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The T cell as a therapeutic target

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I. INTRODUCTION

There is a constellation of diseases whose pathology is a direct consequence of the immune response. Since there has been a failure to identify either an infectious aetiology or a pathognomonic immune abnormality, these diseases are characterized as being autoimmune. Rheumatoid arthritis, systemic lupus erythematosus, myasthenia gravis and multiple sclerosis are all well-known examples. An important therapeutic approach to these diseases is the selective inhibition of immune activity.

The pathological manifestations of the immune response can be inhibited in a variety of ways. Single key mechanisms can be targeted with therapeutically important results so long as that mechanism plays an important role in the disease. Thus inhibition of a single pathway such as prostaglandin biosynthesis has been found to be of great utility in ameliorating pain and inflammation of arthritis even though it leaves multiple aspects of arthritic disease untreated. The utility of interfering with other mechanisms of tissue damage such as cell infiltration, enzyme release, antibody formation, immune complex deposition, etc., or other mediators such as interleukin 1 (IL-1), superoxide, leukotrienes, tumour necrosis factor or γ-interferon, needs to be determined. In contrast, the T cell plays a central role in the overall orchestration of the immune response. It provides factors which are necessary for its full development, for its targeting and regulation. Its central role makes it a suitable therapeutic target for control of diseases characterized by multiple immune mechanisms.

The central role of the T cell in autoimmunity has been strongly supported by studies in animal models. For example, it has been shown that autoimmune diseases can be established in normal animals by the transfer of T cells from diseased animals. This means, first, that T cells must carry the molecular information which determines disease occurrence; second, T cells must have the ability to recruit sufficient effector cells to cause tissue damage; and, finally, they must have sufficient intrinsic replicative capacity to sus-

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tain chronic disease. Thus T cell-targeted therapy should be useful for the treatment of chronic autoimmune disease.

II. AN OVERVIEW OF THE T CELL

The T cell is composed of lymphocytes characterized by the expression of the T cell antigen receptor-CD3 complex on their surface[1]. The T cell antigen receptor (TcAR) is composed of a disulphide-linked 90 kD heterodimer in which there is an α and a β chain. The TcAR is a member of the immunoglobulin supergene family and confers specificity to the T cell in its reactions. After the TcAR reacts with antigen, the CD3 complex is believed to transmit a signal to the cell interior. A second TcAR has also been identified and designated as a γ, δ heterodimer[2,3]. Both TcAR and CD3 must be present to have a functional T cell.

The T cell is not a single, uniform, functionally homogeneous cell. The identification and characterization of functionally distinct subsets of CD3 T cells has been made possible by monoclonal antibodies and by the production of T cell clones. Monoclonal antibodies have allowed the identification and purification of T cells on the basis of cell surface antigens. T cell clones have enabled the study of the biological activities of a single homogeneous T cell phenotype. These two techniques have led to the subdivision of T cells into two major classes called CD4 and CD8 (formerly T4 and T8) in human cells and L3T4 and Lyt 2 in murine T cells respectively. CD4 T cells are characterized by a requirement to interact with antigen in the context of major histocompatibility complex (MHC) type II antigens, i.e. the immune response gene products such as DR, DP and DQ in man, Ia and Ie in the mouse, often generally referred to as IA. It is important to note that many autoimmune diseases show genetic linkage to the immune response genes. Since immunogenic peptides which induce T cell-mediated immunity have been shown to bind to the immune response gene products[4,5], it has been speculated that the genetic linkage of disease with the immune response genes comes through their antigen presentation properties[6,7]. CD8 T cells, by contrast, react with antigen in context with MHC class I gene products, i.e. the classical HLA transplantation antigens in man and the H-2 transplantation antigens in the mouse. It is believed that both CD4 and CD8 molecules augment T cell avidity for antigens by binding to the respective antigen binding class II or I MHC molecules[8].

The major functional activity of CD4 T cells is the synthesis of factors to help B cells to produce antibodies (i.e. IL-4 and IL-5) and to help T cells to expand, proliferate, and mature (i.e. IL-2, IL-4, IL-5) to carry out their functions. CD4 T cells also produce other important factors such as γ-interferon and lymphotoxin. Gamma-interferon, for example, may have a significant role in autoimmunity by inducing increased expression of IA molecules on other cell types. Increased IA increases the presentation of autoantigens to immune cells and may, thereby, increase the occurrence of autoimmune

disease[9,10]. Recently, it has been shown that CD4 cells can be further subdivided by function into two classes, TH1 and TH2[11,12]. The TH1 cells can be distinguished by the presence of the T200 antigen (CD45R) which is also designated as OX22 in rats[13] and as 2H4 in man[14]. The TH1 cells produce IL-2, γ-interferon and lymphotoxin, and they require IL-2 for autocrine growth. They are involved in delayed hypersensitivity and the transfer of autoimmune disease to naïve recipients. TH2 cells, by contrast, are involved in helper functions. Thus they synthesize IL-4 and IL-5, both of which are major helper factors for antibody production, and require both IL-1 and IL-4 for autocrine growth[15].

T cells bearing CD8 demonstrate two functional characteristics. First they have the ability to lyse target cells such as virus-infected cells and thus act as antigen-specific effector cells. The lytic activity of CD8 cells appears to require the synthesis of IL-4 to induce T cell activation for killing and synthesis of protease[16]. It is not clear whether the killing activity of T cells plays a major role in tissue damage in autoimmune disease, although it has been implicated in experimental autoimmunity. CD8 T cells also can act to down-regulate T cell activity and, when carrying out that function, have been called T suppressor cells. Suppressor cell regulation may play a significant role in preventing the occurrence of autoimmune disease.

