic treatment (● – – – ●) 5 mg 33B3.1/d; (○——○) 10 mg/d; in treatment of ongoing rejection (■ – – ■) 20 mg/d for the first 2 d and 10 mg/d for the last 8 d.

rat IgG2a directed at the 55 Kd alpha chain of human IL-2-receptor. Various doses were used in recipients of a first cadaveric kidney allograft in a prophylactic and curative protocol against graft rejection episodes. An update of our experience in these two clinical conditions is presented.

Prophylactic use of 33B3.1 in kidney allograft

Importance of the dose

In all presented results concerning the prophylactic use of anti-IL-2-R, the MoAb was given in a peripheral vein (bolus) for 14 d starting on the day of surgery. In addition, the following were given: (1) prednisone (P) at 1 mg/kg the first week reduced to 10 mg/week, and (2) azathioprine (A) at 2 mg/kg (adapted to the blood white cells count) for the first month, progressively decreased and finally stopped. Cyclosporine A (Cy-A) was administered at 8 mg/kg/d and then adapted according to blood trough levels at day 14 (i.e. immediately after the end of 33B3.1 treatment). The patients were thus receiving Cy-A monotherapy around days 40–45.

Nine patients were initially treated with 5 mg of 33B3.1 per day. Three of them experienced a mild rejection episode (all easily reversed by anti-thymocyte globulins (ATG) used as rescue treatment [3]). The second group included recipients who received 10 mg/d of 33B3.1 and were either treated in a pilot study [3, 7] or in a still ongoing randomized trial of 33B3.1 against rabbit ATG. Four rejections occurred during 33B3.1 treatment in these first 58 patients. Figure 1 shows that trough blood levels of the MoAb reached a plateau averaging 1.5 µg/ml at day 6. If the blood level

Table 1. *Incidence of rejection episodes in 80 recipients receiving either 33B3.1 or ATG*

Rejection	33B3.1 ($n=40$)	ATG ($n=40$)
Days 0–14	10% ($n=4$)	0%
Days 15–30	2.5% ($n=1$)	5.5% ($n=2$)
Days 31–60	6% ($n=2$)	6.6% ($n=2$)
Days 61–90	10.7% ($n=3$)	18% ($n=5$)
Total (0–90)	29.2 ($n=10$)	30.1% ($n=9$)

achieved with 10 mg/d is compared (see below), the large difference suggests that not enough drug was given, since the rejection episodes observed could not be explained by an early rise in host anti-33B3.1 antibodies [3].

Prevention of early rejection in patients receiving 10 mg/d of 33B3.1

Table 1 gives the rejection frequency found in the first 40 randomized patients compared to their 40 ATG-treated counterparts. The two randomized groups included first cadaveric graft recipients and did not differ in mean age, anti-lymphocyte immunization, HLA matching and sex. Rejection episode frequency was 10% (all fully reversible) during anti-IL-2-R therapy. There was no rebound effect within the 2 immediate weeks following 33B3.1 treatment, although obviously there was also no deletion of the clones committed against the donor as indicated by the occurrence of late rejection episodes (Table 1). At 3 months, frequency of rejection was similar in 33B3.1- and ATG-treated recipient groups. Interestingly, infections (viral, but also bacterial such as urinary tract infections) were less frequent in the 33B3.1 group. Although samples are still too low to allow definitive conclusions, anti-IL-2-R therapy was well tolerated with almost no clinical side effects [3]. Two patients died in the ATG group (massive gastric bleeding, day 6, and CMV plus legionaires' disease day 120) and one in the anti-33B3.1 group (cryptococcosis meningitis, day 120). Graft survival was 95% and 85% in anti-IL-2-R and ATG group respectively with a mean follow up of 6 months in both groups.

Virtually no circulating lymphocytes bearing IL-2-R or bound 33B3.1 molecules could be detected during anti-IL-2-R treatment [8], indicating a lack of expansion of the activated lymphocyte population, unlike that found at the peripheral level in unmodified monkey kidney recipients [2]. Monitoring of circulating 33B3.1 trough levels (Figure 1) indicated that a dose of 10 mg/d led to high blood levels averaging 4.5 μg/ml ($\sim$30 nM) at day 6. These levels were far above what a simple arithmetic projection would have suggested. These concentrations, around 40 times the Kd of the 33B3.1 [6], suggests that a dose of 10 mg/d is required to saturate the binding of the monoclonal antibody on soluble-IL-2-R (S-IL-2-R) [9], on macrophage Fc receptors, and to allow a large excess of the free MoAb. Thus the results so far obtained in the prevention of rejection in the ongoing controlled study seem to confirm and extend those previously suggested by the pilot study [3].

Table 2. *Anti-33B3.1 immunization*

	IgM	IgG	None
Prophylactic treatment			
5 mg/d	66.6%	77.7%	33.3%
($n=9$)	(23 ± 5)[1]	(24 ± 8)	
10 mg/d	87.2%	79.6%	10.2%
($n=48$)	(17.3 ± 7)	(18.9 ± 7	
Rejection treatment			
20 mg (first 2 d)	100%	66.6%	0
($n=6$)			

[1]Time of peak anti-33B3.1 (day $\pm$ SD).

Almost all treated recipients developed IgG and IgM [10] antibodies against the drugs (Table 2). However, only a few patients exhibited a sufficiently early response to interfere with the treatment during the first 2 weeks following transplantation. Interestingly 3/4 patients who experienced a rejection episode during 33B3.1 treatment belonged to the group of patients who presented both an early rise in anti-33B3.1 and a concomitant drop in 33B3.1 trough circulating levels. Although it has not yet been possible to detect unambiguously anti-idiotypes, these results suggest that other mechanisms of neutralization of anti-IL-2-R MoAb by the host antibody response might operate by sharply decreasing the drug circulating levels, which would be likely to occur because of formation of insoluble immune complexes rapidly cleared from the circulation.

Treatment of ongoing acute rejection episodes with 33B3.1 MoAb

The effect of 33B3.1 was also studied in 10 recipients of a primary kidney graft experiencing a common acute cellular rejection episode [11]. In this protocol the MoAb was given at 20 mg/d (instead of 10 mg in prophylaxis) for the first 2 d and 10 mg/d for 8 additional d. The dose of the first 2 d was modified in order to reach a circulating trough level as early as day 2 instead of day 6 as obtained in the prophylactic protocol [3]. Cy-A and any other drugs were not modified during the 10-d course of MoAb treatment. Prednisone was given at 1 mg/kg at the end of the 33B3.1 course and then was slowly reduced each week. In contrast to its effect in the prophylaxis of rejection, 33B3.1 did not appear to be a valuable agent in reversing declared kidney rejection episodes. Although in six cases the rejection crises were cured solely by MoAb therapy, in four of these six cases, creatinine levels remained at a plateau during all 10 d of 33B3.1 treatment, with a slow (but nevertheless complete) decrease when the steroid was increased to 1 mg/kg according to the protocol indicated earlier. In two cases (out of the six) the creatinine decreased immediately after 33B3.1 treatment. In three other cases creatinine continued to rise during the first 5 d of MoAb treatment and a rescue treatment (methylprednisone boluses) had to be given. A last patient did not respond to either anti-IL-2-R or to various rescue therapies including corticoid boluses or ATG. In none of the cases could anti-33B3.1 host immune

response or circulating MoAb trough levels (which were >6 μg/l at day 2) account for the resistance of these episodes to the MoAb therapy. Thus, even though it was a pilot study, we cannot suggest that this MoAb would have any advantage over our current practice of traditional steroid bolus treatment given in Cy-A-treated patients experiencing an acute rejection; therefore, we decided not to pursue further trials.

Conclusion

Monoclonals directed to lymphocyte surface determinant, such as strong immunogens, are not likely to be a definitive approach in immunosuppression. These agents are nevertheless of major value in targeting the proper molecules to be interfered with in blocking the allogeneic response of graft recipients. As suggested by theory and by animal experiments, we have shown that the high affinity binding site for IL-2 on its specific membrane receptor is one of these major targets. Anti-IL-2-R MoAb must evidently interfere with this high affinity site of IL-2-R in order to work [4, 5], in accordance with the fact that anti-IL-2-R MoAb against Tac chain does not modulate IL-2-R at the cell surface membrane and cannot efficiently activate human complement. It is thus likely that this MoAb works primarily by inhibiting, through competitive interaction, the growth signals required by clonal expansion of recipient T-lymphocytes committed against donor HLA antigens. This model fits with the efficiency of this agent in preventing early rejection episodes in clinical studies and with the lack of circulating cells bearing IL-2-R in treated recipients. It is likely that T-lymphocyte clonal expansion in these patients cannot develop and that therefore rejection cannot occur unless there is an early rise in recipient anti-33B3.1 neutralizing antibodies. This host immune response is, however, not the only factor capable of inhibiting anti-IL-2-R. S-IL-2-R is commonly found [sometimes high levels >50 nM; unpublished] in recipient sera and may further increase during rejection episodes [12]. Its ubiquitous presence in recipient blood samples continues to be a major difficulty for assaying anti-idiotypes and may require the use of cumbersome biochemical purification of host IgG deprived of S-IL-2-R to assess unambiguously the anti-idiotypic component of the recipient anti-33B3.1 immune response. S-IL-2-R also makes it difficult to differentiate free circulating 33B3.1 from its complexed form with S-IL-2-R, which is inactivated. This S-IL-2-R should behave exactly like circulating anti-idiotype antibodies, and may account for the high 33B3.1 dose required in the prophylactic protocol. It should strongly 'cooperate' with the recipient response to neutralize anti-IL-2-R MoAb effects and allow rejection to occur when both inhibiting agents are present at sufficient levels. It is thus important in the future to measure the level of the 'free' fraction of circulating MoAb to have a relevant monitoring indication of MoAb efficacy.

Although 33B3.1 was efficient in preventing graft rejections, with fewer infectious episodes and virtually no side effects, making it very valuable in prophylactic use, this antibody had only a delayed and inconsistent effect on ongoing acute rejection episodes. Several hypotheses may account for this relative inefficacy: (1) it is likely that anti-donor clonal expansion had already developed in the graft and that IL-2 might not, at this stage, be absolutely required for effector functions; (2) in addition, host-activated lymphocyte trafficking is probably not as high as in the first days after

transplantation, and graft-invading effector cells may be less exposed to the MoAb interaction. This is suggested by the finding that in the rat only a low percentage ($<2\%$) of ^{132}I labelled anti-IL-2-R enters a rejecting allograft [13]; (3) S-IL-2-R might be produced in higher quantities during rejection [12] and interact with the MoAb; (4) other lymphokines may drive alternative activation pathways of graft invading cells; and (5) cytotoxic T cells might not be the major target to interact with in acute rejection since they can heavily infiltrate a non-rejected kidney [14]. In conclusion, it may be said that antibodies interacting with the high affinity binding site of IL-2 on its membrane receptor appear to be a target of choice in preventing rejection. However, these agents have much less efficiency when used in ongoing rejection. Their mechanisms of action and the choice of relevant monitoring parameters are however still uncertain.

The circulating levels of excess free anti-IL-2-R (i.e. unbound to soluble IL-2-R) and the presence of host anti-idiotypic antibodies are likely to be critical. It is thus important to set up simple methods allowing routine measurement of these factors. These methods, which have yet to be designed for anti-IL-2-R because of the problem of soluble IL-2-R, should further increase the clinical efficiency of this monoclonal.

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Therapy of Autoimmune Diseases with Monoclonal Antibodies to Class II Major Histocompatibility Complex Antigens: The Role of T Lymphocytes

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Introduction

The major histocompatibility complex (MHC) is a critical region in the control of various immunologic functions. Class II MHC antigens play a central role in lymphoid cell interactions and are encoded by I-region genes referred to as immune response gene in the mouse. Class II antigens have been shown to mediate antigen presentation *in vitro* and *in vivo* in various experimental models. Genetic studies have demonstrated a polygenic basis for susceptibility to autoimmunity in most experimental models [1]. At least one major component for susceptibility usually maps within the MHC, as exemplified by the clear association of many autoimmune diseases with Class II MHC alleles in human as well as genetic studies in animals [1].

Immunosuppressive effects of anti-Class II antibodies

Antisera and monoclonal antibodies (MoAbs) directed against Class II MHC antigens have been shown to block antigen presentation to T lymphocytes as well as primary or secondary antibody responses *in vitro* [2–5]. *In vivo* treatment with anti-Class II monoclonal antibodies has been shown to interfere with helper T-cell function, with differentiation of I-restricted T cells in the neonatal period [6, 7] and with antibody production to multichain synthetic polypeptide antigens in the mouse [8]. Suppression of humoral immune response in this last model was haplotype-specific since the only response inhibited by anti-I-A^k MoAbs was that under control of I-A^k in F1 mice with the genotype H-$2^{k/b}$. Such haplotype specificity was observed in cases of immunization with antigen in aqueous solution but not in immunization in complete Freund's adjuvant [8]. *In vivo* treatment with anti-Class II antisera and

MoAbs have further been shown to inhibit delayed-type hypersensitivity (DTH) reactions against tumoral antigens in the mouse [9]. Suppression of DTH reactions in anti-Class II treated mice in this model was dependent on T cells, since spleen T cells from treated mice were capable of transferring protection into syngeneic recipients [10]. The T cells responsible for transferring protection were sensitive to low dose cyclophosphamide. Protection against allograft rejection has been obtained in the mouse using anti-Class II antibody treatment as well [11, 12].

Prevention of experimentally induced autoimmune diseases by anti-Class II antibodies

Both the role of Class II MHC genes in the genetic control of autoimmune diseases and the blockade of immune reactions to foreign antigens *in vivo* by anti-Class II antibodies provide a rationale for treating autoimmune diseases with monoclonal antibodies directed against Class II antigens. Successful prevention of autoimmune phenomena was obtained in experimental allergic encephalomyelitis (EAE) [13], experimental myasthenia gravis [14], Type II collagen arthritis [16] in which well defined autoimmune diseases are induced by immunization with autoantigenic preparations in complete Freund's adjuvant. Prevention in these models was obtained by *in vivo* injections of monoclonal antibodies to I-A gene products [13–16]. Treatment of ongoing disease has even been obtained in SJL/J mice (H-2^s) induced to develop EAE by treatment with anti-I-A^s [13]. Decreased accumulation of radiolabelled lymph node cells in the central nervous system was demonstrated in mice so treated [17] and it was shown that treatment of (SJL/J × BALB/c) F1 mice combining the SJL/J (H-2^s) high responder strain to the BALB/c (H-2^d) low responder strain was haplotype-specific, since suppression of EAE was only seen in F1 mice immunized with antigen in adjuvant and injected with anti-I-A^s MoAb and was only partially prevented by injecting anti-I-A^d MoAb. Antibody to the low responder allele was ineffective in preventing passive transfer of myelin-basic protein-sensitized lymphocytes [18].

