

New Reagents on the Horizon for Immune Tolerance

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Abstract

Recent advances in immunology and a growing arsenal of new drugs are bringing the focus of tolerance research from animal models into the clinical setting. The conceptual framework for therapeutic tolerance induction has shifted from a “sledgehammer” approach that relies solely on cellular depletion and cytokine targeting, to a strategy directed toward restoring a functional balance across the immune system, namely the different populations of naive cells, effector and memory cells, and regulatory cells. Unlocking the key to tolerance induction in the future will likely depend on our ability to harness the functions of T regulatory cells. Also, dendritic cells are strategically positioned at the interface between innate and adaptive immunity and may be subject to deliberate medical intervention in a way that can control a chronic inflammatory response. Many reagents with tolerance-inducing potential are currently undergoing clinical testing in transplantation, autoimmune diseases, and allergic diseases, and even more that are on the horizon promise to offer enormous benefits to human health.

Immune tolerance: balanced cellular and humoral immune response that enables tolerance of harmless molecules while preserving immunity to potentially harmful microbial invaders

Deletion: mechanism of tolerance achieved through lymphocyte death (apoptosis)

Anergy: mechanism of tolerance defined by a state in which T lymphocytes are rendered functionally nonresponsive

TCR: T cell receptor

APC: antigen-presenting cell

INTRODUCTION

The ability of the immune system to distinguish self from nonself and harmful from harmless molecules can be operationally defined as immune tolerance. A breakdown in tolerance is a common thread underlying many human diseases. Indeed, much of our understanding of the regulation of the immune system has come from the investigation of conditions featuring impaired tolerance to self (e.g., autoimmune diseases) or otherwise harmless molecules (e.g., allergic diseases), as well as the induction of tolerance following transplantation of an allogeneic organ or tissue. In fact, the ability to suppress aberrant or simply unwanted immune responses in a specific and permanent manner, without compromising host defense—in other words, to create a state of immune tolerance—has been the “holy grail” of immunology ever since tolerance induction was first demonstrated in mice more than 50 years ago (1).

This review describes the molecular pathways that maintain a tolerant state and discusses the rapidly expanding armamentarium of potential tolerance-inducing reagents that are now making their way into clinical investigation. Rather than exhaustively describe the dozens of novel protolerogenic drugs in development, this short review focuses on key agents in the later stages of clinical development across the diverse fields of transplantation, autoimmunity, allergy, and asthma.

MECHANISMS OF IMMUNE TOLERANCE

T and B cell tolerance can be established (or lost) either centrally, at the site of primary lymphocyte development in the thymus or bone marrow, or peripherally, at locations of antigen recognition and processing in the secondary (e.g., central lymph nodes and spleen) and tertiary (e.g., intestinal Peyer's patch) lymphoid tissues. Both T and B cells navigate tightly regulated developmental pathways that depend on the sum of positive and

negative signals delivered through the antigen receptors and other cell surface signaling molecules (2, 3). Although the majority of self-reactive T and B cells are deleted through apoptotic mechanisms or receptor editing (in the case of B cells) (4), self-reactive clones may escape deletion and vigorously respond if challenged by infection, self-antigen in the context of innate or adaptive help, or a self-mimicking foreign antigen (5).

In the peripheral immune system, the key mechanisms that establish and maintain tolerance include clonal deletion, anergy, ignorance, and regulation (**Figure 1**). For instance, T cell stimulation by foreign antigen can promote active cell death to prevent excessive expansion of T cells. Aging MRL-*lpr* mice with a deficiency in apoptosis caused by the insertion of a retroviral transposon in the *Fas* gene develop a profound lymphadenopathy and a lupus-like disease (6). Similarly, humans with *Fas* mutations develop an autoimmune lymphoproliferative disease characterized by defective T cell apoptosis, massive lymphadenopathy, splenomegaly, autoantibody production, autoimmune hemolytic anemia, thrombocytopenia, and neutropenia (7). Thus, dysregulated T cell homeostasis is an important checkpoint in some cases of autoimmunity.

In addition to receiving a signal through the T cell receptor (TCR), the T cell requires a second activation signal to enable a productive immune response. The most important costimulatory pathway for T cell activation involves CD28 expressed on T cells and its ligands B7.1 (CD80) and B7.2 (CD86) on antigen-presenting cells (APCs). Engagement of CD28 by B7 molecules lowers the threshold for TCR signaling required for T cell activation, amplifies cytokine responses, and promotes T cell proliferation, survival, and differentiation (8). In several animal models of autoimmune disease and organ transplantation, blocking this interaction following TCR and coreceptor (CD4 or CD8) engagement results in extensive cell death, anergy induction and robust tolerance (9).