While this brief listing of T cell activities is by no means comprehensive, it illustrates two major roles which the T cell may play in autoimmune disease: (i) they provide factors which recruit, activate and help other cell types to carry on immune activities in an antigen-specific fashion; and (ii) they regulate the overall immune activity in both an antigen-specific and non-specific manner.

(1) Evidence for a role of T cells in autoimmune disease

The importance of T cells in autoimmune disease has been established by three principal methodologies: the study of pathological tissues, the study of susceptibility of animals with defined genetic constitutions to autoimmune disease, and the examination of the conditions under which disease can be transferred from affected animals into normal animals. The study of tissues from affected patients or animals allows the identification and characterization of T cells in pathological lesions as has been done in rheumatoid arthritis[17-19]. In man, an apparent deficiency has been shown for the suppressor-inducer ($CD4^+$, $2H4^+$) T cell subset in multiple sclerosis[20], systemic lupus erythematosus[21], juvenile arthritis[22] and rheumatoid arthritis[23,24]. The reproducible diminution of this T cell subset in these autoimmune diseases is suggestive of pathological significance. Similarly in the diabetes-prone BB/W rat, the loss of the $RT6.1^+$ T cell is correlated with disease[25,26]. In collagen-induced arthritis in animals, the appearance of activated $CD4^+$ T cells is one of the earliest pathological changes[27-29]. In experimental allergic encephalomyelitis (EAE) brain lesions, 49% of

infiltrating cells are positive for the Lyt 1 marker and 9% for the Lyt 2 marker[30]. In murine models of systemic lupus, i.e. the NZB/W mouse, developmental abnormalities in the thymus and in T cell differentiation are found[31]. A role for T cells has also been deduced in streptococcal arthritis by the failure to induce chronic autoimmune disease in animals in which T cell reactivity is compromised either by the use of genetically T deficient animals[32,33] or by the use of cyclosporin A to inhibit T cell activity[34]. In other induced models of autoimmune disease, adjuvant arthritis for example, T cells cloned from diseased animals have been shown to transfer the disease to naive animals[35]. Those T cells have recently been shown to have antigenic cross-reactivity between a component of *Mycobacterium* and the proteoglycan core protein of the rat[36]. In collagen arthritis[37-39], experimental thyroiditis[40] and experimental allergic encephalomyelitis[41], T cells from an affected animal similarly transfer disease into normal animals. These T cells also bear antigen-specific reactivity to collagen, thyroglobulin or myelin basic protein, respectively. Moreover, T cell clones derived from diseased animals bearing specificity for the autoantigen can transfer disease to naïve recipients[42,43]. T cell transfer of disease also occurs in chemically induced autoimmunity[44]. Taken together, these data suggest that the T cell is a carrier of key information critical to the onset and perpetuation of autoimmune disease. Actual tissue damage is not necessarily T cell-mediated, but may be[45]; other cell types may be recruited and effector molecules of non-T cell origin may cause the pathological changes. Tissue damage by infectious insult[46,47] or antibody[39,48] may be required for full expression of disease.

(2) Effectiveness of T cell-targeted therapy: animal studies

Several approaches have been taken to demonstrate that suppression of T cell function can lead to amelioration of autoimmune disease. Perhaps the most direct way to explore T cell-targeted therapy is to alter the function or to specifically delete selected T cells. This has been done effectively in animals with the use of antibodies, toxins and drugs directed to various components of the T cell system. Although none of the therapeutic approaches can unambiguously be shown to act on T cells without affecting other additional immune pathways, taken together they form an impressive body of evidence suggesting T cell therapy may be of benefit in autoimmune disease.

(a) Anti-T cell therapy

Therapy directed towards the removal and suppression of all T cell function is effective in many autoimmune conditions. Such broadly based therapy also inhibits essentially all facets of the immune response critical for a successful response to an infectious insult. Rat anti-Thy 1.2 has been successfully used to treat spontaneous murine lupus in the MRL/lpr mouse[49]. While it was suc-

cessful in the MRL/lpr mouse, it was without beneficial effect in the NZB/W mouse, where the development of antibody against the rat anti-Thy 1.2 antibody may have augmented the nephritis by increasing deposition of immune complexes in the kidney. In BXSB mice at 3 months of age, a single injection of anti-Thy 1.2 retarded many signs of autoimmunity such as anti-DNA antibody, but failed to prolong life[50]. Furthermore, continuous treatment with the rat anti-Thy 1.2 antibody led to fatal anaphylaxis. The presence of anaphylactic reactions and immune complex disease suggests limits on the continuous administration of heterologous antibodies, but does not limit use for one time for amelioration of acute immune crises. However, in animals with spontaneous autoimmune disease such as the NZB/W mouse where T cell function may be already seriously compromised pan-T cell therapy may not be as therapeutically useful as a selective T cell therapy targeting a specific functional imbalance. In this case, more general non-specific immunosuppressives such as cyclophosphamide may be advantageous since they block B cell function as well.