Prevention of spontaneous autoimmune diseases by anti Class II antibodies

Besides experimentally induced autoimmune disease, evidence has been obtained indicating anti-Class II MoAbs were able to prevent the development of spontaneous autoimmune diseases *in vivo*. It was first shown that long-term treatment with anti-I-A^s prolongs survival and clinical nephritis in (NZB × NZW)F1 mice [19]. The same therapeutic approach was applied to the BB rat model, in which prevention of insulin-dependent diabetes mellitus (IDDM) was achieved by *in vivo* treatment with a monoclonal antibody to I-E equivalent (D-encoded) antigen. No prevention was obtained in BB rats treated with MoAb to I-A equivalent (B-encoded) antigen, indicating that the effect of anti Class II MoAb in this model was locus-specific. The BB rat disease is characterized by association of diabetes with other autoimmune phenomena, in particular thyroiditis. It is noteworthy that prevention of thyroiditis was obtained on injection with the same anti-I-E MoAb as that preventing the occurence of IDDM, while anti-I-A MoAb injections had no preventive effect on thyroiditis. Although genetic susceptibility to thyroiditis has not been studied in the

BB rat model, these data indicate that either similar genetic background or similar immunological effector mechanisms are likely to be involved in both thyroid and islet cell disease in the BB rat [20].

The effect of anti-Class II antibodies in NOD mice

A new model for autoimmune IDDM has recently been developed in the non-obese diabetic (NOD) mouse. Spontaneous disease is predominantly observed in female mice from the 10–12th week of age [21]. Earlier disease can be induced in females and males on injection of 150 mg/kg of cyclophosphamide, twice at 2-week intervals [22]. The role of autoimmunity in this model is clearly demonstrated by prevention of diabetes upon neonatal thymectomy [23], treatment with anti-L3T4 MoAb [24–26], or treatment with cyclosporin A [27]. The disease is characterized by an infiltration of the islets by mononuclear cells, predominantly $CD4^+$ T cell [28]. Adoptive transfer of overt diabetes has been obtained by injecting splenic T cells, including $L3T4^+$ and $Lyt2^+$ cells, from diabetic NOD mice, into either NOD neonates [29] or adult 8-week-old male recipients [30, 31]. Transfer with bone marrow cells has recently been obtained as well [32]. Susceptibility to IDDM in this model has a polygenic basis, one genetic component mapping to the MHC [33, 34]. The NOD mouse exclusively expresses I-A antigens which show a unique pattern characterized by five consecutive nucleotide changes on I-A β leading to two amino acid substitutions in position 56 and 57. The serine at position 57 differs from the aspartic acid present in all non-diabetic strains studied so far, including the non-obese normal (NON) strain [1, 35, 36]. The NOD strain expresses no I-E antigen. Expression of I-E antigens in (C57BL/6 × NOD)F1 in which the parent C57BL/6 transgenic mouse was prepared by microinjection of $E\alpha^d$ gene sequence has been shown to prevent the development of insulitis [37].

We recently obtained evidence that spontaneous IDDM was prevented in the NOD mouse by injecting 1.2 mg/week of purified MoAb specific for $I\text{-}A^{NOD}$, starting treatment at either 3 weeks of age or at birth, providing that continous treatment was maintained up to the end of experiments [38]. This observation provides a new illustration of the prevention of a spontaneously occurring autoimmune disease by anti-Class II MoAb treatment. In order to study further the mechanisms of protection, we attempted to determine whether treatment with anti-Class II MoAb could be used to protect against diabetes transfer into syngeneic recipients. Evidence was obtained indicating that treatment of neonatal recipients with anti-Class II MoAb did protect against the transfer of diabetes by spleen cells from diabetic mice. By contrast, treatment of MoAb starting at birth did not achieve protection against diabetes transfer into 8-week-old male recipients, despite maintenance of anti-Class II MoAb treatment after the cell transfer [38]. It was thus hypothesized that anti-Class II MoAb treatment could only provide protection if administered to immunocompetent hosts (e.g. non-irradiated recipients) suggesting that irradiation abrogated protection induced by anti-Class II MoAb injections [38].

The role of T cells

Next we evaluated radiation-sensitive mechanisms mediating anti-Class II MoAb-induced protection. These mechanisms were investigated using the adult model of

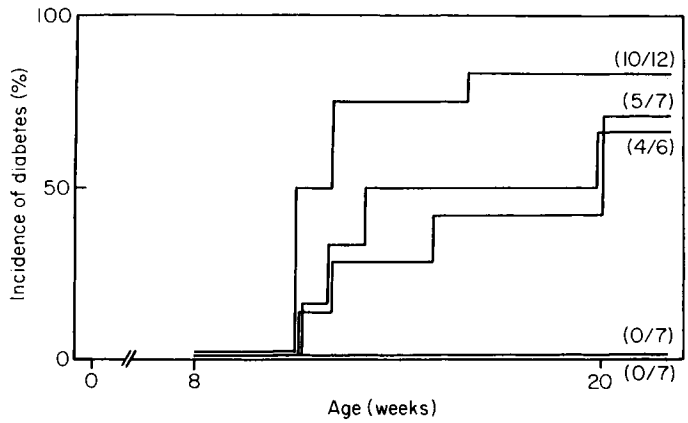

Figure 1. Transfer of diabetes into 8-week-old male recipients. Eight-week-old male NOD recipients were irradiated (750 rads) on day 1 and injected with spleen cells from diabetic NOD animals either totally (10/12), or treated with complement alone (5/7), or with 3.155 (anti-Lyt2$^+$) MoAb plus complement (0/7), or with 172.4 (anti-L3T4$^+$) MoAb plus complement, or reconstituted by addition of anti-L3T4$^+$ depleted and anti-Lyt2$^+$ depleted subsets (4/6) as previously reported [29]. Numbers in parenthesis indicate the total number of diabetic animals over the total number of recipient. All recipients mice received a total of 20×10^6 treated or reconstituted spleen cells.

diabetes transfer previously mentioned [30, 31]. In order to set up this model, we verified that the transfer of diabetes by spleen cells collected from diabetic NOD female mice into 8-week-old pre-irradiated (750 rad) male recipients required both L3T4$^+$ and Lyt2$^+$ cells in the experimental conditions that we used (Figure 1) as previously reported [31].

In order to test if irradiation indeed abrogated the protection induced by anti-Class II MoAb injections, we set up experiments in which irradiated 8-week-old male recipients were reconstituted with spleen cells from anti-Class II MoAb-treated non-diabetic NOD donors prior to transfer of spleen cells from diabetic NOD mice. When male recipients are treated from birth with anti-I-A^{NOD} MoAb prior to 750 rad irradiation (Table 1) and are subsequently transferred with total spleen cells from diabetic NOD mice (Group A), the number of diabetic recipients does not differ significantly as compared with that of male recipients treated with a control MoAb (Group B). By contrast, restoration of irradiated male recipients with spleen cells from 8-week-old NOD mice treated from birth with anti-I-A^{NOD} MoAb 24 h prior to transfer of spleen cells from the diabetic mice afforded effective protection (Group C). Irradiated control male recipients (Group D) restored with spleen cells from 8-week-old NOD mice treated from birth with control MoAb did develop diabetes upon transfer of spleen cells from diabetic NOD mice. Subsequent experiments showed that spleen cells from NOD mice treated with anti-NOD Class II MoAb at age 21–56 d prior to injection into 8-week-old irradiated male recipients, were as effective as spleen cells from NOD mice treated from birth in restoring anti-Class II-induced protection. However, spleen cells from mice only treated for 7 d prior to transfer were ineffective (Table 2).

As suggested in Figure 2, T cells appear to be responsible for restoring anti-Class II MoAb-induced protection against diabetes transfer in NOD male syngeneic

Table 1. *Restoration of protection by spleen cells from anti-Class II MoAb treated NOD mice in 8-week-old irradiated NOD recipients*

Group	MoAb treatment[1]	Restoration by spleen cells[2]	Number of diabetic animals over total number of recipients[3]		
			4 weeks[4]	8 weeks[4]	12 weeks[4]
A	10-3-6	−	5/9	8/9	8/9
B	MKD6	−	9/12	11/12	11/12
C	10-3-6	+	1/9	1/9	1/9
D	MKD6	+	0/8	2/8	5/8

[1]All donors of spleen cells from anti-I-A^{NOD} Class II (10-3-6) or control (MKD6) MoAbs were treated from birth up to 8 weeks of age with 1.2 mg i.p. purified MoAb.

[2]50×10^6 spleen cells collected from anti-NOD Class II or control MoAbs were injected i.p. into irradiated NOD male recipients 24 h after irradiation (750 rads).

[3]All recipients were injected i.p. with 20×10^6 total spleen cells from diabetic NOD mice 48 h after irradiation.

[4]Post-diabetic cell transfer.

Table 2. *Duration of anti-Class II MoAb treatment prior to restoration of protection in 8-week-old irradiated NOD recipients*

MoAb treatment[1]	Age period of treatment[2]	Number of diabetic animals over total number of recipients[3]		
		4 weeks[4]	8 weeks[4]	12 weeks[4]
10-3-6	1 to 5 days	0/9	1/9	1/9
10-3-6	22 to 56 days	0/12	0/12	2/12
10-3-6	50 to 56 days	3/9	7/9	7/9
MKD6	1 to 56 days	5/11	9/11	10/11

[1]All donors of spleen cells from anti-NOD (10-3-6) or control (MKD6) MoAbs were treated with 1.2 mg/week i.p. of purified antibody.

[2]50×10^6 spleen cells collected at 60 days of age from MoAb-treated donors prepared as indicated in ([1]) were injected i.p. into irradiated NOD male recipients 24 h after irradiation (750 rads).

[3]All recipients were injected with 20×10^6 spleen cells collected from diabetic NOD mice 48 h after irradiation.

[4]Post diabetic cell transfer.

recipients, since treatment of protective spleen cells by anti-thy1 MoAb plus complement abrogates the protection.

Present evidence for induction of suppressor T-cell treatment with anti-Class II MoAb must be considered in the light of previous observations based on *in vitro* assays in models of active immunization against viral or allogeneic antigens. One should also recall that inhibition of DTH reactions against tumoral antigens by anti-Class II antibody treatment has been transferred *in vivo* by spleen cells obtained from anti-Class II antibody-treated mice. The transfer of protection in this last model is

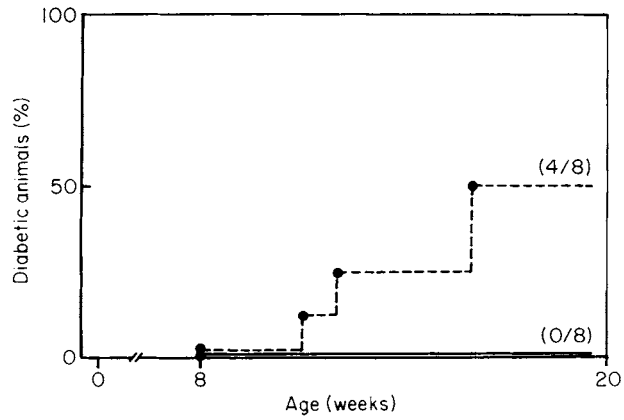

Figure 2. Restoration of anti-Class II MoAb-induced protection against diabetes transfer by spleen T cells from 8-week-old NOD mice treated with anti-IA^{NOD}. Eight-week-old male NOD recipients were irradiated (750 rads), restored 24 h later with 50×10^6 spleen cells either treated with complement alone (continuous line) or with 4.221 (anti-Thy $1,2^+$) MoAb plus complement (dotted line), as previously reported [29] then transferred with 20×10^6 total spleen cells collected from diabetic NOD mice. Numbers in parenthesis indicate the number of diabetic animals over the total number of recipients in each group.

abrogated by T-cell depletion and sensitive to low dose cyclophosphamide [10]. In all previously mentioned models including our own NOD mouse model, the mechanism of induction of the suppression phenomenon remains elusive.

A direct effect of anti-Class II MoAb treatment on T cells is very unlikely. Induction of suppressor T cells in this model may be a secondary phenomenon to a primary action of anti-Class II MoAb on antigen-presenting cells e.g. functional impairment or blockade of antigen presentation. There is some evidence *in vitro* that a direct effect on antigen-presenting cells may indeed be the first step to subsequent induction of suppressor T cells, as in T cells primed to sperm whale myoglobin [39–41].

Interestingly, major modifications of circulating lymphocytes induced by anti-Class II MoAb *in vivo* include a decrease in B cells and to a lesser extent of $L3T4^+$ cells. Comodulation of I-A and I-E antigens on B-cell membranes has been reported on anti-I-A or anti-I-E MoAb treatment *in vitro*. The significance of these B-cell modifications in the biological effect of anti-Class II antibodies *in vivo* and the possible consequences on helper T cells remain unknown [42–45].

A direct action of anti-Class II antibodies on target cells of immune reactions must also be considered, but the significance of aberrant Class II antigen expression on target cells is still a matter of debate, as is its role in eliciting autoimmunity [46].

Other previously mentioned models have shown that anti-Class II MoAb-induced suppression is I-locus specific and haplotype-specific. The question must however be raised in our model regarding the antigenic specificity of the suppression observed. One should recall that the specificity of the protection induced by anti-Ia antibodies in regard to the antigen used for immunizing spleen cell donors has been strongly suggested in the case of immunization against tumoral antigens and delayed-type hypersensitivity to alloantigens.

