

The momentum for harnessing tolerance processes is now unstoppable. At this stage, we really do not know how far we can go on the route to reprogramming the immune system. At some point, we will need to take stock and decide whether the patient's interests can best be served by maintaining them on a safe dose of drugs, or whether we aim for complete tolerance with the possibility that some stochastic event may disturb that process and so jeopardize the transplanted organ. Perhaps the compromise will be that of aiming for tolerance by weaning patients off their minimal drug regimens.

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36. H.W. and S.C. are founders and shareholders in Tolerrx, Inc. H.W. is nonexecutive director of the company and chairman of its Scientific Advisory Board. S.C. acts a consultant to the company.

#### REVIEW

# Immune Therapy for Autoimmune Diseases

Lawrence Steinman

Our increasing understanding of the pathophysiology of autoimmune disease has revealed a number of checkpoints that can be targeted with immune therapy, including key mediators of lymphocyte adhesion and migration, destructive cytokines involved in tissue damage, and the complex of molecules critical in the presentation of self-antigen and the activation of autoaggressive T lymphocytes. In many organ-specific autoimmune diseases, the identity of the molecules attacked by T cells and autoantibodies is known and attempts are under way to tolerize the immune system with a high level of specificity to these targets.

The immune system, normally efficient in defeating external threats from the microbial world, at times directs its potent arsenal against the body's self-constituents, causing autoimmunity. Collectively, autoimmune diseases affect about 5% of North Americans and Europeans, two-thirds of whom are women (1). The diseases often involve distinct anatomic regions. For example, the immune system attacks the synovial lining of the joints in rheumatoid arthritis (RA), the thyroid gland in thyroiditis, the insulin-secreting beta cells of the pancreas in type 1 diabetes mellitus (T1DM), and the myelin sheath of the brain and the spinal cord in multiple sclerosis (MS). In systemic lupus erythematosus (SLE), there are protean manifestations with involvement of skin, kidneys, joints, and brain.

Although the causes of autoimmunity remain largely unknown, a prominent theory suggests a central role for infectious microbes in the etiology of these diseases. Thus, be-

cause certain microbes bear similar molecular structures to the molecules of self, a response to such a region on a microbe might also provoke autoimmunity—a concept termed molecular mimicry (1). Because the thymus is intimately involved in tolerance of T cells to self, some theories also invoke a failure in central thymic tolerance to explain autoimmunity (1). In some cases, the causes of autoimmunity are more apparent. For example, experimental therapy in one autoimmune setting can provoke yet another. Thus, treatment of MS patients with a monoclonal antibody to CD52, although showing some promise in ameliorating MS, has led to autoimmune hyperthyroidism in a third of the MS patients (2). Rarely, nonautoimmune diseases like cancer can also provoke autoimmunity. One example of this is in the paraneoplastic syndromes, in which an immune response to breast or ovarian cancer triggers an autoimmune reaction in other organs, including brain, retina, peripheral nerve, and muscle (3). In other instances, our natural physiology can prove the cause of an autoimmune response, such as in myasthenia, which may be transmitted to the fetus or

newborn through the passage of autoantibodies across the placenta (1). On the other hand, physiological changes such as pregnancy can suppress such autoimmune conditions as MS and RA (4, 5).

Despite detailed knowledge of the targets of attack and the cells perpetrating the autoimmune damage (Table 1), immunologists have so far had limited success in developing highly specific countermeasures to suppress pathologic responses without globally interfering with the immunity in the body. Nevertheless, some treatments have had considerable success. For example, blocking antibodies and soluble receptors specific for the inflammatory cytokine tumor necrosis factor (TNF) have had notable therapeutic efficacy in the treatment of RA (6). Similarly, antibodies that interfere with homing of white blood cells to the site of autoimmune inflammation in the brain have been used in treating sufferers of MS (7). Examples of more nonspecific therapy of autoimmunity now encompass recombinant cytokine molecules, such as  $\beta$ -interferon ( $\beta$ -IFN), for treatment of MS (8). In addition, familiar medications already widely used to treat other conditions, such as hypercholesterolemia (8), hypertriglyceridemia (9), allergy (10), and hypertension (11), also inhibit some of the main pathways in autoimmune inflammation. Such drugs have already shown promise in preclinical models of autoimmunity, as well as in early-stage clinical trials. This review explores the considerable progress in treating autoimmunity with cytokines or their antag-

Department of Neurological Sciences and Neurology, and Interdepartmental Program in Immunology, Stanford University, Stanford, CA 94305, USA. E-mail: steinman@stanford.edu

onists and with antibodies to homing molecules and discusses new approaches aimed at inducing antigen-specific tolerance in the context of autoimmune diseases (Fig. 1).