(b) Anti-CD4 therapy

Treatment of mice with anti-CD4 antibody (Gk1.5 anti-L3T4 antibody) prior to immunization with autoantigen leads to the inhibition of disease in collagen arthritis[51], EAE[51-54] experimental myasthenia gravis[55], diabetes in the non-obese mouse (NOD)[56] and NZB/W lupus nephritis[57]. If anti-T cell therapy can be administered before the onset of disease, it can be effective in suppressing the autoimmune response with a short course of therapy. This is, of course, a general immunosuppressive regimen and demonstrates that $CD4^+$ T cells are required for a full immune response. As $CD8^+$ T suppressor cells are not affected, it is possible that antigenic stimulation in the presence of anti-CD4 antibody might shift the CD4/CD8 balance to a more tolerogenic signal. Unlike anti-IL-2R antibody (see below), anti-CD4 antibody does not appear to require the lytic activity of complement in order to be effective, since, for example, it still prevents the influx of T cells into delayed hypersensitivity lesions in C5-deficient mice. The CD4 molecule enhances the efficiency of antigen presentation with IA bearing cells and thus plays an important role in antigen recognition[12]. Further it has also been shown that in systems which bypass the CD4 requirement for binding to antigen presenting cells, anti-CD4 antibodies still suppress the induction of a proliferative response[58,59] suggesting a further possible action of these antibodies. Alternatively, FcR or C3b interaction directed by anti-L3T4 antibody may be sufficient to inhibit T cell function. Thus it is an open question if, in the NZB/W mouse where anti-T cell therapy is less effective, anti-L3T4 acts because it suppresses L3T4 cell function, or whether, by suppression of L3T4 function, it allows the activity of $Lyt\ 2^+$ cells to be dominant.

The effectiveness of this therapy depends, in fact, upon the isotype of anti-CD4 antibody used[60]. The fluorescence-activated cell sorter was used to select variant anti-CD4 (W3/25) isotypes IgG1, IgG2b, and IgG2a all from the same clone, and all therefore with apparently identical binding specificities for CD4. Both IgG1 and IgG2a were superior to IgG2b antibodies in preventing EAE. They found it was sufficient simply to bind the antibodies to the CD4 receptor; frank depletion of CD4 cells was not necessary for a therapeutic effect[61]. This is consistent with work using anti-CD4 (OX35) antibody Fab fragments in EAE of the rat[62] and using anti-L3T4 Fab fragments in the mouse to induce long-term tolerance[63]. This suggests that the mechanism of anti-L3T4 is most consistent with interference with antigen presentation. That function alone is effectively carried out by Fab anti-L3T4 antibodies.

The use of anti-L3T4 antibodies appears to circumvent one of the major problems of using monoclonal antibodies. Whereas anti-Thy 1.2 therapy elicited a strong immune response with anaphylaxis and death in the BXSB strain, this did not occur with anti L3T4. The strength of the suppressive and tolerogenic signal given by anti-L3T4 antibodies may prevent an immune response to the antibody. Finally, use of this reagent suggests that even if immune pathways other than T cells play a major role in autoimmune disease, those pathways must be directly dependent upon the CD4 T cell for expression of their activity.

(c) Anti-CD8 therapy

Although the evidence presented above on CD4 therapy suggests its general usefulness, some reservations must be stated based upon the nature of the disease being treated. For example, if viral infection may be important for the continuation of the disease or viral infection may be a side-effect of therapy as in transplantation (see below under anti-CD3), the removal of CD4 cells may exacerbate disease. Thus, in Theiler's virus-induced demyelination[64], treatment with anti-L3T4 around the time of viral inoculation leads to increased demyelination, encephalitis and death in the majority of animals tested. No effect was noted on established disease. By contrast, treatment with anti-Lyt 2.2 (CD8) antibody led to decreased demyelinating lesions irrespective of whether the therapy was administered early or after the disease was established, suggesting that the CD8 T cell was in some manner directly involved in demyelination. While these results are not applicable to all autoimmune diseases, they do emphasize that autoimmune diseases should not be put into a single category for therapeutic treatment, and that caution must be used in extrapolating from one autoimmune disease to another.

(d) Anti-IA therapy

Antigens must be presented to T cells in the context of IA in order for them to respond. Moreover, in many autoimmune diseases, disease susceptibility is genetically linked to the IA phenotype. Therefore blocking or removal of the specific IA antigen by use of anti-IA antibody might be a method for selective immunosuppression and therefore a candidate for blocking autoimmune disease. In EAE, where disease induction is regulated by the H-2^s gene, the use of specific anti-IA^s antibody prior to immunization with myelin basic protein inhibits onset of clinical disease[65]. Although clinical disease was inhibited, the induction of disease as measured histologically was not prevented, although its severity was much less. Thus T cells were found to have been autoimmunized to myelin basic protein (MBP), and the T cells were shown histologically to have gained entry to the CNS. Moreover, when mice were treated with anti-IA antibody on the first signs of acute paralysis, a dramatic reversal of paralytic signs occurred, sometimes in as short a time as a few hours[66]. These results are not consistent with a primary effect on the induction of the immune response. They suggest an alternative mechanism such as inhibition of endothelial expression of IA with inhibition of cell migration or induction of suppressor T cell activity[67]. Administration of anti-IA^s antibody prior to administration of ^{51}Cr-labelled MBP-primed T cells clearly diminished the migration of the T cells into the CNS tissue[68]. Anti-IA therapy was also effective in blocking chronic relapsing EAE in mice. Weekly therapy with the antibody started after the first attack of paralysis diminished the number of attacks of paralysis, and eliminated the mortality over a 4 1/2 month treatment period. Similarly in F_1 animals (H-2^sxH-2^d) where disease is dominantly linked to the H-2^s gene, only H-2^s antibody effectively protects against disease induced by passive transfer of T cells. However, antibody against the weaker disease linkage haplotype is also effective in preventing disease induction upon immunization with myelin basic protein[69]. In a spontaneous model of lupus, the NZB/W mouse, treatment of the mice with anti-IA^z was associated with protection from renal disease. That anti-IA^d was effective, but much less so, suggested perhaps a tighter linkage of IA^z with disease[70]. In both of these F_1 cases, no absolute linkage of one haplotype with disease was found. In experimental models such as in the response to (HG)-A-L where a non-responder was crossed with a responder to form a F_1 hybrid, complete haplotype-specific suppression by anti-IA was found[71]. This should be the case in which disease is tightly IA-linked. If immunization was carried out in complete Freund's adjuvant, haplotype-specific suppression in this system was lost. Anti-IA therapy was also found to be effective in autoimmune thyroiditis[72], collagen arthritis[73] and acetylcholine receptor disease[74] in animals. These results suggest that haplotype-specific suppression of autoimmunity is possible if there is a close disease linkage, but it may be more anti-inflammatory than immunosuppressive in its effects. These results also suggest that anti-IA therapy with antibody is not