Finally, the dramatic effect of anti-Class II MoAb treatment in preventing various autoimmune diseases must be discussed in the light of the clear association observed

between most autoimmune diseases and Class II MHC alleles. The mechanisms by which Class II MHC antigens may interfere with immune tolerance to self antigens are unknown. Recent indications have shown a clear association between the development of IDDM and an amino acid substitution in position 57 on DQβ in human diabetic subjects. The same amino acid substitution is found on NOD I-Aβ although not found on the β chain encoded by the Class II D region in the BB rat. Anti-Class II MoAb may provide a powerful tool for interfering with epitopes involved in disease susceptibility. Such interference may lead to emergence of suppressor cells rather than activation of self-reactive helper T cells. Thus, in addition to potential therapeutic implications, treatment with anti-Class II MoAbs may cast light on the mechanism of HLA association with autoimmune disease in humans, and the role of suppressive T cells in the development of autoimmune phenomena [1].

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Idiotype Selection is an Immunoregulatory Mechanism which Contributes to the Pathogenesis of Systemic Lupus Erythematosus

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Several experiments are reviewed to support the following suggestions: (1) Idiotypes can be used to identify pathogenic autoantibodies in murine and human systemic lupus erythematosus; (2) Those idiotypes are targets for immune upregulation which causes them to expand and dominate immunoglobulin responses; (3) Those idiotypes require autoreactive T-cell help; and (4) Both T cells and B cells participating in this response are polyclonal.

These observations are used to construct an hypothesis to explain the sustained production of pathogenic autoantibody subsets.

Introduction

Idiotypes (Ids) serve as targets of some immunoregulatory circuits. They can be either upregulated or downregulated by anti-Ids, and by T-lymphocytes of both helper and suppressor functional classes [1, 2]. Public or cross-reactive idiotypes occur on multiple antibodies with different epitope specificities and are often targets of regulation. Private idiotypes are confined to a single B-cell clone with one epitopic specificity.

Many antibodies which participate in the pathogenesis of human and murine autoimmune diseases are characterized by public Ids. Examples include rheumatoid factors in paraproteinemias, antibodies to acetylcholine receptors in myasthenia gravis, antibodies to thyroglobulin in thyroiditis, and antibodies to DNA in systemic

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lupus erythematosus (SLE). Several investigators have suggested that such public Ids are upregulated selectively, resulting in sustained synthesis of pathogenic autoantibodies.

Several public Ids have been identified on antibodies to DNA in murine and human lupus [3–10]. Ids located near the epitope binding site of Ig, which therefore inhibit binding of DNA antigen by Id+ antibody, include the 16/6 and 35/15 described by Isenberg and colleagues, [8] and the IdX, IdGN1 and IdGN2 described by us. All of these public Ids are found in patients and mice with SLE. The 31 Id described by Solomon, Diamond and colleagues [7] is a framework Id and is found only in humans. Serum levels of 16/6 correlate with disease activity. All of these public Ids occur on Ig which is not DNA-binding, and all occur in healthy individuals as well as in patients with SLE and their family members. However, the Ids are increased in both frequency and quantities in patients compared to family members or healthy controls.

Based on the work done on these idiotypes in human and murine SLE, we can make the following statements: (1) Some public Ids are markers of pathogenic autoantibodies. (2) Antibody responses may be restricted to or dominated by those idiotypes. (3) Antibodies bearing those Ids are regulated by autoreactive helper T cells. (4) The helper T cells and the responding Id-producing B cells are polyclonal.

Public idiotypes as markers of pathogenic antibodies

Several experiments in the 16/6 Id system of Isenberg, Shoenfeld *et al.* [8] and in the IdGN system of Hahn, Ebling *et al.* [5] have shown that these Ids are present on immunoglobulin (Ig) subsets which are pathogenic. The 16/6 Id was found on Ig deposits in glomeruli and dermal/epidermal junctions of patients with SLE [9]. Furthermore, immunization of normal C3H.SW female mice with a human 16/6+ monoclonal antibody (MoAb) to DNA resulted in development of anti-Id, anti-anti-Id, circulating anti-DNA and other autoantibodies. In addition, these normal mice developed immune glomerulonephritis [11].

We have shown that two public Ids, IdGN1 and IdGN2, are found on a large proportion of the Ig eluted from glomeruli of NZB/NZW F_1 mice [12]. This idiotype restriction is similar to that in the new SNF_1 mouse model of SLE, in which glomerular Ig contains large quantities of IgG2b antibodies to DNA, with cationic subpopulations, all dominated by two public Ids [13]. Recent studies in our laboratory showed that IdGN2 is the dominant Id in the glomerular Ig deposits in patients with SLE [14, 15]. IdGN1 and IdGN2 were both significantly more frequent in glomeruli of SLE patients than in glomeruli of patients with non-lupus immune complex glomerulonephritis (GN). The IdGN2+ Ig eluted from glomeruli of SLE patients who died with lupus nephritis, when compared to the IdGN2-negative Ig, was enriched in the complement-fixing IgG1 isotype, and in high-avidity antibodies to DNA. These data are summarized in Table 1.

In several regulatory experiments, we have shown that suppression of IdGNs (and of the related IdX) results in delayed onset of GN in BW mice [15, 16]. In contrast, upregulation of IdGN2 accelerates the appearance of autoantibodies and clinical GN in young BW mice (data not shown).

Table 1. *Idiotypes define a restricted antibody response and pathogenic autoantibody subsets in murine and human SLE*

In the NZB/NZW F1 mouse model of SLE:					
Proportions of total serum Ig bearing each Id:		IdX	IdGN1	IdGN2	All
	6-week-old mice	4	6	9	14[1]
	35-week-old mice	43	33	33	85[1]
Proportions of total glomerular IgG bearing each Id:					
	35-week-old mice	<5	30	16	50
In patients with SLE:					
Quantities of Ids in serum: (expressed as mean units based on μg of binding to a fixed quantity of MoAb anti-Id)					
	SLE patients	4.3[2]		2.8[2]	
	Non-SLE GN	2.1		1.5	
	Healthy	1.8		0.9	
Proportions of patients with Id in glomerluli: (Measured by indirect immunofluorescence)					
	SLE patients	6	45[2]	76[2]	
	Non-SLE GN	60[2]	6	6	

[1]There is slight cross-reactivity between the Ids. The total is determined by sequential absorption of sera or glomerular eluate on anti-Id columns.

[2]Significantly different from other groups, $P < 0.05$ or better. See references 12 and 14 for details.

In summary, there is evidence in at least two idiotypic systems, both of which are found in murine and human SLE, that Ig bearing those Ids participates in the pathogenesis of the disease.

Public idiotypes as targets of immune restriction

Restriction of public Ids as the animals age is characteristic of both BW and SNF_1 mice [6, 12, 13]. Our laboratory has shown that 6-week-old BW females have IdX, IdGN1 and IdGN2 on approximately 14% of their total serum Ig. In contrast, by 35 weeks of age, 85% of the Ig bears one of those three Ids. This was an unexpected finding, since the spectrotypes of IgG antibodies to DNA are expanding broadly during that same time period [17, 18]. The public Ids in the glomerular Ig deposits of nephritic BW mice are even more restricted, with IdGN1 and IdGN2 accounting for 45–50% of the total Ig. This restriction suggests that the pathogenic idiotypes are targets for specific upregulation in SLE.

Autoreactive T cells regulate expression of pathogenic autoantibodies

In preparation for studies of idiotypic regulation, Dr Ando in our laboratory performed experiments to determine the T-cell dependence of the production of

Table 2. *The helper T cell response of NZB/NZW F_1 mice is dominated by autoreactive TH-2 cells which can support production of IgG antibodies to DNA*

T-cell clone/line	Lymphokines secreted	Auto or cognate	IgG anti-dsDNA produced by BW B cells (measured as AFC/10^5 cells in an Elispot assay)
None			1+1
30.8	IL-4, IL-5	Auto	56+7[1]
27.6	IL-4, IL-5	Auto	60+5[1]
30.7	IL-4, IL-5	Cognate	12+4
Line 27	IL-2		3+2
LPS			132+13[1]

[1] Significantly increased compared to unstimulated cultures.
See references 19, 20 and 22.

IgG2a anti-dsDNA in BW mice. Those antibodies contain pathogenic subsets which deposit in glomeruli and cause the GN characteristic of these mice. That response requires help from $L3T4^+$ T cells [19].

Helper T cells were cloned from the spleens of nephritic BW mice. The clonal populations were dominated by $L3T4^+$ T cells which were *bystander* rather than cognate [19]. That is, most of the T-cell clones could help antibody responses to KLH as well as to DNA, and could drive B cells with non-identical MHC markers to make anti-DNA. These T cells had to be activated by antigen-presenting cells bearing self I-A. Therefore, the cells could be activated by self MHC Class II antigens, and they released differentiation and growth factors for B cells that were not restricted by antigen or MHC genes. In normal mice, such non-specific, autoreactive T helper cells are probably part of the preimmune repertoire, and they can provide help during immune responses.

Our early results suggested that the majority of the T-cell clones were of the TH-2 type, as defined by Mosmann *et al.* [21]. TH-2 murine T-cell clones secrete IL-4, whereas TH-1 cells secrete IL-2 and IFNgamma. As shown in Table 2, the autoreactive TH-2 clones could support the production of IgG anti-dsDNA. Clones which were cognate TH-2 (i.e. helped only the anti-DNA response activation) did not support anti-DNA production very well. A T-cell line which was TH-1 (secreting Il-2 rather than BCGF) did not help anti-DNA production at all. Furthermore, B cells isolated from BW spleen produced IgG anti-dsDNA in response to our preparation of IL-5, but not after stimulation by recombinant IL-2 or IL-4 [22], indicating that T cells secreting IL-5 (and possibly IL-6) are required for synthesis of these pathogenic autoantibodies [22]. The requirement for IL-5 to drive BW B cells to make anti-DNA was demonstrated in similar experiments by Herron *et al.* [23].

In constructing hypotheses that apply to both murine and human lupus, it should be noted that TH-1 and TH-2 classes of cloned helper T cells have *not* been described in humans. However, human clones do not secrete equivalent amounts of

IL-2, IFN, and BCGF (B-cell growth factors) in every instance, so there may be relative skewing of lymphokine secretions toward the TH-1 or TH-2 phenotype.

Sainis and Datta [24] have also shown the importance of autoreactive helper T cells in the production of pathogenic autoantibodies in murine lupus, using their SNF^1 model. Furthermore, they demonstrated that production of the $Id^{SN}F_1$ idiotype which dominates the glomerular deposits of IgG2b cationic Ig in this mouse is dependent on their autoreactive T-cell lines. An interesting finding was that some of the supportive T-cell lines were $CD4^-$, $CD8^-$, in addition to the $CD4^+$, CD8 – lines that were predicted.

Helper T cells and responding B cells are polyclonal

One of the explanations for the ability of certain individuals to make pathogenic subsets of autoantibodies is that either T-cell or B-cell clones, or both, escape from regulation and dominate the immune response. Since our data suggested that autoreactive TH-2 helper T cells could account for much of the ability of BW B cells to make IgG2a anti-dsDNA, we investigated the clonality of those T cells. Southern blots of DNA extracted from five of the autoreactive TH-2 cells were cut with several restriction enzymes and hybridized with probes for different C_{beta} gene segment subfamilies of the murine T-cell receptor. Each of the clones displayed different C_{beta} gene segment RFLP patterns. This suggested that the T cells were not derived from a single clone, despite similar proliferative reactivity to Ia.

To determine clonality of the responding B cells, we studied Ig gene usage in autoantibody-secreting hybridoma B cells made from spleens of BW mice [25]. Twenty-two B cells making different MoAbs were derived from a single nephritic mouse, and several others were derived from mice of different ages. Northern blots of RNA extracted from these cells were incubated with cDNA probes for eight different V_H gene segment subfamilies and 10 different V_K gene segment subfamilies. Results of experiments with the V_H gene segment are shown in Figure 1. In the 22 MoAbs obtained from a single mouse, at least six of the 10 known V_H families were used. A few clones did not hybridize with any of the eight available probes. J558, the largest family [26], was used most frequently. Similarly, at least five V_K gene segment subfamilies were used by the same B cells. Therefore, no evidence of clonality emerged, even in multiple antibodies from the same mouse.

Nine of the MoAbs from the nephritic mouse were similar to the pathogenic Igs that can be eluted from glomeruli, $IdGN2^+$ IgG2a anti-dsDNA. Contributions from both heavy and light Ig chains are required for expression of IdGN2. Those nine MoAbs used three different V_H and three different V_K gene segment subfamilies, again suggesting no clonality of response.

B-cell hybridomas from other BW mice of various ages showed similar, wide usage of different Ig V region genes [27]. Therefore, we could find no convincing evidence of unique genes or of clonality contributing to the majority of IdGN+ MoAbs. These findings support our hypothesis that activation of B cells is polyclonal, and could result from non-specific responses to BCGF.

The enigma of the absence of adequate suppression

An explanation for the absence of suppressor signals is also needed to understand why polyclonal activation of T and B cells drives the production of pathogenic

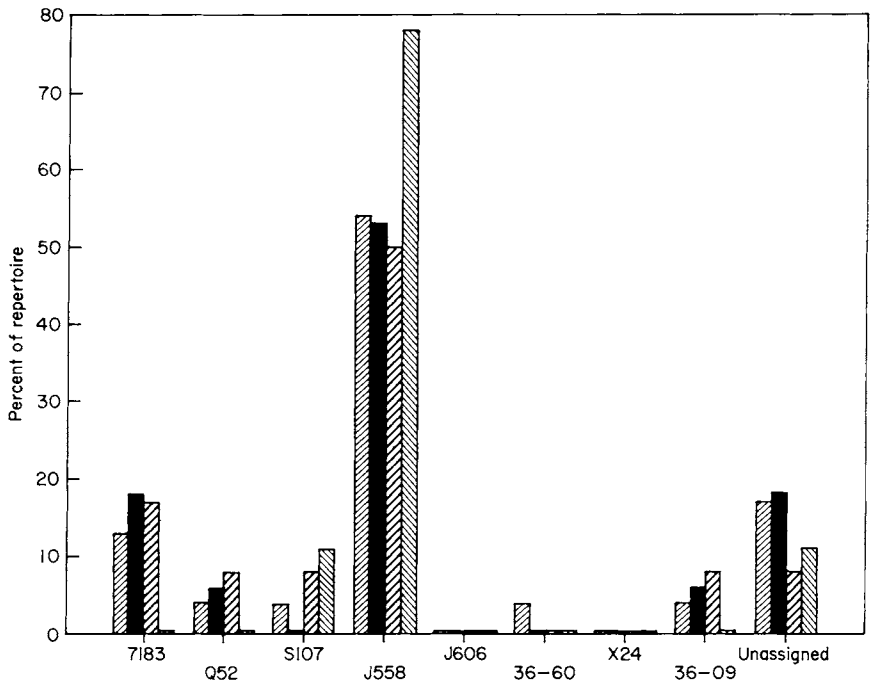

Figure 1. Usage of V_H immunoglobulin genes by 22 different monoclonal antibodies from a single nephritic NZB/NZW F_1 mouse. The families are listed according to estimates of their location on germline DNA, the most 3′ to the left and 5′ to the right. For all B-cell hybridomas secreting MoAbs, including those with anti-DNA activity, those bearing IdX, and the pathogens bearing IdGN2, J558 was used most frequently, but multiple families were used. Similarly, in experiments not shown here, multiple V_K gene families were used by the same antibodies. Similar wide distribution of gene usage was found for several other hybridoma B cells obtained from BW mice of various ages. See reference 25. ▨, all MoAbs; ■, anti-DNA; ▨, IdX; ▧, $IdGN_2$.

antibodies in SLE. The skewing of T-cell responses to help rather than suppression could result from dominance of TH-2-like cells, with resultant decreases in IL-2 and IFN gamma secretion. Recent studies [28] have shown that a suppressor precursor T cell (Tsp) cannot differentiate into a T suppressor-effector unless IFN gamma is present. The IGN signal can be augmented by IL-2. In fact, preliminary data suggests that in human SLE, $CD8^+$ precursor cells develop into $CD8^+$ amplifier cells (rather than suppressors), which can increase IgG synthesis [29].