### Immune Therapies Targeting Cytokines and Their Receptors

Early research with synovial cultures from RA patients revealed that TNF was a critical mediator in the inflammatory response (6), and immunohistochemical studies of inflamed synovium from RA patients revealed TNF and TNF receptor at the site of disease. Furthermore, preclinical studies provided direct evidence that antibody to TNF could ameliorate arthritis, at least in mouse models (6). More important, however, administration of chimerized anti-TNF, or a soluble recombinant TNF receptor-immunoglobulin G (IgG) fusion protein, led to remarkable improvement of function in RA patients (12, 13) and has also proved beneficial in inflammatory bowel disease (14). Blockade of other inflammatory cytokines, such as interleukin-1 (IL-1), IL-6, and IL-15, has also proven a successful strategy in ameliorating RA, although the current experience with such drugs appears far less extensive than that seen with TNF blocking agents (15, 16).

Notwithstanding the success of TNF treatments, the use of anti-TNF drugs has revealed some problems. For example, it is not understood why about 50% of patients with longstanding RA do not respond to TNF inhibitors (6) and why blockade of proinflammatory cytokines can place some individuals at increased risk of infections such as tuberculosis (6). Strangely, while TNF blockade has been successful in improving function and clinical status in RA and Crohn's disease, the same medicines worsen MS (6, 17). This is most likely explained by the fact that, although excessive TNF is injurious for myelin-producing cells in the brain and spinal cord, it is also an important growth factor for these same cells (18). Many inflammatory cytokines are thus akin to the two-headed god, Janus: On the one hand they are mediators of damage, yet they have beneficial roles in repair as well (17).

Other approaches that target certain T cell populations with monoclonal antibodies to CD4 and to T cell receptors have been less impressive in RA and in MS (1). New enthusiasm is being generated for depleting B lymphocytes with antibodies to CD20, an approach that has been successful for treating lymphoma. Initial trials in RA are promising, and trials are being initiated in MS and SLE (19). Another hopeful approach for treatment of RA lies in the inhibition of the molecules present at the immune synapse between T cells and antigen-presenting cells, which are involved in antigen-specific activation of T cells. The synapse includes stimulatory molecules such as CD28, which interacts with CD80 and CD86 on the presenting

cell. Blockade of this interaction with a fusion protein-cytotoxic T-lymphocyte-associated antigen 4-IgG1 (CTLA4Ig), which also binds CD80 and CD86 with a high affinity, can prevent T cell activation and has been shown to improve the symptoms of RA in early clinical trials (20).

### Cytokines for Treatment of Autoimmunity

Interferon (IFN) has been used in treating MS, with the rationale that because these cytokines have antiviral activity, and because MS might be triggered by some as yet unknown virus, they could exert a therapeutic influence by this means. However, because administration of IFN $\gamma$  to MS patients has been found to exacerbate disease, the trial using this cytokine was halted. Subsequently, testing of  $\beta$ -IFN proved more successful, reducing the relapse rate of MS by 30% and slowing disease progression to a modest degree, with a measurable reduction in the number and volume of injured areas in the brain, as assessed by magnetic resonance scans (21, 22). Although  $\beta$ -IFN has both antiviral activity and a therapeutic effect in MS, direct proof that a particular viral pathogen

crobes at almost any location in our bodies. Once activated, lymphocytes migrating through blood vessels arrest their movement and bind to Velcro-like receptors along the vessel walls to initiate the process of penetrating the target organ (1, 26). It was hypothesized that lymphocytes express specific adhesion molecules that recognize other proteins that represent particular anatomic "addresses." Thus, because the key homing molecules—integrins and selectins—display a high degree of diversity, the particular integrin or selectin expressed is critical for entry to a particular anatomic site. As a consequence, it is conceivable that blocking a particular ligand/receptor interaction would be sufficient to abolish pathologic homing, yet leave lymphocytes free to move elsewhere. This has indeed turned out to be a very promising avenue, at least in preclinical models of autoimmunity (27). For example,  $\alpha$ 4-integrin on T lymphocytes allows these cells to recognize vascular cellular-adhesion molecule 1 (VCAM-1), facilitating migration to the central nervous system (27). Although VCAM-1 is usually not expressed at high levels on blood vessels in the brain, in animal models and in