fully effective in preventing immunization with antigen, perhaps because, unlike anti-CD4 therapy, there is no tolerogenic signal or direct suppression of T cell function. Thus any lack of complete suppression of the response to antigen response or escape of IA from blockage finds the immune system fully capable of responding to antigen.

(e) T cell line therapy

Antigen-specific T cell lines have been developed which will transfer autoimmune disease to naïve recipients. Thus, as noted above, T cell lines against type II collagen wil transfer collagen arthritis, *Mycobacterium*-specific T cell lines will transfer adjuvant arthritis, thyroglobulin-specific T cell lines will transfer thyroiditis, and myelin basic protein-specific T cell lines will transfer EAE. These T cell lines can be altered by treatment to render them able to tolerize against disease induction. Thus, after irradiation, a myelin basic protein reactive T cell line which normally transfers disease can be administered to naïve animals, and they are rendered tolerant to disease induction by subsequent administration of myelin basic protein in adjuvant[75]. Using cyclosporin A, suppressor cell lines could be developed from recovered EAE rats. These suppressor lines ($CD4^+$) could also be used to protect against EAE in naïve animals[76]. Similar results have been obtained for adjuvant arthritis[35], thyroiditis[77] and collagen arthritis[78,79]. In adjuvant arthritis, high-pressure treatment has been used to alter T cell lines which normally transmit adjuvant arthritis into T cell lines able to vaccinate against the induction of adjuvant arthritis after exposure to complete Freund's adjuvant. The tolerance mechanism is not immediately clear, but studies of tolerance induced by administration of neuraminidase-treated allogeneic cells suggest the procedure may lead to the elimination or functional compromise of responding T cell clones[80]. Perhaps not surprisingly, tolerance is elicited only to antigen exposure. These T cell lines do not tolerize to the administration of other disease-inducing T cell lines of the same specificity.

The applicability of therapy based upon vaccination with T cell lines appears problematical at this time. First, T cell vaccination appears most effective when given before disease onset. Therefore the likely place to target therapy would be early treatment of genetically susceptible individuals. Even if such a population could be defined with assurance, the next step, producing appropriate T cell clones, would need to be undertaken.

(f) Antibody against the T cell antigen receptor (TcAR)

Anti-idiotypic antibody therapy has been undertaken in a number of experimental autoimmune diseases to block production of a dominant autoimmune antibody. This has been attempted, for example, against the anti-DNA antibody of NZB/W lupus[81], the anti-myelin basic protein antibody of

EAE[82], and the anti-acetylcholine receptor antibody of experimental autoimmune myasthenia gravis[83]. Although favourable results have been obtained, the overall success with passive anti-idiotypic therapy has been limited, perhaps because of the induction of additional pathogenic antibodies which express other idiotypes, or because the single idiotype may make only a small contribution to the total disease. Thus, for example, in NZB/W nephritis, treatment with antibody against the dominant idiotype of anti-double-stranded DNA (dsDNA) antibody suppressed the dominant dsDNA idiotype. That idiotype was, however, quickly replaced by another idiotype. Nonetheless, therapeutic effects have been obtained in cases where the primary idiotype has been used as an immunogen[84]. These results suggest that a limited autoantibody repertoire should exist before anti-idiotypic antibody can be fully effective. Moreover, even in cases where there is extremely strong evidence for a primary antibody mediation of disease, i.e. in experimental autoimmune myasthenia gravis (anti-acetylcholine receptor disease), there is good evidence that T cell involvement is required in disease because of both the IA linkage[85] and because of the demonstrated requirement for T cell help in antibody formation[86,87]. This raises the possibility that anti-TcAR idiotype antibodies might be a better approach to modifying autoimmune disease: first, because the TcAR appears to have a much more limited repertoire than antibody; second, because it appears to be the T cell which contributes to the chronicity of the disease; and third, because the possibility that manipulation of the TcAR-stimulated T cell will result in a tolerogenic signal.

Some evidence has been elicited which suggests that such an approach may have validity. It has been observed that immunization using T cell lines reactive with autoimmune antigen could protect against subsequent disease upon exposure to specific antigen. This suggests direct use of the TcAR in the protection against autoimmunity. Use of anti-idiotype antibodies against the TcAR might give similar results. For example, immunization of Brown Norway rats with syngeneic T lymphoblasts reactive with renal tubular antigen led to an anti-Id antibody (TB-anti-Id)[88] which was competitively cross-reactive with anti-idiotype antibody prepared against renal eluate antibody (RE-anti-Id). The RE-anti-Id could also react with the tubular antigen-specific lymphoblasts[89]. Both antibodies were also inhibited by tubular antigen, suggesting that the binding was to regions of the antigen-binding variable region of both the anti-tubular antigen antibody and the TcAR. Both antibodies gave partial inhibition of renal tubular disease. Since, in this disease, Lyt 2 cells cause renal injury, inhibition of disease is likely to be due in part to inhibition of nephrogenic Lyt 2 cells via the TcAR.