Hypothesis

Although we have made a strong argument for the importance of autoreactive, polyclonal T- and B-cell activation in SLE, it is unlikely that upregulation of autoantibodies is purely random, with all memory B cells activated by unbalanced T-cell help. This could not explain the remarkable idiotypic restriction that occurs in BW and SNF_1 mice, or the dominance of IdGN2 in human lupus nephritis. Therefore, we postulate that T helper cells deliver help preferentially to subsets of B cells expressing IdGN and other public Ids characteristic of SLE, and that most pathogenic autoantibodies are derived from a limited number of these public Ids. This theory is illustrated in Figure 2. Idiotypic selection could result from (1) activation of

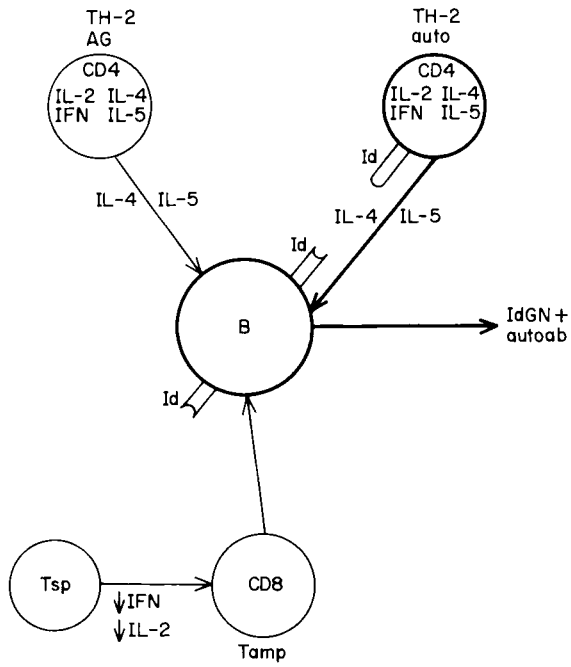

Figure 2. An hypothesis regarding abnormal upregulation of Id-producing B cells in murine and human SLE. The B cell producing Ig bearing public Ids which contain pathogenic autoantibody subsets is central to the scheme. All T cells are acting as helpers/amplifiers with few suppressor signals. The dominant T cell is the $CD4^+$ autoreactive TH-2-like cell which secretes more BCGF (IL-4, IL-5) than T-cell growth factors (IL-2, IFN gamma). The antigen-responsive $CD4^+$ cell may also stimulate, but less so. The relative paucity of IL-2 and interferon gamma resulting from the dominance of the TH-2-like cell pushes the evolution of T suppressor precursor cells (Tsp) toward amplifying $CD8^+$ cells (Tamp) rather than suppressor cells. The dominant $CD4^+$ autoreactive TH-2-like cell drives the activated central B cell which secretes antibodies bearing selected idiotypes. Such selection could result from (1) activation of a resting B cell by linking of surface Id by anti-Id, (2) specific recognition of Id on an activated B cell by T helper cells which subsequently serve as bystanders, or (3) association between certain Ids on B cells and high numbers of receptors for BCGF, making those cells more responsive to bystander, TH-2-like help.

an Id-bearing B cell by cross-linking of Id by anti-Id, followed by non-specific growth following helper T-cell stimulation; (2) recognition of the Id on activated B cells by helper T cells followed by specific upregulation; or (3) increased density of B-cell growth and differentiation factor receptors on Id-bearing B cells, making the cell responsive to non-specific growth signals.

In summary, SLE is a disease of abnormal immune regulation in which autoreactive T-cell populations arise which are skewed toward production of IL-4 and IL-5 (B-cell differentiation and growth factors) rather than IL-2 and IFN gamma. These TH cells selectively expand B cells carrying certain idiotypic markers. Within the antibodies bearing certain public idiotypes, such as IdGN, are the subpopulations which are pathogenic and contribute to disease.

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Induction of SLE-like Disease in Naive Mice with a Monoclonal Anti-DNA Antibody Derived from a Patient with Polymyositis Carrying the 16/6 ID

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The role of a pathogenic anti-DNA idiotype (16/6 Id) is presented. An SLE-like disease was induced in naive BALB/c mice by immunization with a human IgM monoclonal anti-DNA antibody which was derived from a patient with polymyositis (SA_1). The antibody carries the 16/6 Id and binds to DNA. A parallel human IgM monoclonal antibody derived from the same patient while in remission (SA_2), served as a control.

The SLE in the mice was characterized by serological markers, including high serum titers of anti-DNA, anti-Sm/RNP, anti-Ro and La, and anti-histone antibodies. In addition, high levels of murine anti-16/6 and 16/6 Id antibodies were demonstrated. The clinical findings entailed increased erythrocyte sedimentation rates, leukopenia and proteinuria.

These findings support our previous studies on the importance of the 16/6 Id as a pathogenic idiotype in SLE.

Introduction

Systemic lupus erythematosus (SLE) is considered to be the prototype of autoimmune diseases [1]. This assumption is based on the diversity of autoantibodies detected in the sera of SLE patients and on the immune-mediated injury observed in multiple organs. Yet, this clinical condition does not fulfill the criteria for definition as a clear cut autoimmune state. The pathogenic autoantibody has thus far not been identified (e.g. how do anti–DNA antibodies induce tissue injury?); the autoantigen is still obscure [2, 3]; the disease could not be induced in experimental animals either by DNA immunization (DNA is not immunogenic [4]) or by anti-DNA autoantibodies, even with 'pathogenic' monoclonal anti-DNA antibodies [5].

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Following the production of human monoclonal anti-DNA antibodies [6, 7] and the delineation of the common anti-DNA idiotype, 16/6 Id [8], a better categorization of SLE disease as an autoimmune condition was achieved.

The 16/6 Id is harbored on anti-DNA antibodies [8, 9], but not exclusively [10, 11]. Autoantibodies with the 16/6 Id seem to have a pathogenic role in SLE. Although appearing in other autoimmune diseases and conditions [11–16], high titers of this idiotype are mainly found in sera of patients with clinically active SLE [17]. Furthermore, its deposition in afflicted kidneys and skin of patients with SLE was confirmed [18, 19].

The best evidence for the pathogenic role of the 16/6 Id was recently shown by us [20]; immunization of naive mice with 1 μg of the 16/6 Id induced an SLE-like disease [20]. The experimental SLE-like disease was characterized by serological as well as clinical parameters. In all mice increased erythrocyte sedimentation rate (ESR), leukopenia and severe proteinuria were observed. These findings were associated with increased titers of anti-DNA, anti-Sm, and anti-cardiolipin antibodies. Furthermore, in the affected mice high concentrations of anti-16/6 Id antibodies as well as 16/6 Id (of murine origin) were recorded. Electron microscopy and immunohistochemistry have demonstrated the deposition of immune complexes containing the 16/6 Id (most probably of murine origin) in the mesangial tufts of the kidneys [20]. We could not demonstrate any increase in antibody titers against bovine serum albumin, (T,G)-A-L[poly(Tyr,Glu)-poly(DLAla)-poly(Lys)] thyroglobulin in the sera of the immunized mice.

The induction of SLE in mice was found to be strain-dependent [21]. Thus, the disease was most easily induced in BALB/c ($H\text{-}2^d$), C3HSW ($H\text{-}2^b$), AKR ($H\text{-}2^k$) and SJL ($H\text{-}2^s$) mice, while C57BL/6 ($H2^b$) and C3H/He ($H\text{-}2^k$) mice were resistant to the induction of the disease. These results suggest that susceptibility to SLE induction is non-H-2 linked. It is noteworthy that sensitivity to the induction of the disease is directly correlated with the ability to respond to the 16/6 Id by production of anti-Id antibodies. The pathogenic role of 16/6 Id was further demonstrated in SLE prone mice such as NZB/W F_1 in which the females spontaneously develop proteinuria and high titers of anti-DNA antibodies around the fourth month of life. Immunization of this strain with 16/6 Id brought on the disease earlier, and after 3 months 5/5 immunized mice developed the disease (in comparison to one out of five non-immunized mice); after 9 months all five died while in the control immunized mice only 1/5 died [21].

The induction of SLE serology is much more impressive in females as compared with males [21], while in castrated BALB/c males treated with estrogen, the disease may be seen even 2 months following immunization [22].

The disease can be induced also by immunization of mice with mouse monoclonal anti-16/6 idiotypic antibody (m-anti-16/6 Id). Furthermore, the kidney damage in mice immunized with the anti-Id antibody is earlier than in the 16/6 Id immunized mice, suggesting the central role of the anti-16/6 Id antibody in the induction of the disease [23].

We describe here the induction of SLE-like disease in BALB/c mice following immunization with the antibody SA_1, which is a human monoclonal IgM derived from a patient with polymyositis. The antibody binds to dsDNA and carries the 16/6 Id [24].

Table 1. *Autoantibody reactions of mice immunized with the human monoclonal SA_2 (16/6 Id+)*

Mouse serum	Immunization with MoAb	Anti 16/6	16/6	ssDNA	dsDNA	Poly (I)	poly (G)	Cardiolipin
NMS ($n=4$)	–	47 ± 7	35 ± 4	74 ± 9	65 ± 4	47 ± 3	58 ± 5	45 ± 4
S_1 ($n=7$)	SA1	1042 ± 69	1487 ± 74	1043 ± 78	1454 ± 81	1247 ± 66	1003 ± 74	847 ± 72
S_2 ($n=7$)	SA2	84 ± 11	69 ± 8	82 ± 7	71 ± 8	58 ± 7	74 ± 8	66 ± 7
S_3 ($n=5$)	BR2	57 ± 5	63 ± 7	77 ± 8	64 ± 6	72 ± 8	69 ± 9	75 ± 7

Antibody binding of serum from female mice immunized with the human monoclonal IgM-SA1 carrying the 16/6 idiotype, the human monoclonal IgM SA2 or BR2 (human MoAb derived from PBLs of a patient with breast cancer). An ELISA assay was performed 4 months after booster. Results are expressed as mean $M \pm SD$ $O.D._{405} \times 10^{-3}$. All sera are shown in this table at a dilution of 1:200. NMS, normal mouse serum; MoAb, human monoclonal antibody. *n*, number of mice immunized.

Table 2. *Antibody responses to nuclear determinants of mice immunized with the human monoclonal SA1 (16/6 Id+)*

Mouse serum	Immunization with MoAb	Sm	RNP	SS-A (Ro)	SS-B (La)
NMS ($n=4$)	–	15 ± 5	27 ± 5	37 ± 4	25 ± 3
S_1 ($n=7$)	SA1	1343 ± 84	1249 ± 63	1004 ± 52	978 ± 41
S_2 ($n=7$)	SA2	47 ± 5	55 ± 3	74 ± 8	67 ± 9
S_3 ($n=5$)	BR2	64 ± 8	79 ± 6	84 ± 9	66 ± 7

See Table 1 for details.

Materials and methods

Mice

BALB/c female mice were obtained from the Tel Aviv University repository and were used at the age of 2–3 months.

Monoclonal antibodies

The production, purification, and ligand binding of the monoclonal antibodies SA_1 and SA_2 were reported in detail elsewhere [24]. Briefly, the human IgM monoclonal SA_1 was generated by the human hybridoma technique from the peripheral blood lymphocytes (PBL) of patient with active polymyositis. The antibody was found to bind to ssDNA, dsDNA, poly(I), and poly(G) and to carry the common lupus anti-DNA antibody idiotype (16/6 Id). The SA_2 human IgM monoclonal antibody was produced by similar methods from PBLs of the same patient while in remission. The SA_2 lacked the ligand binding capacities of SA_1 and did not have the 16/6 Id. The SA_2 was therefore employed as a control antibody. BR_2 is another human monoclonal IgM generated by the hybridoma technique from lymph node lymphocytes of a patient with breast cancer.

Immunization

Three groups of BALB/c female mice were immunized with 1 μg/ml of affinity-purified human monoclonal antibodies in CFA in the hind footpads and were given booster injections with the same amount in PBS solution 3 weeks later. Ten mice in each group were immunized with 1 μg of either affinity-purified SA_{y1} or SA_2 in complete Freund's adjuvant (CFA; Difco) intradermally into the hind footpads.

The following measurements were taken at 3, 4, and 5 months: erythrocyte sedimentation rate, white blood cell counts, and quantitation of protein in the urine. In addition, the titers of the following antibodies were determined: anti-ssDNA, dsDNA, poly(I), poly(G), cardiolipin, SS-A(Ro), SS-B(La), Sm, RNP, and histones and their subfractions, as well as the titer of the 16/6 Id and anti-16/6 Id. All the above determinations were carried out by ELISA as has been detailed previously [6, 7, 20, 21, 25].