**Table 1. Specific targets of the immune response in organ-specific autoimmune disease.**

| Disease                 | Immune target                                |
|-------------------------|----------------------------------------------|
| Myasthenia gravis       | Acetylcholine receptor                       |
| Multiple sclerosis      | MBP, MOG, PLP                                |
| Pemphigus vulgaris      | Desmoglein-3                                 |
| Rheumatoid arthritis    | Filaggrin                                    |
| Hashimoto's thyroiditis | Thyroid peroxidase, thyroglobulin            |
| Graves' disease         | Thyrotropin receptor                         |
| Type 1 diabetes         | Glutamic acid decarboxylase, insulin, HSP 60 |

triggers MS itself has not so far been forthcoming (8, 17, 21, 22). Thus, other mechanisms of action for  $\beta$ -IFN's success in this disease might be considered, including reduction of metalloprotease activity, which is required for lymphocyte migration to the brain (23), or reduction of major histocompatibility complex (MHC) class II expression in the brain, a prerequisite for triggering myelin-reactive T cells (24).

Systemic administration of recombinant cytokines has also been tried with some success in other autoimmune conditions. For example, in early clinical trials with the autoimmune skin disease psoriasis, clinical improvement and amelioration of skin lesions was observed with administration of IL-4 (25). Within the lesions, the number of (presumably pathogenic) IFN $\gamma$ -producing T cells was markedly reduced, and the levels of two cytokines involved in psoriasis, IL-8 and IL-19, were also reduced in the lesions.

### Promising Results of Clinical Trials with Antibodies Against Homing Receptors

Cells of the immune system represent highly mobile elements, primed to defend against mi-

human MS its expression is increased (27). Indeed, administration of  $\alpha$ 4-integrin in experimental autoimmune encephalomyelitis (EAE) was able to ameliorate disease and block encephalitogenic T cell clones from entering the brain (27). In the sequence of events,  $\alpha$ 4-integrin interacts first with VCAM-1 on the surface of the blood vessel and then with the secreted cytokine osteopontin, which is expressed in the extracellular matrix (28). This interaction is critical in the migration of lymphocytes into the brain itself and suggests that osteopontin may also play a critical role in MS and in autoimmune demyelination (29, 30, 31).

The  $\alpha$ 4-integrin is also critical for homing to a range of organs in other models of autoimmune disease, including spontaneous T1DM in nonobese diabetic mice, as well as in models of RA, ulcerative colitis, and asthma (32). Thus far, however, the strategy for treatment of autoimmune disease by blockade of homing molecules has not shown the level of exquisite specificity originally envisioned. To achieve this, further refinements, such as the development of drugs able to react with specific regions

on  $\alpha 4\beta 1$ -integrin rather than, for example,  $\alpha 4\beta 7$ -integrin, may allow for improved specificity in blocking homing to the brain rather than to the intestine.

At the same time, antibody to  $\alpha 4$ -integrin has shown some degree of success in phase 2 clinical trials of MS and in inflammatory bowel disease (7, 32). Because the same antibody blocks the ability of lymphocytes bearing  $\alpha 4\beta 1$ -integrin to bind to VCAM-1 and enter the central nervous system, as well as the ability of  $\alpha 4\beta 7$ -integrin to bind to the intestinal mucosal addressin cell adhesion molecule 1 (MadCAM-1), the treatment can influence pathology in both organs. In inflammatory bowel disease, the remission rate was twice as high in individuals receiving the antibody as in those receiving placebo (33), and in MS, the antibody decreased the fre-

quency of clinical relapses by 50%, also reducing the frequency of injured areas on magnetic resonance scans (7).