As greater evidence of T cell antigen receptor specificity and structure is developed, and evidence is produced of limited variable region repertoires in diseases such as arthritis[90], anti-idiotypic therapy directed against the TcAR may become a new therapeutic possibility.

(g) Factors to shift Ts/Th activity balance

To the extent that T suppressor cells regulate the expression of autoimmunity, and T helper cells facilitate the expression of autoimmune disease, factors which shift the balance from T help to T suppressor activity may be useful in disease treatment. Anti-L3T4 (see above) and cyclosporin A (see below) may help shift the helper/suppressor balance in favour of suppressor cells. We have also found a factor in conditioned media which will increase the relative proportion of Lyt 2^+ T cells in peripheral blood and spleen of mice (Laux, D, Otterness, I *et al.*, unpublished). Whether a factor that increases CD8 levels would be of value for controlling autoimmune disease needs to be tested.

(h) Inhibition of T cell homing

T lymphocytes have recognition structures for specific homing receptors that regulate their migration from the circulation and lymph into lymphoid organs. For example, Mel-14 is a monoclonal antibody which recognizes a T cell homing structure for lymph node high endothelial venues (HEV) and, when bound to the T cell surface, can block its adherence and entrance into the lymph node[91]. Submitogenic activation of lymphocytes increases the expression of Mel-14[92] and thus the circulation through the lymph node. Mel-14 has been used to prevent lymphadenopathy in MRL/lpr mice. Although Mel-14 prevents homing into the lymph nodes, the aberrant cells accumulate in the spleen instead[93]. These data clearly show the potential to change lymphocyte trafficking and localization patterns. If, as has been suggested, there are specific homing receptors in areas of inflammation, inhibition of such homing receptor activity should keep T cells from circulating through the inflamed area and thereby prevent their continuous recruitment. This is an important area for further research.

(i) Anti-IL-2 receptor therapy

Resting populations of T cells express little IL-2 receptor (IL-2R) when it is measured by antibody against the low-affinity receptor (p55, also called TAC in man). In cells recently stimulated to proliferate, IL-2R expression is significantly enhanced. Thus targeting removal of IL-2R bearing T cells offers a mechanism for selectively deleting activated T cells. Presumably, during active disease most of the activated T cells would be disease-related. Thus normal, non-activated T cells would be left intact and able to be later recruited to other functions. Administration of monoclonal anti-IL-2R antibody M7/20 was shown to suppress insulitis in autoimmune non-obese spontaneous diabetic (NOD) mice[94]. In NZB/W lupus mice, the anti-IL-2R antibody protected from renal injury as measured by a decreased incidence of pathologic proteinurea. Moreover, deposition of both gp70 and immunoglobulin com-

plexes in the kidney was inhibited. Studies of the mechanism of inhibition suggest that both complement and inhibition of IL-2 binding to the receptor appeared to be required. Anti-IL-2R antibody was ineffective in blocking delayed hypersensitivity in C5-deficient strains of mice, as was a second monoclonal anti-IL-2R antibody (7D4) which failed to block IL-2 binding[95]. Interestingly, *in vitro* 7D4 blocks T cell proliferation by preventing IL-2R internalization[96].

IL-2 itself has been made into a chimeric fusion protein with toxin[97]. Similar substances have been made by chemical linking of toxin with antibodies[98]. As such it has been shown to block delayed hypersensitivity reactions[99]. Presumably it will also be effective in treatment of autoimmune disease. It is targeted, just as is anti-IL-2R antibody, by its binding to the IL-2R, whereupon it can destroy the activated T cells.

In a comparison made at the time of immunization, anti-IL-2R antibodies were as effective as anti-L3T4 antibodies in abrogating delayed hypersensitivity[100]. This suggests that the use of IL-2R antibodies possesses a large advantage in that it eliminates only the responding T cell clones and leaves T cells of other specificities intact and fully capable of responding to infectious insult.

(j) Inhibition of MHC class II antigen expression

The linkage of the majority of the autoimmune diseases with a particular MHC antigen suggests that the presentation of antigen by IA to T cells is an integral part of the disease[101]. γ-Interferon plays a major role in enhancing the expression of IA[102] in the mouse and DR[103] in man. This facilitates the presentation of antigens to T cells. For example, presentation of myelin basic protein by astrocytes in EAE is γ-interferon-dependent[104,105]. In some autoimmune diseases, such as diabetes in the NOD mice[9], lupus in the NZB/W mouse[106], and experimental thyroiditis[107], it has been shown that disease induction is accelerated by γ-interferon. Moreover, in EAE recovery is associated with the suppression of γ-interferon production by T cells[10]. In NZB/W mice, treatment with γ-interferon exacerbates disease and treatment with anti-γ-interferon antibodies improves disease survival[106]. These data suggest that therapy targeted to removal of γ-interferon in order to decrease antigen presentation might be a useful therapy in man.

Contrary data do however exist. Thus γ-interferon has been shown to have a direct anti-inflammatory effect *in vivo* when applied systemically[108]. This appears also to be the case for EAE where treatment with γ-interferon improves survival, and treatment with anti-γ-interferon causes more severe disease[109]. Moreover, in cases where the autoimmune disease might have an infectious aetiology, γ-interferon might have a therapeutic benefit in its own right.