Table 3. *Anti-histone subfractions antibodies in serum of mice injected with SA1*

Mouse serum	Immunization with MoAb	Total histones	Histone subfractions				
			H_1	H_{2a}	H_{2b}	H_3	H_4
NMS ($n=4$)	–	37±5	27±2	42±7	36±5	52±8	41±3
S_1 ($n=7$)	SA1	808±55	901±31	213±15	249±17	198±21	847±27
S_2 ($n=7$)	SA2	47±3	112±13	78±8	83±7	91±8	124±12
S_3 ($n=5$)	BR2	56±3	124±14	86±9	93±9	77±6	131±15

See Table 1 for details.

Results

Tables 1–3 summarize the serological results and show autoantibody titers determined in the groups of animals immunized with SA_1 and those with the control monoclonal antibodies. The titers are expressed in O.D. units ($\times 10^{-3}$). As can be seen for all autoantibodies, the titers were significantly higher in the group of mice injected with SA_1 that carries the 16/6 idiotype *vs* the other group immunized with SA_1 and BR2 that lack the 16/6 Id. No increased titers were recorded against bovine serum albumin.

Similar results were achieved when the titers of the 16/6 Id as well as the anti-16/6 Id were determined (Table 1). The clinical findings, e.g. increased ESR, leukopenia, and the degree of proteinuria, paralleled the serological findings.

Tables 1 and 2 represent the antibody titers in serum from the immunized mice 4 months after booster injection. These antibody titers were detected only in mice immunized with SA_1 antibody and which carry the idiotype 16/6, and not in SA_2-IgM or BR_2-IgM mice. The titer of the anti-idiotypic antibodies against the idiotype 16/6 (anti-16/6) reached a maximum level 1 month after booster injection and remained stable. High levels of antibodies bearing the idiotype 16/6 (anti-anti-idiotype specific antibodies) were produced in the mice immunized with SA_1 antibody (Table 1). Data shown in Table 1 indicate high levels of anti-ssDNA, dsDNA, poly(I), poly(G) antibodies. In addition to high antibody levels to nucleotide determinants, elevated levels of anti-cardiolipin antibodies were observed. Table 2 shows the antibody levels against nuclear determinants Sm, RNP, SS-A(Ro) and SS-B(La). Mice immunized with SA_2 carrying the 16/6 idiotype showed high levels of antinuclear antibodies.

Table 3 summarizes the increased titers of autoantibodies reacting with total histone preparation and with histone subfractions. The highest O.D. readings were recorded when H_1 and H_2 histone subfractions were employed as autoantigens.

Discussion

Previously, we showed for the first time that an SLE-like syndrome can be experimentally induced in naive mice not spontaneously prone to develop SLE [20]. This was achieved following immunization with the 16/6 Id which is a common human anti-DNA idiotype. This antibody seems to have a pathogenic role in SLE and especially in kidney involvement [18]. In the current study, we have extended our experience to another human antibody, the SA_1. This antibody, although carrying the 16/6 Id [24], differs slightly from the original 16/6 human anti-DNA antibody by its stronger binding to dsDNA. These facts may explain the earlier appearance of the disease (e.g. proteinuria) in the injected mice (3 months *vs* 4 months). However, it is very likely that it is not the DNA binding activity which is important in inducing the disease but the presence of the idiotype. We are currently trying to induce the disease with anti-DNA antibodies, without 16/6 Id and with antibodies carrying the 16/6 Id that do not bind to DNA. In the current study, we had an ideal negative control for the SA_1, namely SA_2, a monoclonal hybridoma IgM which was generated by identical methods to SA_1 and was derived from the same patient while in remission, albeit the IgM does not bind to DNA and does not harbor the 16/6 Id.

How could a human anti-DNA idiotype induce the emergence of a murine pathogenic idiotype, which is deposited in the kidneys? And how could an anti-DNA antibody induce the emergence of such apparently unrelated autoantibodies as anti-Sm or anti-histone?

The 16/6 Id is an anti-DNA antibody [6–8]. Experimentally induced anti-16/6 Id antibodies were previously shown by us to bind to the DNA binding site (AbB_2) thus expressing the 'internal image' of the autoantigen, namely DNA [8]. Although DNA is not by itself immunogenic [4], an anti-idiotype having the DNA three-dimensional structure might be much more immunogenic. Thus, the mouse anti-16/6 seems to react with the original human 16/6 Id as with the experimental one [23], therefore inducing a pathogenic mouse 16/6 Id which is a mouse anti-DNA and can be deposited in the kidney and thus induce the defects in kidney function (e.g. proteinuria).

Regarding our second question, it seems that we may be dealing with the 'Oudin enigma' [26], namely that all the autoantibodies appearing in SLE, although differing in their binding properties, are related via a common ancestor, a family with the same V gene, which diversified via a somatic mutation [27]. Supporting this notion, Kaburaki and Stollar have recently reported on human antibodies which bound to DNA, RNP, Sm, and SS-A and still bore the 16/6 Id [28]. Similar results were noted by Milgiori *et al.* [29] in MRL/*lpr*/*lpr* mice.

Our studies may also point to the origin of spontaneous SLE in human and animal models. Thus, an exogenous antigen harboring the 'internal image' of a pathogenic idiotype induces dysregulation of common 'ancestor idiotype' via the Id anti-id cascade, thus leading to the emergence of diverse autoantibodies such as anti-Sm and anti-histone antibodies.

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Cellular Mechanisms in Immune Tolerance and Treatment of Autoimmune Disease: Studies Using Total Lymphoid Irradiation (TLI)

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A major goal in organ transplantation and in autoimmune disease is to induce specific immune tolerance to alloantigens and self-antigens respectively. Total lymphoid irradiation (TLI) is a technique which has been used to induce specific immune unresponsiveness and treat autoimmune diseases in laboratory animals and humans. Following TLI, there is a marked depletion of mature T and B cells and their associated immune responses in the peripheral lymphoid tissues. In patients with cadaveric renal allografts, there is a gradual return of immune responses to third party alloantigens, but not to donor alloantigens. Similarly, in patients with severe lupus nephritis, there is a gradual return of immune responses to exogenous antigens and mitogens, but autoantibody formation and spontaneous immunoglobulin secretion remain markedly reduced.

Introduction

Although TLI was originally developed as a therapy for Hodgkin's disease [1], during the past several years, modifications of this radiotherapy procedure have been used as an immunosuppressive regimen in patients with organ transplants and with autoimmune disease [2–15]. Five centers have reported on the use of TLI in more than 100 primary or secondary renal transplant recipients [2–6]. Similarly, five centers have reported on the use of TLI in over 100 patients with intractable rheumatoid arthritis [7–13]. Single center studies have examined the effect of TLI in multiple sclerosis [14], lupus nephritis [15, 16], and autoimmune polyneuritis [17].

In all instances, megavoltage radiation usually from a linear accelerator has been targeted to the lymphoid tissues in the neck, chest, abdomen and groin, such that the cervical, axillary, hilar, para-aortic, pelvic and inguinal lymph nodes and thymus were included in the fields. Non-lymphoid tissues which usually included the thyroid and heart were shielded with lead blocks. In most studies, the spleen was

included in the field. Multiple single treatments (fractions) of 100–200 rads each were given until the targeted cumulative dose was achieved. The total dose varied considerably with some centers using as little as 750–800 rads for patients with transplants or rheumatoid arthritis [2, 10], and others using as much as 3,000–4,000 rads [3, 8].

TLI and organ transplantation in laboratory animals and humans

The rationale for the use of TLI in organ transplant recipients was derived from animal studies which showed that this radiotherapy regimen could be used in rodents, dogs and primates to induce specific transplantation tolerance [2, 18–21]. Fractionation and shielding techniques similar to that used for humans can be used to deliver 3,400 rads (17 fractions of 200 rads each) to the lymph nodes, spleen, and thymus of adult mice without substantial morbidity and mortality [18]. 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 [22]. Thus, allogeneic bone marrow injected intravenously into mice within one week after TLI results in permanent chimerism without the development of graft-versus-host disease (GVHD) [20, 23–25]. The resultant chimeras are specifically tolerant of the tissues of the marrow-donor strain and will accept permanently donor-type skin grafts and promptly reject third-party grafts [20, 23–25].

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 1 to 2 d after the completion of irradiation, only about 50% will accept grafts transplanted 7 d after irradiation. By day 21, none of the recipients will accept marrow grafts [26]. 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 [26, 27]. A cumulative dose of at least 3,000 rads is required to achieve tolerance in a high proportion of marrow recipients [26, 28]. Although the major lymph nodes above and below the diaphragm as well as the spleen must be irradiated in order to prepare mice for successful marrow transplantation, irradiation of the thymus is not esential [26]. Rats given TLI have also been successfully tolerized to alloantigens, and permanently accept skin and heart transplants following marrow transplantation [29].

Tolerance in outbred dogs and baboons given TLI

The use of TLI alone (1,800 rad total dose) or in combination with a brief course of other immunosuppressive agents was studied in adult mongrel dogs given heterotopic heart allografts from unmatched donors [21]. Maximum allograft survival with TLI alone was 28 d, and with six intramuscular injections of rabbit anti-dog thymocyte globulin (ATG) alone was 33 d. However, marked synergy was observed with the combination of the two regimens such that 50% allografts survived 200 d or more. Forty percent of dogs given TLI and ATG maintained their allografts with minimal or no cellular infiltrate during the entire observation period (360–495 d) and rejected a third party heart within 2 weeks. Thus, the latter recipients were

specifically unresponsive to the initial allograft despite the withdrawal of all immunosuppressive reagents after day 10.

Long-term acceptance of liver and kidney allografts in outbred baboons treated with TLI in the absence of marrow transplantation has been reported by Myburgh and his colleagues [30]. The most successful regimen involved the use of enlarged radiation fields which included the whole abdomen as in previous rodent studies, but with a cumulative radiation dose of 800 rad given in 100-rad fractions. Using this procedure, unmatched kidney allografts survived more than 200 d without post-transplant immunosuppressive drugs in 80% of recipients [30]. Some animals in these studies were followed for as long as 4 years with intact kidney allografts [30]. Specific unresponsiveness in the latter graft recipients was demonstrated by the rapid rejection of a third party graft transplanted one year after the initial graft [30].

The first human study of TLI in renal transplantation combined pretransplant radiotherapy with maintenance prednisone and azathioprine in patients who had rapidly rejected a previous cadaveric renal allograft [31]. One-year graft survival was improved by approximately 30% as compared to historical controls. A comparison of the outcome of similar patients treated with cyclosporine and prednisone or TLI showed similar graft survival over 3 years [3]. Subsequently, two centers reported that renal transplant recipients given TLI could be maintained on low-dose prednisone ($\approx$ 10 mg/day) as the sole immunosuppressive drug [4, 6]. Nine of 11 patients in one of these studies [6] developed specific unresponsiveness to donor alloantigens in the mixed leukocyte reaction [32]. Two of these patients were withdrawn from all immunosuppressive therapy and maintained good graft function during an observation period of 10 to 14 months [S. Strober, *et al.*, manuscript in preparation]. Recently, pre-transplant TLI has been combined with maintenance low dose cyclosporine and prednisone [2]. Graft survival in the latter group was superior to that with cyclosporine and prednisone alone [2].

Treatment of intractable rheumatoid arthritis with TLI

Five centers have reported on the use of TLI in intractable rheumatoid arthritis [7–13]. A total of 108 patients were studied in three uncontrolled and two controlled trials. In all studies, the patients' joint disease was not controlled adequately with non-steroidal anti-inflammatory drugs, gold compounds and D-penicillamine prior to TLI. A subset of these patients had failed therapy with azathioprine, methotrexate and/or steroids also. There was substantial variation in the total dose of TLI given, since patients received as little as 750 rads excluding the spleen in one study [10] to as much as 3,000 rads including the spleen in another [8]. The mean age of the patients also varied considerably, such that the oldest group of patients (mean age 66 years) [9] differed from the youngest group of patients (48 years) by 18 years [7]. In addition, the mean severity of disease (functional class by American Rheumatism Association criteria) at entry also differed widely between studies.

All five studies showed a statistically significant improvement in joint disease activity during a 6 or 12 month follow-up period when the parameters before and after irradiation were compared. The controlled studies reported that patients receiving a total dose of 2,000 rads were statistically significantly improved as compared to those given 250 rads [33], but the efficacy of 750 rads was similar to that of

2,000 rads [10]. Long-term follow-up studies indicated that improvement after TLI persists at least 4 years in most patients [7]. However, a gradually increasing proportion develop recrudescence of disease after 2 years requiring the use of adjuvant drug therapy such as methotrexate in order to maintain improvement [7].

Although there was agreement within these reports concerning the efficacy of TLI in intractable rheumatoid arthritis, there were marked differences in the severity of side effects and complications. The incidence of Herpes zoster varied between zero [10] to 36% [11], and that of severe bacterial infections from zero [10] to 33% [8]. It is of interest that the study with the lowest incidence of viral and bacterial infections used the lowest dose of irradiation (750 rads excluding the spleen) [10], and that the study with the highest incidence of systemic staphylococcal infections used the highest dose (3,000 rads including the spleen) [8].

The mortality rates in the studies also varied considerably from zero [10, 11, 13] to 36% [9]. Overall, 13 deaths in 108 patients were reported in the published studies from 1979 through 1987 [7–13]. The highest mortality rate (36%) was observed in the study of Nusslein *et al.* [9], which had the highest mean patient age (66 years). When the Stanford University study of 32 patients was analyzed, three of four deaths occurred in patients at least 66 years of age (7 of 32 patients) [7]. The effect of TLI on the mortality rate in intractable rheumatoid arthritis is difficult to ascertain, since a control population matched for at least age, sex and severity of disease would have to be studied for comparison. Recent studies have shown that age and severity of disease are important variables which determine mortality rates [34, 35].

In view of the uniformity of efficacy for at least 6 months, and the variability of side effects of the rheumatoid arthritis patients given TLI, it is difficult to draw conclusions as to the role of TLI in the treatment of this disease. As in the case of drugs, it is critical to develop an optimized regimen in order to assess the benefits and indications for the therapeutic intervention. Judging from the published studies an optimized risk:benefit ratio may include reducing the total dose of irradiation to 750 rads with *inclusion of* the spleen in a single sub-diaphragmatic field, and limiting the age of the patients to 62 years and the severity of joint disese to less than functional Class III. The reduction of radiation dose, patient age, and disease severity is likely to reduce side effects substantially, and still maintain efficacy [10]. Although the exclusion of the spleen from the irradiation field resulted in few side effects in a previous study [10], the duration of efficacy may be substantially reduced.