This “two-signal” model of T cell activation is replicated across numerous different T cell subsets under different activation conditions and tissue milieu, and also depends on a variety of other cell surface receptor:ligand interactions, including CD154/CD40, 41BB/41BBL, ICOS/ICOSL and OX40/OX40L, to name a few.

Interestingly, the costimulatory pathways have both positive and negative regulatory elements that control immunity. Two key examples are CTLA-4 and PD-1. CTLA-4 binds to the same ligands as CD28, namely CD80 and CD86, but rather than promoting activation, differentiation and effector function, CTLA-4 attenuates T cell activation. In fact, this pathway is so critical that blockade early in life results in massive lymphoproliferation and death in mice (10). In humans, when anti-CTLA-4 antibodies are used for cancer therapy, they often provoke autoimmune diseases of the endocrine organs, gastrointestinal tract and skin (11). PD-1 is expressed on activated T cells as well as B cells and myeloid cells. PD-L1 and PD-L2 engagement of PD-1 on activated T cells has been found to inhibit T cell proliferation and cytokine production (12). Blockade of this pathway also results in severe autoimmune manifestations. Thus, agonist drugs targeting negative regulators of this type can potentially have profound effects on tolerance.

The CD40:CD154 costimulatory pathway has also been widely studied in animal models of autoimmunity and transplantation. CD40 is a member of the TNF receptor superfamily that is constitutively expressed on APCs such as B cells, macrophages, and dendritic cells (DCs). Its ligand, CD154, is rapidly expressed on the surface of activated T cells following TCR engagement. The interaction of CD40:CD154 is known to facilitate both humoral and cellular immune responses, driving B cell proliferation and enhancing T cell responses. Blocking this interaction effectively abrogates autoimmune disease in a variety of murine experimental models—including experimental autoimmune encephalomyeli-

tis, type 1 diabetes in the NOD (nonobese diabetic) mouse, collagen-induced arthritis, uveitis, thyroiditis, and lupus (reviewed in Reference 13)—and can induce long-term tolerance to skin, islets, bone marrow and other transplanted organs and tissues (14). In addition, CD40 ligation induces maturation of DCs, prolongs the presentation of MHC-peptide complexes, and promotes release of inflammatory mediators (15).

In the past several years, we have gained an increased appreciation of how the balance of various T cell subsets determines the outcome of immunity. T cells can differentiate into multiple states: Th1 cells produce primarily IFN γ and IL-2 to promote intracellular immunity, whereas Th2 cells generate IL-4, IL-5, and IL-13 that drive humoral immunity and allergic responses (16). Th2 cells are thought to predominate in conditions such as chronic graft-versus-host disease (GVHD), systemic sclerosis and allergic diseases. More recently, two other T cell subsets have been described that contribute to the fine balance of the immune system. Th17 cells, so named because they produce IL-17, participate in the control of certain infectious diseases and autoimmune syndromes (17). In addition, thymic-selected and peripherally derived CD4⁺CD25⁺FoxP3⁺ regulatory T cells (Tregs), as well as Tr1 cells and Th3 cells, dominantly suppress T cell immunity by mechanisms that depend on cell:cell contact, probably involving CTLA-4 and/or membrane-bound TGF- β and secretion of IL-10 and TGF- β , depending on the regulatory T cell subtype and immune setting (18). Interestingly, each of these subsets is controlled by unique transcription factors (Tbet–Th1; GATA-3–Th2; ROR α –Th17; and FoxP3–Tregs).

Indeed, there is growing evidence that CD4⁺CD25⁺FoxP3⁺ T cells are powerfully engaged in the negative control of pathologic autoimmune and allergic responses (19). The negative regulatory role of Tregs is strikingly illustrated by the clinical phenotype of individuals with the FoxP3-deficient,

DC: dendritic cell

NOD: nonobese diabetic

GVHD: graft-versus-host disease

Regulatory T cells:

T cells that suppress immune responses by a cell:cell contact-dependent mechanism or secretion of cytokines such as IL-10 and TGF β

X-linked immunodeficiency syndrome IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome), which causes autoimmune disease in multiple organs (e.g., type 1 diabetes, hemolytic anemia, and thyroiditis), inflammatory bowel disease, atopic dermatitis, and fatal infection (20). A recent study has shown that FoxP3 mutations in patients with IPEX are characterized by heterogeneous clinical phenotypes and regulatory abnormalities, including not only a lack of CD4⁺CD25⁺ Tregs but also a dysfunction of these cells. In some of these patients, the effector CD4⁺CD25⁻ T cells show reduced susceptibility to suppression (21). New avenues are being considered for therapeutic intervention focused on harnessing the regulatory functions of these cells.