(3) Drug therapy, animal studies

The traditional method of treating disease has been by the use of drugs, that is, small organic chemicals targeted to specific mechanisms. Non-specific immunosuppressive agents exist that have little T cell selectivity[110], but to date, few drugs have been found that are specifically directed at the T cell. Cyclosporin A (CsA) is the best-known T cell-directed drug, and its primary use has been in human transplantation (see below). CP-17,193 was also found to be selective for the T cell limb of the immune response[111,112], and did not act through the cyclosporin binding protein cyclophilin (Handschumacher, unpublished). Unfortunately, in species other than the mouse, hepatoxicity was found to limit its utility[113]. FK-506 is a new immunosuppressive[114] and has been reported to be a T cell-selective agent.

(a) Cyclosporin A

Cyclosporin A (CsA) has been shown on the basis of *in vitro* studies to be a drug largely selective for the inhibition of T cell function. It inhibits the synthesis of the T cell lymphokines IL-2[115,116], IL-4[117,118], IL-5[117] and γ-interferon[119,120]. Although several authors have claimed that IL-2R expression on T cells is inhibited by CsA[121-123], other groups have reported that CsA does not prevent IL-2R expression[124-126]. This difference may be explained by data which show that low- but not high-affinity IL-2R expression on human lymphocytes is inhibited by CsA[127]. Numerous explanations have been marshalled to explain the inhibition of T cell activation by CsA. While it has been suggested that CsA interferes with the binding of Ca^{2+} by calmodulin[128], by displacement of prolactin from the T cell surface[129,130], by inhibiting an enzyme in the activation of ornithine decarboxylase[131] or by blocking of Ca^{2+} influx-dependent Na^+/H^+ exchange[132], the evidence presented for CsA acting through cyclophilin[133], a novel cyclosporin binding protein, appears the most compelling.

CsA has been shown to suppress or modulate both spontaneous and induced autoimmune disease in animal models in which T cells play an important role[134]. For example, CsA prevented lymphadenopathy and expansion of T cell subsets without altering autoantibody production in the MRL/1pr mouse[135]. Likewise in the NZB/W lupus mouse model, CsA inhibits the spontaneous renal disease, prevents immune complex deposition[136] but fails to reduce circulating immune complexes[137]. CsA also prevents the early rise of autoantibody titres and causes a fall in the high titres of autoantibody in old NZB/W mice[138]. It also prevents the spontaneous onset of diabetes in the BB rat[139].

Early treatment with CsA also prevents development of disease in several animal models of induced autoimmune disease. Adjuvant arthritis[140,141], streptococcal cell wall arthritis[142], experimental autoimmune uveitis[143], EAE[144,145], and collagen II arthritis[146,147] are all suppressed by

CsA treatment around the time of immunization. By contrast, if treatment is begun after the disease is established, the therapeutic effect may be minimal or, in the case of rat collagen arthritis, disease exacerbation has been reported.

These results suggest CsA is most effective in blocking *de novo* immunization, a finding that suggests CsA acts most efficiently at the time of antigenic triggering of lymphocytes[148]. In some systems it appears to be partially selective for the activation of helper/inducer T lymphocytes and may spare the suppressor T lymphocyte population[149,150].

(b) FK506

With the success of CsA, a fungal metabolite, in suppressing the development of immunity to organ grafts in man, fermentation broths have been screened as a source for better-tolerated immunosuppressant compounds for transplantation. A strain of *Streptomyces tsukubaensis* yielded FK506 as a novel immunosuppressant[114]. This substance, a neutral macrolide, demonstrated good immunosuppressive effects in *in vitro* immune systems. It suppressed the mixed lymphocyte reaction[114,151], T cell proliferation, generation of cytotoxic T cells, production of T cell-derived soluble mediators such as IL-2, IL-3 and γ-IFN, and IL-2 receptor expression[114]. The IC_{50} values of FK506 in these *in vitro* immune systems were approximately 100-fold lower than CsA.

The *in vivo* immunosuppressive properties of FK506 were shown in experimental transplantation. FK506 was found to prolong skin graft survival in rats[152], indefinitely prolong survival of cardiac allografts in rats[153], and prolong the life of canine kidney transplants[154].

FK506 has been tested in the rat collagen arthritis model and was shown to suppress arthritis, but only when administered during the induction phase of the disease[155]. Anti-type II collagen antibody formation and skin responses to type II collagen were also suppressed. The effect of FK506 in this model was similar to that of CsA, except that FK506 did not exacerbate the disease when started during the immediate preclinical phase.

As with CsA, FK506 apparently affects the early stage of T cell activation; data suggest that FK506 affects the biochemical actions post-Ti/T3 complex triggering[156]. Whether the immunosuppressive activity of FK506 will be of value for human transplantation or for treatment of autoimmune disease remains to be determined.

(4) Regulation of T cell function in the clinic

(a) Inhibition of T cell function

So far, the only major use of T cell-specific antibodies for disease therapy has been in transplantation. Suppression of T cell function is necessary for pro-

longed graft survival. Anti-thymocyte globulin was used as an immunosuppressive agent and as an acute suppressant during rejection crises[157,158]. Anti-thymocyte globulin has now been largely replaced by monoclonal anti-CD3 antibody for reversal of acute graft rejection crises[159]. It was found effective in reversing the rate of rejection, reducing the rate of retransplantation, and lowering patient mortality[160-162]. It could also be used for sparing of the use of cyclosporin in patients with poor renal function.