Comparison of the risks and benefits of an optimized TLI regimen with those of currently used chemotherapeutic agents such as methotrexate in controlled trials would provide important information concerning therapeutic alternatives in patients with intractable rheumatoid arthritis. In addition, an optimized TLI regimen may provide an alternative for patients who have experienced intolerable side effects from methotrexate (i.e., hepatotoxicity, stomatitis, etc.) such that discontinuation of the drug resulted in a recrudescence of disease activity.

Treatment of lupus nephritis with TLI

Recent controlled studies indicate that the combination of cyclophosphamide and steroids are more effective than steroids alone in treatment of severe lupus nephritis [36, 37]. The efficacy of the combination therapy is best appreciated when the patient

base is stratified according to the pretreatment renal biopsy findings. Patients with little or no glomerular scarring and tubular atrophy are adequately treated with steroids alone, and only a small percentage go on to renal failure during a follow-up period of at least 5 years [38, 39]. On the other hand, the majority of patients with a moderate to high degree of scarring and atrophy (chronicity index >1) progress to renal failure after treatment with steroids alone. The combination of cyclophosphamide and steroids significantly reduces the progression to renal failure as compared to steroids alone in this group of patients [36, 39].

Severe side effects associated with the use of combined intravenous or oral cyclophosphamide and steroids include bacterial sepsis, alopecia, cystitis, sterility, bladder cancer, lymphoma and leukemia [40]. Radiotherapy (TLI) has been used as an alternative to cyclophosphamide and/or azathioprine in an attempt to find an effective therapy for severe lupus nephritis with fewer side effects [15, 16]. Previous studies of the use of alkylating agents in the treatment of malignancies have documented an increased risk of secondary lymphoma, leukemia, and bladder cancers [41, 42]. On the other hand, TLI alone has not been associated with an increased risk of secondary lymphoma, leukemia and bladder cancer in over 1,500 patients in follow-up studies of up to 10 years [43–46]. The combination of TLI and chemotherapy has been associated with an increased risk of lymphoma and malignancy, but the risk is not significantly different from that associated with chemotherapy alone (43–46). The incidence of sterility in patients with Hodgkin's disease treated with TLI alone has been reported to be less than 10% in studies of more than 1,000 patients (1).

Twenty patients with severe lupus nephritis and nephrotic syndrome whose disease was not adequately controlled with steroids alone or in combination with cytotoxic drugs were treated with TLI (total dose 2,000 rads) at the Stanford University Medical Center. Almost all of the patients fell into the poor prognosis category based on the pretreatment renal biopsy chronicity index of Austin *et al.* [39]. The results of the outcome of 15 patients followed for up to 6 years have been reported previously [16]. Figure 1 shows that there was a statistically significant improvement in the mean levels of serum albumin, proteinuria, serum anti-DNA antibodies and C3 of the 20 patients during the first 3 years. The mean levels of serum creatinine did not change significantly during the same time interval. There was a substantial reduction in the mean daily dose of prednisone such that during the second and third years after radiotherapy the dose was $\leqslant 10$ mg/d.

The mean number of spontaneous IgG plaque forming cells (PFC) was more than 10 times higher in lupus patients before TLI than that of normal controls [47]. The mean number of IgM PFC was also elevated as compared to normal controls, however, the difference was not as striking. Spontaneous immunoglobulin secretion was reduced to normal levels in eight patients assayed between 32 and 78 months after TLI [47].

Changes in the number of total (Leu-4^+), helper/inducer (Leu-3^+), and cytotoxic/suppressor (Leu-2^+) T cells were studied before and after TLI [47]. The mean values for all three cell populations before TLI was reduced as compared normals. The Leu-3^+ subset was affected most severely. Comparison of the levels of Leu-2^+, Leu-3^+, and Leu-4^+ cells before and after TLI showed that only the Leu-4^+ level was reduced significantly using the paired t-test ($P<0.02$) during the first

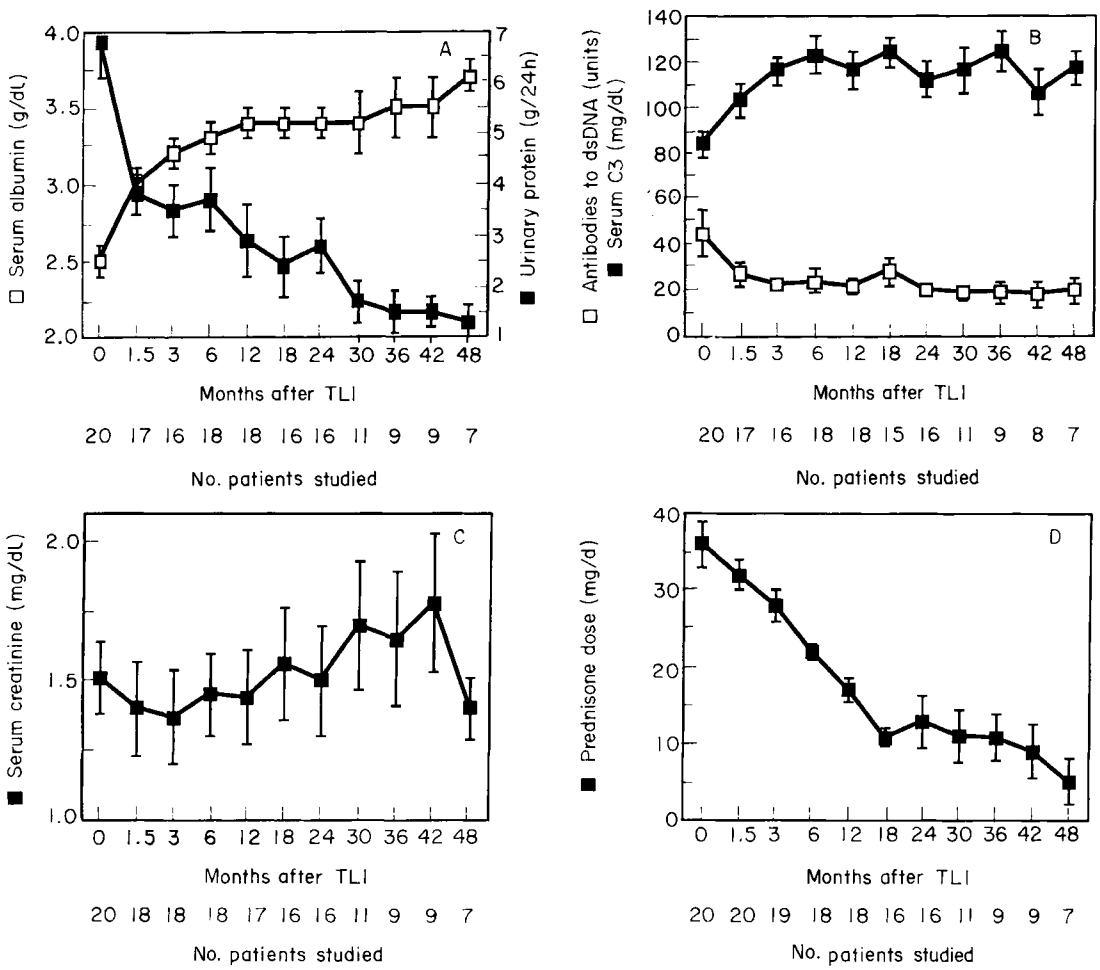

Figure 1. Kinetics of the changes in disease activity and steroid therapy in 20 patients during the 3-year interval after TLI. Graphs show the mean values and standard errors for all the patients before treatment and the mean of the data closest to the given time point after the start of TLI. Panel A, changes in the concentration of serum albumin g/dl (□) and excretion of urinary protein g/24 h (■). Panel B, changes in the concentration of antibodies to double stranded (ds) DNA units (□) and C3 in the serum mg/dl (■). Panel C, changes in the concentration of serum creatinine mg/dl (■). Panel D, changes in the daily dose of prednisone mg/d (■).

year. In subsequent years, the Leu-4$^+$ levels were not significantly reduced ($P > 0.05$). At the 4-year point, the mean level of Leu-2$^+$ cells was not statistically significantly different from normal, but the level of Leu-3$^+$ and Leu-4$^+$ cells remained significantly reduced ($P < 0.002$; unpaired t-test) [47].

A similar pattern was observed in the *in vitro* proliferative response of peripheral blood mononuclear cells to mitogens [phytohemagglutinin (PHA), concanavalin A (Con-A), pokeweed mitogen (PWM)] and to allogeneic leukocytes [47]. The mean response fell during the first year after TLI, and gradually recovered during the second to fourth years. During the fourth year, the mean values were not statistically significantly different from the pre-TLI values for all four mitogens ($P > 0.05$,

Table 1. *Complications associated with TLI and cyclophosphamide in the treatment of lupus nephritis*[1]

Complication	TLI	Oral cyclophosphamide[2]	Intravenous cyclophosphamide[2]
Major infection	0/20	3/18	2/20
Minor infection[3]	3/20	–	–
Herpes zoster	5/20	6/18	5/20
Amenorrhea	1/15[4]	5/7	5/11
Hemorrhagic cystitis	0/20	3/18	0/20
Neoplasia	0/20	3/18[5]	0/20
Deaths	2/20	1/18	2/20

[1]Fraction of patients.
[2]Balow *et al.* [40].
[3]Bronchitis, lower extremity cellulitis, gluteal abscess.
[4]Patients with ovarian lead shields.
[5]Carcinoma of cervix, carcinoma of bladder, polycythemia vera.

two-tailed paired Student's *t*-test). However, during the first year, values from Con-A, PHA and the mixed leukocyte reaction (MLR) were significantly reduced ($P < 0.01$; paired *t*-test).

During the follow-up period (up to 90 months) one of the 20 patients died due to an apparent suicide and another due to stroke and pancreatitis. Two progressed to renal failure and are currently on dialysis. Complications observed during the study are listed in Table 1. Three patients developed minor bacterial infections and five developed localized Herpes zoster. Amenorrhea occurred in one of 15 patients with ovarian lead shields. Table 1 also shows the complications reported by Balow *et al.* [40] in the study of the use of oral or intravenous cyclophosphamide in lupus nephritis performed at the National Institutes of Health. The follow-up time of the patients of Balow *et al.* [40] was substantially greater than that of the Stanford study. Therefore, direct comparisons of risks per patient per unit time cannot be made. Nevertheless, the data is consistent with the lower risk of secondary malignancy and sterility associated with the use of TLI in the treatment of lymphoma as compared to that with alkylating agents [41–46]. In order to compare adequately the risks and benefits of TLI with cyclophosphamide in lupus nephritis, it is clear that controlled randomized trials are required in which a stratified patient base is observed for a sufficient period of time to assess progression to renal failure and the development of secondary malignancies.

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Maintenance of Autoimmunity

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Borrowing from the carcinogenesis field, the natural history of Sjögrens syndrome (SS) is analyzed from an evolutionary and multistage point of view. SS is divided into three stages called initiation, promotion and progression. The first stage may be asymptomatic and characterized only by serum autoantibodies (e.g. anti-Ro). The second stage is clinical autoimmune exocrinopathy with potential for lymphocyte aggressive behavior, extraglandular tissue infiltration and pseudolymphoma. The final stage is malignant lymphoma. This sequence is compared with the natural history of human immunodeficiency virus infection which also progresses from an asymptomatic state through AIDS-related complex to AIDS. Indeed, some HIV-infected patients have developed a clinical picture resembling SS due to salivary gland lymphoid infiltrates. This relatively new finding suggests a possible viral etiology for SS.

The Epstein Barr virus must be considered in this context in view of (1) the history of infectious mononucleosis in many SS patients, (2) the production of an autocrine B-cell growth factor by B-cell lines established from SS patients, and (3) the development of EBV-related lymphomas in AIDS patients. If not itself the initiating agent, EBV may yet play an important role in maintaining the state of B-cell proliferation which progresses through autoimmunity into lymphoma in SS.

Introduction

Progress in understanding the pathogenesis of autoimmune diseases may be hastened if we borrow concepts from the carcinogenesis field and begin to analyze our problem from an evolutionary and multistage point of view. Carcinogenesis is often divided into three stages called initiation, promotion and progression. Autoimmune diseases, particularly as encountered in the clinic, can also be divided into stages. At the very

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Table 1.

1.	Autoimmunity
	A. Asymptomatic
	B. A normal consequence of aging
	C. Potentially reversible
2.	The immune response is both
	A. Inner directed (introspective)
	1. MHC genes required for antigen recognition
	2. Idiotype network
	B. Outer directed (extroverted)
	1. Host defense
3.	Autoimmune disease
	A. Aberrant expression of Class II MHC
	B. Molecular mimicry

least, the factors that induce autoimmune disease should be considered separately from factors which act to maintain and promote disease progression. This concept will be discussed using Sjögrens syndrome (SS) as a model autoimmune disease.

In clinical medicine, autoimmunity and autoimmune disease are quite different (Table 1). Autoimmunity, i.e. the presence of serum autoantibodies, can be totally asymptomatic. Autoimmunity is practically physiologic in the most senior of our citizens. Thirty to fifty percent of octogenarians may have serum rheumatoid factor or antinuclear antibodies without any disease associated with those autoantibodies in younger individuals.

Autoimmunity is also reversible. The autoantibodies that appear in subacute or chronic infections disappear when the infectious agent is eradicated. Furthermore, numerous drugs induce autoantibodies rather commonly although true autoimmune disease induced by these same drugs is a rare event. As an example, 60% of cardiac patients taking procainamide in conventional doses for one year will develop antinuclear antibody; only 5% of these individuals will develop symptoms of drug-induced lupus. Upon stopping the offending drug, the symptoms will disappear rapidly although the positive serology may persist for months. This experience with drug induction demonstrates both the distinction between autoimmunity and autoimmune disease as well as the duration required for reversibility. The asymptomatic autoimmune state is more easily reversed than is the drug-induced autoimmune disease.