At this point, there are two main concerns with the above approach. As discussed, the importance of the  $\alpha 4$ -integrin in homing to more than one anatomic site means that there is at least a theoretical concern that recipients of the therapy would become generally compromised in their ability to fight infection. This concern has been borne out in a phase 2 trial in MS, in which an increased rate of pharyngitis, a form of upper respiratory tract infection, was observed (7). A second concern is that upon cessation of blockade of integrins, cells might again home to the site of disease. This is something that has been observed in some MS patients after discontinuation of the drug (7).

### Commonly Used Drugs Can Provide Cost-Effective Potential Therapies for Autoimmunity

Statins lower cholesterol by virtue of their ability to inhibit one of the critical enzymes, HMG-CoA reductase, and are taken by millions of individuals worldwide. Remarkably, in animal models of autoimmunity such as EAE and collagen-induced arthritis, these drugs have also proven protective (8, 34). Along with their other effects, statins reduce the inducible expression of MHC class II molecules and block the expression of costimulatory molecules necessary for the induction of autoaggressive T cells (34). They can also shift the balance of cytokines produced by autoaggressive T cells from T helper-1 ( $T_H1$ ) proinflammatory cytokines, such as  $IFN\gamma$  and TNF, to  $T_H2$ -type cytokines, including IL-4, IL-5, IL-10, and IL-13 (31). Encouragingly, in pilot trials using statins

to treat MS, inflammatory lesions were measurably reduced (35), and large-scale controlled trials of statins are now under way for both MS and RA.

Similarly, other drugs, such as peroxisome proliferator-activated receptor  $\alpha$  (PPAR- $\alpha$ ) agonists (widely prescribed to treat hypertriglyceridemia) and blockers of angiotensin used in treating hypertension, have demonstrable anti-inflammatory properties. Furthermore, these drugs have been shown to inhibit clinical disease and pathology in models of autoimmunity such as EAE (9, 11). As with statins, PPAR- $\alpha$  agonists are capable of shifting the cytokine profile from  $T_H1$  to  $T_H2$ , and early experiments with angiotensin blockers have indicated that they have similar properties (9). Histamine-1 receptors are found on  $T_H1$  T cells, and administration of histamine antagonists can block the development of EAE, again with an altered cytokine balance from  $T_H1$  to  $T_H2$  (10).