Treatment with anti-CD3 leads to the disappearance of detectable levels of T cells in the blood within 1-2 minutes of administration of as little as 1-2 mg of antibody. Virtually all patients experienced a febrile response. Whether this is due to a release of lymphokines triggered by the anti-CD3-CD3 interaction is not known. However, after a single 7-10-day course of antibodies an immune response develops to the idiotypic determinants on the antibody molecule[163,164]. Because of the spectre of serum sickness and the possibility of anaphylactic reaction, the administration of a second course of antibody is unattractive. Moreover, the immune response to the antibody would limit its effectiveness. However, anti-thymocyte globulin or a different isotype or idiotype anti-CD3 antibody can be used after the primary anti-CD3 treatment.

Both the effectiveness of graft prolongation and the number and severity of infectious episodes appear to be related to the number of circulating T cells and the CD4/CD8 ratios[159]. A high CD4/CD8 ratio is associated with a higher incidence of graft rejection. Conversely, viral complications are associated with a lower CD4/CD8 ratio including a possible virally-based renal dysfunction. Anti-CD4 therapy may also be effective in transplantation based upon studies in monkeys[165]. However, human studies, including autoimmune disease, are only now being carried out, and results are not yet published. The results with anti-CD3 antibodies suggest there is no bar to acute therapeutic use of monoclonal antibodies in human disease.

Other more general T cell depletion techniques, such as thoracic duct drainage[166], have been used in arthritis. Thoracic duct drainage was shown to be effective; however, the technique was not practical for more than experimental use. Similarly to anti-CD3 treatment in transplantation[167], as the T cells return in number after depletion by thoracic duct drainage[168], the CD8 cells come back first followed by the CD4 T cells. The observation that disease improved in thoracic duct T-depleted individuals suggests that the disease is at least partially T cell-dependent, and that the disease does not have an active viral component kept in check by T cells.

(b) Gamma-interferon

Gamma-interferon (γ-IFN) may find a role in the treatment of autoimmune disease if the right disease target and the right treatment regimens can be established. In a phase I study in rheumatoid arthritis (RA) patients, a favour-

able response was reported with low-dose γ-IFN[169]. In a 28-day study, γ-IFN was safe and well-tolerated with fever being the most prominent side-effect[170]. Significant improvement was noted on joint swelling and pain. However, it was not a double-blind placebo-controlled trial. In other open phase II clinical trials[171,172], relatively good short-term efficacy and toleration was indicated. Resting and motion pain, and articular pain, along with general mobility, were improved. Side-effects included fever which was the most prominent, and were reversible upon dosage reduction. A placebo-controlled, double-blind randomized phase III trial with 91 patients for 28 days showed a 30% reduction in the Ritchie or Lansbury index in responding patients, with a reduction in ESR[173]. Preliminary studies of 111 RA patients treated for 12 months suggest a favourable effect in some patients without untoward side-effects.

The mechanism of γ-IFN in arthritis is unclear. Epstein-Barr virus (EBV) has been implicated as a possible aetiological agent in RA[174]. In contrast to normal individuals, the proliferation of EBV-infected B cells from RA patients is not prevented by autologous T cells[175], in large part due to the failure of lymphokine production, including γ-IFN, by rheumatoid T cells. Alternatively, the effect of γ-IFN on IA expression may result in untoward consequences. In man, exacerbation of multiple sclerosis has been associated with γ-IFN therapy[176]. Increased IA expression caused by γ-IFN has been suggested as an explanation for disease exacerbation. Yet, increased IA expression also enhances cytotoxic responses to pathogens. This could be beneficial if a pathogen is involved in the aetiology of RA.

Gamma-IFN appears to be well-tolerated and relatively safe in rheumatoid arthritis. Whether the long-term effect is sufficiently robust to be meaningful remains to be determined by long-term studies and comparisons with other anti-arthritic agents.

(e) Established therapy

Cyclosporin A (CsA) has been shown to be a selective immunosuppressant agent that appears to act on T cells by inhibiting functional activation and clonal expansion. It influences the early phase of the immune response by blocking the production of IL-2 from T helper cells[116,125,126]. This explains the observation that CsA inhibits T cell-dependent B cell activation[177,178] and unprimed helper and cytotoxic T cell subsets[115,179]. The drug apparently achieves its suppressive effect by interfering with lymphocyte activation and consequently altering the balance of effector and regulatory cells in the earliest phase of the immune response. It is particularly effective in organ transplantation where it can be given prior to antigen exposure[180]. However, in autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus, where the immune response has been established prior to initiation of treatment, it may be less effective. There is, nonetheless, a range of auto-

immune diseases which have been reported to respond to CsA. Numerous examples exist of its therapeutic use in animal models, and these studies are being extended to man.

Successful treatment of experimental uveitis in rats[143] led to the examination of CsA in patients with Behçet's disease. This ocular disease has an immune complex component[181] and, in some patients, T cells demonstrate responsiveness to S-antigen[182], produce γ-interferon[183], and demonstrate abnormal suppressor cell activity and MLR responses[184]. CsA was effective in reducing or preventing ocular attacks in Behçet's disease.

CsA has also been used to treat type I-diabetes mellitus based upon studies in the BB rat[139]. This disease is associated with both humoral[185,186] and cell-mediated[187] autoimmune components. In a human study of 12 patients with type I diabetes of recent onset, CsA treatment produced remissions[188] or at least reduced insulin dosage[188,189].

There are other individual case reports of successful CsA treatment of other autoimmune diseases, i.e. Crohn's disease[190,191], ulcerative colitis[192], and bullous pemphigoid and pemphigus[193].