The immune response is both inner and outer directed. It is inner directed by its dependence on major histocompatibility complex (MHC) genes for antigen recognition and on idiotype network for regulation, and outer directed in its ability to respond defensively to foreign invaders. No one can say which aspect of the immune reponse, introspective or extroverted, is more important in immune physiology. Indeed, dual recognition (foreign antigen associated with a Class II MHC cell surface molecule) is required for the initiation of an immune response. So too in autoimmune disease, the dual nature of the immune response must be considered. Autoimmune diseases may arise both from an inner direction, e.g. aberrant expression of Class II MHC molecules (as in autoimmune thyroiditis) as well as from an

outer direction, e.g. molecular mimicry (as in rheumatic fever). The role of the immunologic network in the induction of autoimmune disease has been appreciated for over a decade [1].

Sjögren's syndrome

SS is a chronic systemic autoimmune disease associated with the production of rheumatoid factor and other autoantibodies. It is characterized by lymphocytic and plasma cell infiltration and the destruction of salivary and lacrimal glands, giving rise to the characteristic symptoms of dry mouth and eyes. The term 'autoimmune exocrinopathy' is a suitable and descriptive name for this disease [2].

SS, like systemic lupus erythematosus (SLE), occurs almost 10 times more frequently in women than in men. It can occur at any age but is more common in older women whereas SLE is more common in younger women. The female predominance of both SS and SLE is probably attributable to the action of sex hormones. Androgen is a natural immunosuppressive whereas estrogen is immunoenhancing [3]. Genetic studies have figured prominently in recent investigative work into the etiology of SS. Most of these studies have emphasized genes in MHC and, in particular, the Class II genes which occupy the loci DP, DQ and DR. The HLA susceptibility is related to DR3, DQ1, DQ2, and DRw52 (MT2). Individuals with these genes are prone to the development of anti-Ro (SS-A) and anti-La (SS-B) antinuclear antibodies. Some of these patients may develop an SS/SLE overlap syndrome in which one diagnosis may precede the other by many years [4].

Although suspect, a viral etiology for SS has never been demonstrated. However, recent reports of parotid gland enlargement and dry mouth due to salivary gland lymphoid infiltrates resembling SS in HIV-infected patients [5, 6] raise the possibility that a virus is involved in the pathogenesis of SS. Epstein-Barr virus (EBV) is a likely candidate in view of the development of SS in some patients following infectious mononucleosis [7], and evidence for *in vivo* transformation by EBV with production of autocrine B-cell growth factors [8]. Furthermore, the natural history of SS includes a tendency to terminate as a B-cell lymphoma (Figure 1).

Lymphoproliferation and Lymphoma in SS

An increased incidence of non-Hodgkin's lymphoma in patients with SS was first reported over two decades ago [9]. The chronic antigenic stimulation was suggested as a possible trigger for a malignant transformation event. There are now over 200 examples of this association in the medical literature. Indeed, the risk of lymphoma development is 44 times greater in SS than in the normal population. The autoimmune disorder may precede the development of lymphoma by intervals ranging from 0.5 to 29 years. Certain extraglandular disease features (e.g. splenomegaly) as well as parotid swelling are more likely to occur in patients predisposed to lymphoma. The lymphomas are of two major types: either composed of high undifferentiated B cells or highly pleomorphic and sometimes associated with monoclonal macroglobulinemia. In the first type, antibodies tend to disappear and hypogammaglobulinemia may develop when the malignancy appears. The lymphoma may

Benign autoimmune exocrinopathy	Pseudolymphoma	Malignant lymphoma
Clinical		
Xerostomia	Lymphadenopathy	Massive lymphadenopathy
Xerophthalmia	Splenomegaly	Massive salivary gland enlargement
RA (or another systemic rheumatic disease)	Purpura	Wasting
	Pulmonary infiltrates	
	Renal infiltrates	
Pathology		
Benign lymphoid infiltrates confined to glandular tissue	Atypical extraglandular lymphoid infiltrates	B-cell lymphoma
Serology		
Hypergammaglobulinemia	Hypergammaglobulinemia	Hypogammaglobulinemia
Anti-Ro and La (+)	Anti-Ro and La (+)	Loss of autoantibodies
	Monoclonal spike	

Figure 1. Pathenogenesis of Sjögrens syndrome. The natural history of SS shows progression from a benign to a malignant lymphoproliferative disease.

involve salivary glands or major parenchymal organs such as lungs, kidney, and gastrointestinal tract.

Some patients with SS develop a clinical picture suggestive of malignancy which cannot be classified clearly as malignant even after tissue biopsy. Features of autoimmunity are present. The term 'pseudolymphoma' has been applied to such cases. The course of these patients is quite variable, and many of them respond to corticosteroids or immunosuppressive drugs. Some, however, later develop a frankly malignant lymphoma.

Induction of Sjögren's syndrome

The aberrant and excessive expression of Class II MHC gene products on the surface of cells targeted for autoimmune attack has been attributed to lymphokine production stimulated by virus infection [10]. For example, DR cell surface molecules are present on salivary gland epithelium, perhaps induced by gamma interferon (a known inducer of Class II molecules) produced in response to a salivary gland virus.

In close proximity to these DR-positive epithelial cells are the lymphoid infiltrates in which activated T-helper cells are the most prominent population represented. Activated T cells are also prominent in the pseudolymphoma lesions of SS patients with generalized lymphoproliferation. There are fewer B cells but these too are in an activated state. This seems particularly important in light of the subsequent development of B-cell lymphomas in 5–10% of SS patients, and the production of monoclonal immunoglobulins in the SS salivary glands [9].

Table 2. *Analogy between SS and HIV infection*

	Initiation	Promotion	Progression
Autoimmunity	Serum autoantibodies (Ro:SS-A)	Sjogren's syndrome Pseudolymphoma	B-cell lymphoma Immunodeficiency
HIV Infection	Antibodies to HIV	ARC	AIDS

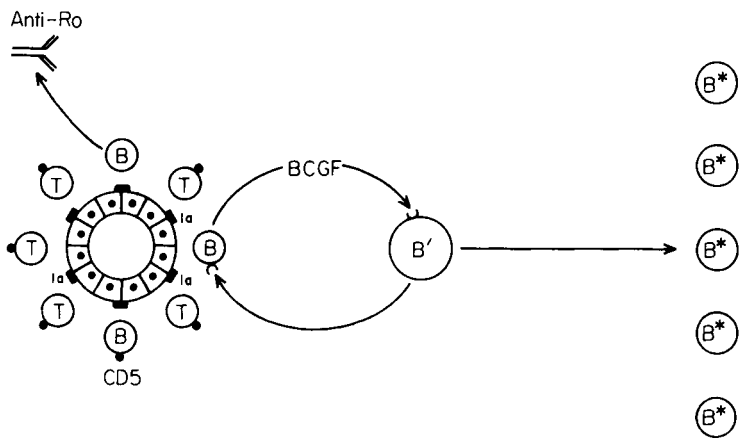

Figure 2. Emphasis is placed on the production of BCGF by EBV-transformed B cells in converting a mild stage of disease initiation (left) into a maintained autoimmune disease with extensive autoimmune tissue lymphoid infiltration and the predisposition to a neoplastic transformation event (right).

Natural killer (NK) cells are important for immunoregulatory functions as well as for natural host defense against malignancy. There is defective NK cell function in the blood of SS patients and absent NK cells in the salivary gland lesions. These results suggest that the salivary gland in SS may serve as an initial nidus for lymphoma development. This hypothesis is supported by an immunohistologic study suggesting that the myoepithelial sialadenitis of SS may contain areas of confluent lymphoid proliferation producing mostly monoclonal IgM/kappa [11]. These lesions were considered 'early lymphomas', analogous to carcinoma *in situ*, which after a variable latent period transform into true lymphomas.

The presence of activated helper T cells and hyperactive B cells without local NK cell defense may be crucial to the malignant transformation event that results in lymphoma development.

Maintenance of Sjögren's syndrome

Evidence of subclinical SS comes from two sets of observations: (1) asymptomatic relatives of SS patients who often have serum autoantibodies, and (2) healthy mothers who have anti-Ro autoantibodies and give birth to babies with congenital heart block. What converts such individuals into symptomatic patients? There must exist

Table 3. *Similarities between AIDS and Sjögren's Syndrome*

Polyclonal B-cell activation
Immune complexes
Increased serum βeta-2-microglobulin
Acid labile interferon
Decreased autologous mixed lymphocyte response
Decreased interleukin-2
Decreased natural killer cells

pathogenetic mechanisms responsible for this conversion. We have searched for such mechanisms by establishing B-cell lines from patients with benign primary SS. Seventeen B-cell lines grew spontaneously from three patients without any stimulation. They all expressed Epstein-Barr virus nuclear antigen and secreted an autostimulatory factor which had properties of a B-cell growth factor [8]. Peripheral blood B cells from SS patients, after culture for 3 d, secreted a similar factor which stimulated the proliferation of established SS B-cell lines. These results suggest that circulating B cells in SS produce an autocrine growth factor that may contribute to lymphoproliferation and ultimately to the emergence of B cell lymphomas.

Conclusion

The natural history of SS suggests a multistage process analogous to carcinogenesis (Table 2). Patients may progress through three stages: (1) initiation, in which isolated salivary gland lesions or perhaps only serum autoantibodies may be present in otherwise asymptomatic patients; (2) promotion into frank SS, perhaps with pseudolymphoma and monoclonal immunoglobulins; (3) progression to B-cell lymphoma with possible accompanying hypogammaglobulinemia and immunodeficiency (Figure 2).

This sequence in SS is analogous to the natural history of infection with the human immunodeficiency virus in which the asymptomatic HIV-positive state is followed by ARC (AIDS-related complex) and then AIDS (Acquired Immunodeficiency Syndrome). Indeed many immunologic features occur commonly in both SS and AIDS patients (Table 3).

SS is not the only situation in which this progression from an autoimmune process to a lymphoma occurs. A similar sequence can be seen in spontaneous autoimmune disease in mice and in mice with experimentally induced chronic graft-*vs*-host disease. A recent study found lymphoproliferative malignancies in 10 of 489 patients (2.2%) with rheumatoid arthritis developing after a mean interval of 11.8 years. As in SS, cytotoxic drugs could not be implicated in pathogenesis and the lymphomas were mostly B cell (non-Hodgkin's) in type [12].

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The Risk/benefit Ratio in Immunointervention for Autoimmune Diseases

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Introduction

The field of application of immunointervention in autoimmune diseases is growing rapidly, in terms of both target diseases and therapeutic agents. Whereas initially only diseases with a threatening prognosis were selected for immunotherapy, now other non-fatal but handicapping diseases are considered for immunosuppressive treatment. At the same time the availability of new immunosupressive agents, more potent but also more toxic than those used in the past, has increased the potential risk of side effects. Consequently, the time has come to make an objective evaluation of the risk/benefit ratio of immunosuppressive therapy in human autoimmune diseases.

Benefits

The increasing spectrum of target diseases

Until a few years ago, only a very limited number of diseases were treated by immunosuppression on a wide scale. Rheumatic and connective tissue diseases represented the primary indication [systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), periarteritis nodosa and other vasculitides]. Less convincing data were obtained in nephrotic syndrome, autoimmune cytopenias, multiple sclerosis, myasthenia gravis and chronic active hepatitis. Except for RA and nephrotic syndrome, all these diseases are associated with a high mortality risk.

More recently other indications have been suggested in diseases with less severe short-term outcome. Immunotherapy has been successfully applied to dermatological psoriasis [1], and in a more preliminary fashion alopecia areata [2] and atopic dermatitis [3]. Similarly, a number of immunosuppressive agents, including cyclosporine, azathioprine and steroids have recently been used in insulin-dependent

diabetes mellitus (Type 1) [4–6], in uveitis, Graves disease [7] and in Crohn's disease [8]. Other indications emerging on the basis of encouraging preliminary data include myocarditis [9], aplastic anemia [10] and chronic polyneuropathy [11].

It should be kept in mind, however, that only severe forms of these diseases represent potential indications of immunosuppression. This comment poses the problem of the expected benefit which is directly linked to the spontaneous outcome of the disease.

Expected benefit

Before discussing the benefits expected from various forms of immunotherapy in autoimmune diseases, one should make a clear distinction between benefit and therapeutic effect. A drug may show a therapeutic effect which is statistically significant but has no real benefit to the patient either because it is too partial (sometimes inferior to that given by conventional treatment), too short lasting, or as we shall see below, is associated with too much toxicity.

Benefits expected from immunotherapy in autoimmune diseases can be classified under three headings: short-term prevention of potentially fatal autoimmunity; long-term prevention of life threatening complications; immediate improvement in the function of a given organ whose deficiency is associated with a major handicap or discomfort.

In the first category, are lupus nephritis and cerebral vasculitis, periarteritis nodosa, aplastic anemia and multiple sclerosis. In all cases the prognosis is sufficiently severe to justify taking some therapeutic risks. Concerning SLE, however, available immunosuppressive therapy has provided dramatic improvement in survival [12], which raises the standards for any new treatments.

The best example of the second category is that of Type I diabetes. Insulin therapy has markedly improved the outcome of this disease, with a majority of patients surviving to over 60 years of age. However, a high percentage of patients still show severe complications, with Type I diabetes representing the leading cause of blindness and of renal failure before 50 years of age [13]. Vascular complications are common (hypertension, cerebral vascular accident). Consequently, an alternative treatment to insulin would be highly welcome, independent of relief from the constraints of daily insulin injections. Similarly, nephrotic syndrome is not fatal in the vast majority of cases but may lead to severe complications due to major hypoalbuminemia and to renal failure, which justifies aggressive preventive therapy.

The third category is that of diseases associated with a major handicap. RA, uveitis and psoriasis are good examples of these. The burden represented by the handicap in question should be evaluated: major disability in RA, blindness in uveitis and major esthetic prejudice in psoriasis. Such patients actively desire a therapy, which physicians have to evaluate in terms of risks and benefits.

The quality of the therapeutic effect

Immunotherapy aims at stopping or decreasing the autoimmune process independently of a direct effect on the symptoms. This clinical improvement will only be manifest if (1) no irreversible lesion has occurred and (2) the immune process is still aggressive.