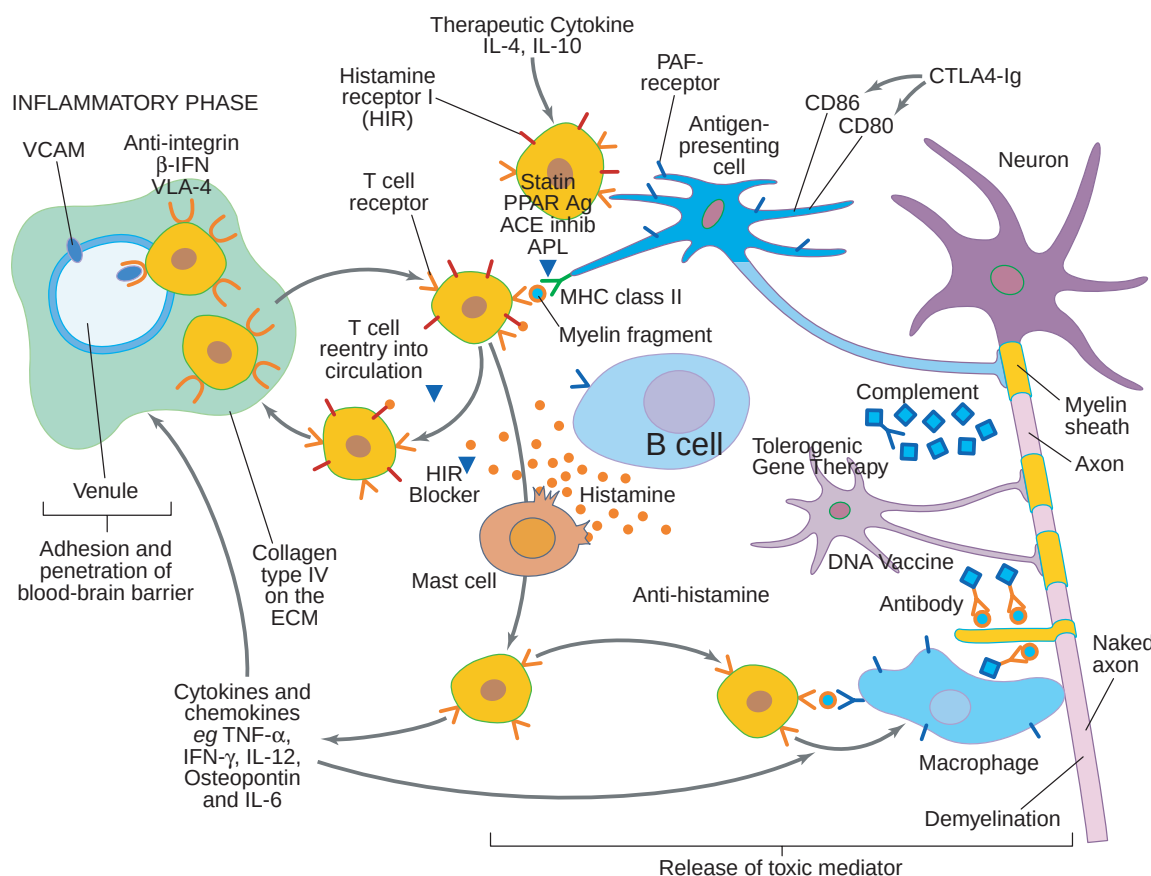


**Fig. 1.** The pathogenesis of multiple sclerosis. There are many similarities between the pathogenesis of MS and of other organ-specific autoimmune diseases such as T1DM, inflammatory bowel disease, and RA. Each step in the process is associated with a potential strategic checkpoint where therapy could be targeted. An antibody to  $\alpha 4$ -integrin blocks lymphocytes from homing to the blood vessel wall, where they bind to VCAM-1 in MS and RA, or to MadCAM-1 in inflammatory bowel disease. In MS,  $\beta$ -interferon inhibits metalloproteases, impeding diapedesis through the extracellular matrix. Extracellular matrix proteins include osteopontin, which is blocked by antibody to  $\alpha 4$ -integrin. Inside the brain, inducible MHC class II is inhibited by statins. Proinflammatory cytokine production is blocked by histamine-1 receptor blockers, statins, PPAR- $\alpha$  agonists, and ACE inhibitors. MS and RA differ in that blockade of TNF is beneficial for RA but worsens MS. In MS, TNF has Janus-like qualities: It is proinflammatory and necessary for the replenishment of myelin. Other strategies aim at delivering suppressive cytokines like IL-4 or IL-10 directly to the immune system or to affected tissues. These strategies induce regulatory T cells,  $T_{reg}$  cells, that are capable of inhibiting autoaggressive T cells. Blockade of costimulation with CTLA4Ig has been effective at blocking activated autoreactive T cells. DNA vaccines and altered peptides tolerate T cells and reduce the spread of autoantibodies. Specific targeting of dendritic cells with antigen or autoaggressive T cells with genes encoding IL-4 and IL-10 has been attempted with some success in animal models.

The importance of these results lies in the fact that antihistamines are widely used for the treatment of allergy and are taken by millions of individuals.

Such results are encouraging, because they suggest that many commonly used medications for a variety of conditions might be effectively combined with new regimes to treat autoimmune disease. Finally, it is interesting to speculate that since atherosclerosis may have an autoimmune component in its pathogenesis (1), the role of angiotensin blockers, statins, and PPAR agonists may be to protect from myocardial infarction and stroke, not only by lowering blood pressure, lipids, and cholesterol but also by blocking the potential autoimmune components of atherogenesis (36).