Systemic lupus erythematosus is characterized by kidney deposition of immune complexes from the circulation and by the formation of immune complexes by binding of antibody to fixed tissue antigen. By decreasing T cell help, antibody synthesis and thereby immune complex formation might be reduced. After treatment with CsA, some patients showed improvements in arthralgia[194,195], arthritis[195,196] and nephritis[195,196]; however, most experienced at least a transient nephrotoxicity with rises in serum creatinine. At a higher dose, more severe nephrotoxicity forced withdrawal of drug[194].

Rheumatoid arthritis (RA) shows evidence of both T and B cell involvement in the disease. Thus non-specific immunosuppressant drugs such as azathioprine, cyclophosphamide and methotrexate have been found therapeutically useful. Studies of CsA in animal models suggested that it might also be beneficial in treatment of RA. In a number of small studies, patients with definite or classic RA who were refractive to second-line drug therapy showed disease improvement as measured by pain, swollen joints, global assessment and Ritchie articular index[197-200]. No significant changes in T cell subsets were seen after CsA treatment[198]. This is not surprising, as immunosuppressants have been shown to change cell function without changing subset proportions[201]. In each of these studies, nephrotoxicity was a major adverse effect. Nephrotoxicity with CsA in renal transplant patients is largely reversible[202]. Renal function in CsA-treated patients was found to normalize within 2 months after drug withdrawal except for older patients (> 50 years) and those with RA[203]. It was calculated that CsA administration for not more than 6 months, and at a maximum dosage of 10 mg/kg for 2 months, leads to an irreversible loss of more than 10% of renal function in RA patients[204]. Although renal function parameters were found to gradually normalize, urinary beta$_2$-microglobulin, which is a marker of renal interstitial disease[205], was still increased after 9 months, indicating renal tubular dam-

age[206]. In patients treated only with NSAID, drug-induced renal disturbances[207-209] are generally reversible upon discontinuing NSAID therapy[208]. This suggests that it is the combination of CsA and NSAID which leads to the irreversible dysfunction[204]. The near-universal use of NSAID for the treatment of RA effectively precludes the general use of CsA in RA.

While CsA is apparently clinically effective in RA, its utility is limited by its side effects. The search for more specific and less toxic CsA derivatives continues. A derivative (Nva^2)-CsA, has very similar properties to CsA but lacks nephrotoxic and hypertensive side-effects[210]. Another, (Val^2)dihydro-CsA, has a different spectrum of activities; it does not suppress humoral immunity and allograft rejection as effectively but still suppresses some types of cell-mediated immune responses which may be involved in autoimmune disease. The clinical utility of these new analogues remains to be determined. Clearly, better compounds in terms of therapeutic ratio must be obtained if CsA-like activity is to become a generally useful treatment of autoimmune disease.

(5) Conclusions

The bulk of the data summarized herein is consistent with the hypothesis that T cells are required for the establishment and maintenance of autoimmune disease. This suggests that the T cell should be a primary therapeutic target. The T cell-specific pharmacological agents developed to date favour inhibition of T cells during the activation process. Thus, based largely on the work in autoimmune disease in animal models, it appears that the immunosuppressive activities of T cell subset-selective antibodies and drugs are less effective in reversing established autoimmune disease than they are in inhibiting autoimmune disease induction. This is understandable from the viewpoint that an established immune response consists of multiple mechanisms, only some of which are dependent on concurrent T cell function. Moreover, many effects may be maintained in memory cells and in immature precursor cells which can be called upon to fill a need if the function of mature effector cells is inhibited. Thus, while a single course of antibody therapy could be used to destroy certain classes of T cells, the broad utility of such therapy is limited by the host immune response to the heterologous antibody. Cyclosporin A acts to prevent T cell activation, but appears to lose much of its therapeutic effectiveness when T cells have already been primed. Thus cyclosporin A has found a useful therapeutic niche in transplantation, but only limited use in autoimmune disease.

By contrast, targeting therapy to the specifically-activated T cell population that is involved in the disease process may be effective in established autoimmune disease. Thus destruction of the small population of IL-2 receptor-bearing T cells by either anti-IL-2R therapy or by using an IL-2-toxin fusion protein appears able to remove acutely responding cells. This provides a method for selectively deleting the T cell circuit activated in disease. By

limiting therapy to times of disease exacerbation, deletion therapy would be carried out at times when the activated T cell population was enriched with disease-related T cells. Alternatively, if a limited T cell antigen receptor repertoire is shown, idiotypic therapy against TcAR would be expected to be effective because it would again eliminate the relevant T cell circuit while leaving the rest of the immune system intact. Here the difficulty lies in identifying the appropriate TcAR clones for deletion. With both of these approaches the problem of how to administer an antibody or immunotoxin fusion protein without eliciting a neutralizing antibody response or allergic reaction would have to be resolved. Methods of tolerization exist in animals, but they have not been adequately developed or tested in man.

Under these circumstances, drugs with new characteristics need to be developed for autoimmune disease. In particular, drugs that would convert an activation signal into a tolerogenic signal would be very useful. Additionally, a drug that is selectively toxic for activated T cells but leaves normal T cells intact would be a significant advance. Such a drug would have to be more selectively targeted than current agents which largely just block cell proliferation. Many of the activated T cells secrete factors, serve as non-dividing effector cells or as precursor cells that are not touched by such therapy. New methods and understanding would need to be developed to discover such agents. As our understanding of the T cell limb of the immune response has grown almost exponentially in the past few years, the many new insights into cellular activation and co-operation cannot but leave one optimistic that solutions will be found to the development of selective therapy for autoimmune disease.

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