The first condition is not observed in advanced stages of diseases involving tissue destruction, such as Type I diabetes (pancreas β cells atrophy), multiple sclerosis (neurone demyelinisation) or glomerulonephritis (glomerular sclerosis). The problem is then to intervene sufficiently early in the course of the disease. Such early intervention requires both an early diagnosis, which is not always feasible and an early decision to treat, which is not always accepted (because risks are less acceptable in less advanced stages). Therein lies a major contradiction in immunotherapy. In the case of Type I diabetes, early diagnosis is difficult because the clinical signs of glucose intolerance (hyperglycemia) occur late, but it is feasible based on genetic (HLA) and immunologic markers (anti-islet and anti-insulin antibodies).

An active immune process is generally present although not always detectable in the absence of parameters to assess immunologic activity, notably in nephrotic syndrome or Crohn's disease.

The quality of the therapeutic effect of immunosuppression in autoimmunity varies according to disease. Spectacular results are obtained in a number of conditions such as RA, lupus nephritis or arthritic psoriasis, nephrotic syndrome and Type I diabetes. In the latter disease patients can stop insulin therapy while retaining good metabolic control, at least as good as previously [4–6]. This effect is linked to the slowing of the autoimmune reaction, but of course the treatment cannot generate more insulin than the preexisting lesions permit. In the case of diabetes the preexisting atrophy of β cells does not allow complete normalization of glucose tolerance (glucose overload still leads to abnormal hyperglycemia [14]). In the case of psoriasis, extensive plaques regress rapidly and totally.

The question remains of the long-term benefit of such striking clinical improvements. One may predict that long-term normalization of glycemia with possible insulin response to glucose stimuli provides better protection from degenerative complications than does fixed-dose insulin therapy in patients without endogenous hormone secretion. This prediction is supported by the lower incidence of such complications in patients with strict glycemic control than in patients with poor control [15] but the formal demonstration of the protective effect mediated by close to normal glycemia is still lacking. The same comment applies to nephrotic syndrome which is often a long-lasting disease where the therapeutic strategy must not only concentrate on the short-term issue but also take into consideration the long-term evolution.

Duration of immunotherapy-induced remissions

Most immunosuppressive agents lose their therapeutic effect when treatment is stopped. This is not really surprising since immunosuppression does not cure the cause of the disease but only limits its development without suppressing the autoantigenic stimulus or the immunodysregulation which underlies the abnormal autoimmune response. Thus in all conditions where cyclosporine has been shown to be efficient, relapses occurred when the drug was stopped, notably in psoriasis [1], RA [16], uveitis [17] and Type I diabetes [18].

Interestingly, the relapses often occur very rapidly (2–4 weeks) after stopping treatment, suggesting that the sensitization (or immunologic memory) had not been erased but rather than cyclosporine had essentially frozen the immune system.

In some unfortunate cases, relapses may occur during treatment. If this occurs when the drug is tapered, one may assume that the dosage has passed below the threshold of effect. If the relapse occurs at unchanged dosage one must look at other hypotheses. The clinical symptoms may be secondary to a burst of autoimmunity. Alternatively, the autoimmune process can still be under control while the lesions progress autonomously after the initial autoimmune attack. This is apparently the case for diabetes relapses which may indeed be seen during cyclosporine therapy. These relapses are not associated with a loss of endogenous insulin production since C peptide levels remain unchanged [18]. In this particular setting, relapse is then more probably due to the appearance of insulin resistance (secondary to chronic insulinopenia) or to an increase in insulin needs (whatever the cause, for example body growth in children).

A therapeutic effect in a chronic disease must persist long enough to justify the drug-associated risks that of course may increase with long-term drug administration.

Fortunately autoimmune diseases are not necessarily chronic and ineluctably worsening. There are several examples of self-limiting autoimmune diseases. This is clearly the case for rheumatic fever and for anti-heart autoimmune disease provoked by sensitization against group A streptococcal antigens that cross-react with heart antigens. It is also the case in some patients with SLE and membranous nephropathies where disease evolutivity may suddenly decrease after several years [19, 20].

In fact, even in the case of Type I diabetes where one does not know the natural history of the underlying autoimmune disease, it cannot be excluded that the evolution could be self-limited in some cases. The anti-islet autoimmune reaction starts several years before the onset of clinical symptoms [21, 22] and it is not known what would be the spontaneous outcome if β-cell destruction were prevented (and thus β-cell autoantigen would still be present). Additionally, little is known about the natural history of the T-cell mediated response which is probably more implicated in the generation of β-cell lesions than the humoral response [23]. In any case one cannot exclude the fact that in some patients the autoimmune anti-islet aggression would stop after some years, making it possible to stop immunotherapy without risking a relapse. The observation of long-term remission of the disease after short-term cyclosporine treatment [24] argues in favor of this hypothesis, even if in other cases the autoimmune reactivity persists for many years, as illustrated by the recurrence of diabetes following a pancreas transplantation performed 10–20 years after the initial onset of the disease [25].

Resistance to immunosuppression

Similar arguments may be envisioned to explain the often relatively high percentage of cases of clinical resistance to immunosuppression. In the case of Type I diabetes, one third of recent onset diabetics do not respond to cyclosporine. The most commonly accepted explanation is that too few β cells are left at the start of cyclosporine treatment; hence the idea of beginning the treatment as early as possible, eventually before insulin therapy, as successfully achieved in a few pediatric cases [26].

Alternatively, the autoimmune response is extremely intense, of secondary (hyperimmune) type and is basically insensitive to cyclosporine (and to most known immunosuppressive methods) [27]. The observation reported by Lafferty that the

anti-islet cell autoimmune response evaluated in an islet allograft model is more sensitive to cyclosporine in young than in old NOD mice [28] argues in this direction.

A last hypothesis is that in some patients, the disease is not immunological in mature. This possibility could apply to a minority of Type I diabetic patients (2–5%) who are neither HLA DR3 nor DR4, show a low incidence of anti-islet antibodies and are clinically resistant to cyclosporine [29].

Perspectives

All the arguments developed above should prompt clinicians to start immunosuppressive therapy in autoimmune disease as early as possible with the double hope of facing a less acute autoimmune reaction (allowing use of smaller doses) and less advanced lesions (more amenable to clinical regression). In many cases early treatment would represent a form of prevention. In diabetes this would indeed be the case if one could select patients at risk long before they show clinically overt glucose intolerance [22]. This is feasible by use of genetic markers (members of diabetic family, diabetes predisposing HLA antigens). The immunological markers (anti-islet and anti-insulin antibodies, activated T cells) focus on patients starting the autoimmune anti-islet cell disease. Metabolic studies (for example decreased early insulin secretion following glucose overload) confirm the onset of infraclinical glucose intolerance. If applied sufficiently early, prevention of the disease can really be envisioned.

The only reservation, already mentioned, is to use aggressive drugs in apparently clinically healthy patients who would probably remain so for several years, if not their whole life span in some cases. This possibility stresses the need for non-toxic but still efficient agents. One may reasonably hope that the active ongoing research on immunomodulators, anti-T cell and anti-Class II HLA monoclonal antibodies or more putatively autoantigen or autoantibody (idiotype) specific manipulation should provide some useful tools in a not too distant future.

Risks

Risks associated with immunosuppressive agents vary considerably with the agent used and the dose and duration of treatment. One should distinguish short- and long-term risks which are essentially different in nature.

Short-term risks (direct drug toxicity)

Short-terms risks are essentially those linked to direct toxicity of the drug. Progress made in the daily use of available immunosuppressors has limited the early risk of infection, which used to be seen with high dose steroids. This applies to cyclosporine which does not promote infection at the moderate doses used in autoimmune disease: we have not seen any opportunistic infections in more than 400 diabetics treated with 5–7 mg/kg/d of cyclosporine continuously for periods up to 18 months [5, 18]. This observation, which considerably facilitates the immunosuppressive approach to autoimmune disease, is not well explained. In fact it is surprising that doses that are immunosuppressive, as assessed by the clinical effect observed in several conditions,

do not alter anti-infectious immune defense. One may assume that such defense essentially involves phagocytes or non-specific immune responses which are hardly affected by cyclosporine, or that the dosage used only provides partial immunosuppression sufficient to mitigate autoimmune reactions but is unable to abrogate all the elements of the anti-infectious defense.

Other than infections the only early side effects of concern are those linked to direct drug toxicity. The wide clinical experience in the careful use of moderate doses of immunosuppressants with or without low-dose steroids has shown that complications were confined to a limited number of clinically significant situations: nephrotoxicity for cyclosporine; sterility for cyclophosphamide; bone marrow hypoplasia for azathioprine; initial acute systemic reaction for OKT3.

Acute nephrotoxicity is commonly observed after a few weeks of cyclosporine administration even at moderate dosage. This toxicity is usually reversible but may also become chronic and partially irreversible. We have systematically studied the factors predisposing to this nephrotoxicity in a large series of diabetic patients treated with cyclosporine alone. The toxicity is only seen at doses >5 mg/kg/d for treatment periods over 4–6 months [30]. When doses remain between 5 to 7.5 mg/kg/d, and treatment is given for periods of 6–12 months or eventually longer, a mild or exceptionally moderate renal toxicity may be observed namely, fibrosis and vascular lesions. It is accepted that fibrosis is a definitive lesion. It is not known, however, if such lesions will evolve or represent a potential risk for the future, particularly if diabetes progresses and is complicated by the onset of nephropathy. This uncertainty represents one of the major unsolved questions of the potential hazards associated with cyclosporine.

As far as monitoring is concerned it is difficult to predict which patients will effectively develop chronic irreversible nephrotoxicity. The cyclosporine dosage is surprisingly not correlated with the onset of toxicity, which is common in patients having received doses over 10 mg/kg/d, but no clear correlation appears among patients receiving doses <8 mg/kg/d. Similarly, long-term very high blood levels have an unfavorable effect but the few patients showing the most obvious toxicity have not necessarily presented unusually high blood levels (>600 mg/ml total trough blood levels). In fact in our experience the best predicting factor is the occurrence of sustained renal failure in the first months of treatment, hence the rule of decreasing the drug dosage when creatininemia increases, irrespective of cyclosporinemia levels [30, 31].

One promising fact is that when the dosage is maintained below 5 mg/kg/d in adults (as can be done efficiently in psoriasis) or below 6 mg/kg/d in children (apparently less sensitive to nephrotoxicity than adults), no toxicity is seen. At slightly higher doses (5–7 mg/kg/d), both in children and adults, nephrotoxicity remains minimal and can be limited by careful monitoring.

Sterility is a common complication of alkylating agents. It appears when cyclophosphamide dosage is over 3 mg/kg/d. It is particularly but not exclusively encountered in children, but it does represent a major risk.

The acute systemic reaction secondary to the initial injection of anti-CD3 monoclonal antibodies has limited the use of OKT3 in autoimmune diseases. Preliminary trials in multiple sclerosis and diabetes have been stopped because of the severity of the reactions observed, greater than that seen in transplant patients. Recent data

obtained in our laboratory have indicated that this reaction was probably linked to the massive release of various cytokines, notably TNFα and IFNγ [32]. One may hope that specific anti-cytokine antibodies will help to prevent this reaction. It should be noted that this acute reaction has not been observed with other monoclonal antibodies, but these other antibodies have not been shown convincingly to be immunosuppressive in autoimmune diseases, if one excepts the preliminary data recently reported in RA with an anti-CD4 antibody [33].

Long-term side effects (malignancies)

The chronic use of immunosuppressive agents essentially exposes to the risk of long-term suppression of immunity. It is rare for individual drug toxicity to be revealed after a very long period of time. Such deleterious effects of long-term immunosuppression essentially comprise infections and tumors.

Infections are rarely observed in the long-term follow-up of immunosuppressed patients not receiving high doses of corticosteroids. One cannot exclude, however, the risk of viral complications when using drugs like cyclosporine or anti-CD3 antibodies which act on T cells. EBV and CMV are particularly feared but it should be emphasized that no infection by any such viruses has been reported so far in patients treated with cyclosporine at reasonable doses. Nor have we observed seroconversion for EBV in diabetics treated with cyclosporine for more than 12 months [34].

The problem of *tumors* is more worrisome. Few studies are available on malignancies which occurred in patients with autoimmune diseases treated with various immunosuppressive agents [35]. There are, however, data incriminating azathioprine in epithelial carcinoma occurring in multiple sclerosis [36], alkylating agents for leukemia in RA [37], cyclosporine for lymphomas in two cases of autoimmune disease. The risk is small (probably $<0.1\%$) but not negligible [38]. It should be stated, however, that based on experience gained in organ transplantation, tumors appear to occur when several drugs are used in combination and the risk of neoplasia closely depends on the nature of agent used, the dosage and the duration of the treatment.

Conclusions

All arguments presented show that immunosuppressive agents can now provide extremely profound immunosuppression which is susceptible to improving clinically a wide and increasing variety of autoimmune diseases. The new agents available are very potent and show minimal direct toxicity; the problem of cyclosporine-induced nephrotoxicity is only significant at relatively high doses. The risk of neoplasia is potentially more troublesome for very long-term treatments. At any rate, it is fair to conclude that the new immunosuppressive drugs can achieve major immunosuppression with minimal risk.

Drug resistance is still observed. The best approach to overcome it is probably to treat patients at an earlier stage of the disease, but the risks are then more difficult to accept in the absence of life-threatening prognosis. Therein lies the real paradox of immunotherapy. In practice one has to weigh in each disease and even more precisely

in each individual case: the consequences of the spontaneous outcome of the disease (under conventional treatment) and the risks associated with the drug to be selected in the conditions of treatment (dosage, duration, drug combination).

Present efforts aim at improving the assessment of these two parameters: (1) by a better understanding of disease pathogenesis; (2) by cautious selection of drug dosage and duration of treatment; (3) by careful monitoring of drug pharmacokinetics and (4) by a systematic survey of complications (early detection of viral infections and of tumors before the point of no return, eventually helped by assessment of immunoglobulin isoelectrofocusing as performed in our group in diabetic patients [34]).

When sufficient progress has been achieved, one may probably be able to treat patients at an earlier stage of the disease which in most cases, notably in Type I diabetes, will lead to increased incidence of response and improvement of long-term effects which in turn will justify taking more risks. In other words one must progressively move out of the vicious circle which prompts clinicians to treat preferentially advanced cases who need high drug dosage and whose clinical response in any case can only be partial. Treating earlier cases who would probably need lower dosage and show a more clearcut benefit for a smaller risk.

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