### Antigen-Specific Therapy for Autoimmunity

The identity of the target antigens in several major autoimmune diseases has been characterized, including T1DM, pemphigus vulgaris (PV), myasthenia gravis (MG), and MS. Thus, in T1DM, the immune system targets four major antigens, insulin, IA-2, glutamic acid decarboxylase (GAD), and heat shock protein 60 (hsp-60). As a consequence, the insulin-producing islets of Langerhans of the pancreas are destroyed. If a child at risk for T1DM, by virtue of having a first-degree relative with the disease, develops antibodies to insulin, GAD, and IA-2, then the probability of that child developing T1DM in the next five years approaches 100% (37). In PV, the immune system attacks desmoglein-3, a protein that allows epithelial cells to adhere to each other in the skin. Antibodies to desmoglein-3 cause widespread blistering by interrupting these intercellular "welds," and if autoantibodies from individuals with PV are transferred to mice, the mice will also develop skin blisters (38). In MG, antibodies are produced against the acetylcholine receptor at the neuromuscular junction (1, 17, 39); in the case of MS, antibodies and T cells are detected that react to various myelin proteins and lipids (1, 8, 17).

Despite such detailed knowledge of the target antigens in many autoimmune diseases (Table 1), immunologists and clinicians have had a difficult time silencing the destructive immune responses directed to self-antigens. A prime reason has been that the immune response "broadens" during the course of disease. Thus, an immune response to a single epitope on an organ-specific antigen at the start of disease can trigger immune responses to neighboring epitopes on the same molecule or to other epitopes on nearby molecules. This process, termed epitope spreading, ultimately draws in a larger repertoire of immune responses toward other autoantigens, ultimately enhancing the level of tissue destruction.

One strategy to combat the increase in breadth of immune response during the pro-

gression of an autoimmune disease would be to devise therapies that would simultaneously tolerize against multiple immune responses. One such solution to the problem of epitope spreading came with an invention made more than 30 years ago. In the 1970s, a random copolymer of the amino acids glutamate, tyrosine, alanine, and lysine, now termed glatiramer acetate, was initially designed to mimic the composition of myelin basic protein (MBP). It was effective in ameliorating EAE and was taken into clinical trials of MS, where it reduced the relapse rate by 30% (40). Fortuitously, the random nature of the copolymer tolerized against a variety of different myelin antigens (40, 41). Glatiramer was perhaps the first example of an altered self-antigen, now termed an altered peptide ligand (APL). For example, one of the combinations in glatiramer, EYYK, is capable of tolerizing mice to MBP and preventing paralysis in the EAE model of MS (42). Another mechanism, not envisioned at the time glatiramer acetate was invented, is its capacity to induce a  $T_H2$ -dominated response to myelin antigens (43).  $T_H2$  responses are associated with allergic-type reactions, and indeed about 10% of individuals taking glatiramer acetate develop allergic reactions, including bronchial constriction and local skin rashes (44). The development of clinical manifestations of allergy, when shifting immunity from  $T_H1$  to  $T_H2$ , is a vivid demonstration of the application of the  $T_H1/T_H2$  "paradigm" in humans (45).

More recently, an APL of MBPp83-99 was constructed by revising the main contact sites that this epitope has with the T cell receptor. APLs can induce a  $T_H1$  to  $T_H2$  shift in cytokine production in animal models of autoimmunity. This is a potentially important protective feature of APL, because  $T_H2$  cytokines have been shown to suppress immune responses to neighboring antigens that arise by epitope spreading. For example, by inducing IL-4, an APL for MBP can reverse ongoing EAE, induced by a different myelin molecule (46). In a phase 2 placebo-controlled clinical trial, an APL was able to shift the response of MBP-specific T cells, favoring  $T_H2$  cytokines over  $T_H1$  cytokine production (44). At lower doses, reduction in brain lesions was seen, while at higher doses, exacerbations of disease were noted in three patients (47). Allergic phenomena including skin rashes were seen following induction of a  $T_H1$  to  $T_H2$  shift in 10% of the patients (44). Avoiding such allergic phenomena will be a challenge in the development of this therapeutic strategy for shifting the balance of dominant cytokines from  $T_H1$  to  $T_H2$ .

Three other trials of antigen-specific therapy for T1DM are under way or have been completed recently. These include phase 2 trials with GAD and with an APL of insulin (48) and of hsp-60 (49). In the trial with the APL for

hsp-60, decreased exogenous insulin use was observed in diabetics, as well as a shift toward  $T_H2$  immunity (49). In a trial of insulin administered either orally or parenterally, no benefit was seen in preventing the development of T1DM in first-degree relatives of patients who had already developed diabetes (50).

Antigen-specific therapy of autoimmune disease has been attempted with oral administration of myelin antigens in MS, collagen in RA, and insulin in T1DM. Despite success in the prevention of disease in animal models of autoimmunity, in which antigen was fed to the animals at the time of disease induction, clinical trials attempting to treat ongoing disease in humans have thus far been unsuccessful (51). It is well established, however, that the oral route is highly effective in animals for desensitization of immune responses (52, 53). Antigen-specific T cells can be activated within 6 hours of feeding the antigen, and repeated administration of low doses of antigen by the oral route can diminish the number of effector T cells in the circulation that are capable of responding to that antigen (52). Finally, regulatory T cells ( $T_{reg}$ ) secreting transforming growth factor beta, IL-10, and other suppressive  $T_H2$  cytokines have been shown to be induced by oral feeding of self-antigen (53).

Such experiments point to the continued promise of using oral delivery to induce tolerance. Thus, despite the lack of success, so far, in using the oral route for tolerance induction in humans, powerful regulatory mechanisms clearly exist at mucosal sites, and it is conceivable that these may prove important to exploit for future immune-based therapy.

Another promising method to induce tolerance to islet cell antigens in T1DM involves the administration of antibodies to CD3. By engaging CD3 in the immune synapse on T cells (17), these antibodies are able to deviate the immune response to islet antigens toward  $T_H2$  cytokine production and to activate regulatory T cells capable of restraining the autoreactive responses (54). In a small trial with antibodies to CD3 in patients with T1DM treated within 6 weeks of diagnosis, an improved metabolic control of glucose was observed over the first year of disease (55). Various strategies, including administration of APL, blocking CTLA4, and use of anti-CD3, have proven efficient at inducing regulatory  $T_{reg}$  cells. The effectiveness of  $T_{reg}$  at restraining autoaggressive T cells in experimental settings (20, 43, 54, 56) suggests that it may some day be possible to harness the regulatory capacity of these cells in the treatment of a range of autoimmune diseases.

Recent studies with large-scale microarrays, designed to analyze the spread of the antibody response in EAE, have demonstrated that as demyelinating disease progresses, antibodies develop toward multiple myelin

proteins (57). This again emphasizes the challenge posed by epitope spreading when investigators try to devise therapeutic countermeasures. One method of tolerizing to multiple myelin components on a large scale has been developed with DNA plasmids encoding several myelin antigens (57). When these DNA plasmids were administered to mice after the first attack of EAE, they reduced the subsequent relapse rate by greater than 50%. At the same time, a reduction was seen in the spreading of antibodies toward multiple myelin antigens, including MBP, proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG) (57). A phase 1 trial with DNA vaccines designed to tolerize against myelin proteins has been approved by the Food and Drug Administration.

Dendritic cells (DC) are already being targeted as a means of boosting immunity to infectious pathogens and cancer antigens, and competing strategies aimed at delivering suppressive cytokines to DC or to T cells are also being used with some success in treating animal models of autoimmunity (58). For example, by using specific receptors expressed by DC, it has been possible to target small amounts of antigen to these specific subsets of the cells without incurring their activation, thus attaining tolerance rather than immunity (59). Such experimental studies suggest that DC may be successfully used in guiding tolerizing therapies for autoimmune disease in the clinic.

### Future Directions

A number of partially effective treatments for major autoimmune diseases have now been developed, and most of the approved treatments have, thus far, brought considerable relief to as many as two-thirds of patients with autoimmune diseases such as RA and MS. Nevertheless, none of the approved

treatments yet have the exquisite specificity to neutralize the highly restricted set of immune responses that characterize most autoimmune conditions. Furthermore, they carry substantial economic cost, with an annual price tag of about \$5 billion in the United States. Although there is clearly a net benefit in alleviating suffering and disease progression, if such "high-tech" drugs turn out to be curative only in synergistic combinations, we may see an even more substantial rise in the fiscal costs of therapy. We should keep in mind that many drugs are generic and already approved for non-autoimmune diseases and that these may prove efficacious when used alone or in combination with the newer drugs designed to treat autoimmunity. Finally, some day a true "cure" for the ailments of autoimmunity may be developed—perhaps based on antigen-specific immune therapy—that might eventually prove as cost effective as vaccination for infectious diseases.

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