TRANSLATING TOLERANCE TO THE CLINIC

Given our present understanding of immune mechanisms (Figure 2), multiple targets for intervention can be envisioned. T cells develop in the thymus and differentiate into T helper cells of the Th1, Th2, and Th17 lineages under the control of professional antigen-presenting cells (APCs) and a variety of cytokines. The activated dendritic cell (DC) and macrophage are the predominant professional APCs and therefore play key roles in host defense and the development of chronic inflammation. In an inflammatory response, IL-12 produced by activated DCs and macrophages promotes the development of Th1 cells, while IL-4 production promotes the development of Th2 cells. TGFβ and IL-6 stimulate the development of Th17 cells. Among other effects, TGFβ upregulates the receptor for CD23, leading to expansion of the Th17 cell subset. Each of these T cell subsets may be a potential target of a tolerance-inducing strategy. Antigen-specific activation of naive T cells requires the binding of the TCR to MHC peptide on an APC (Signal 1) and a costimulatory signal (Signal 2). The more im-

portant second signal derives from binding of CD80/86 (B7) on the APC to CD28 on T cells. Thus, interventions targeting Signal 1 and Signal 2 may rebalance a dysregulated immune system and lead to a tolerant state. Other receptor:ligand costimulatory interactions also can stimulate T cell activation and include CD154:CD40, ICOS:ICOSL, and OX40:OX40L. These costimulatory signals are counterbalanced by inhibitory signals that downregulate T cell function; they include the following receptor:ligand pairs: CTLA-4:CD80/86 and PD-1:PD-L1/PD-L2. In principle, all of these pathways may be influenced using specific agonists and antagonists, and modulate an aberrant immune response.

The extent of T cell activation is delicately counterbalanced by the production of regulatory cells. After developing in the thymus, FoxP3⁺ T regulatory cells enter the periphery, where, under certain conditions, they can suppress the differentiation and function of Th1 and Th2 effector cells. Antigen-specific FoxP3⁺ Tregs also appear to be inducible in the periphery. FoxP3⁺ Tregs suppress T cells in a contact-dependent manner, probably involving membrane-bound TGFβ and CTLA-4. Tr1 and Th3, other Treg subsets, suppress T cells by secreting IL-10 and TGFβ. Tolerance interventions may be aimed at one or more of these checkpoints to restore a balanced immune response without harming the host. For example, tolerance-inducing therapy may (a) boost the numbers or function of regulatory T cells (FoxP3⁺ Tregs, Tr1, and Th3); (b) inhibit the activation of naive T cells by costimulatory blockade; (c) inhibit the function of DCs; (d) negatively signal through the TCR (e.g., anti-CD3), resulting in the induction of Tregs; or (e) decrease the survival of self-reactive B cells by inhibiting BAFF or the BAFF-R. Neutralizing proinflammatory cytokines such as TNFα or IL-6 may promote a tolerance-inducing environment. DCs and other myeloid cells, as well as T and B cells, express Toll-like receptors (TLRs) on their surface, which can be recognized by microbial pathogens, or self-antigens (DNA and RNA),

and provoke an immune response. TLR antagonists may also be useful in downregulating certain types of inflammatory responses, leading to a tolerant state.

However, it is important to emphasize that most of our understanding of tolerance is derived from animal rather than human studies. Perhaps the most significant barrier to the development of tolerance therapeutics in humans is the absence of well-defined biomarkers of tolerance. Without validated biomarkers, studies are limited to the use of clinical endpoints that define tolerance in an operational rather than an immunological sense. Other challenges include difficulties in sampling sites of tissue pathology in vivo (sampling is often limited to the blood), interpreting "tolerance signatures" from the laboratory in the context of concomitant immunosuppressive drug therapy, and the inability to distinguish disease-related, antigen-specific responses in affected versus healthy individuals. Despite these challenges, however, a number of promising protolerogenic strategies are indeed moving into the clinic for testing in transplantation as well as in autoimmune and allergic diseases.

Transplantation

Organ transplantation has become a standard treatment for end-stage disease of the kidney, liver, lung and heart, as well as severe type 1 diabetes mellitus. Short-term survival rates have improved over the past several decades for each of the transplanted organs, but long-term survival rates have remained relatively static in many cases, especially for kidney transplantation (22–24). This is reviewed in detail elsewhere in this volume (J. Tantravahi, K.L. Womer, B. Kaplan, "Why Hasn't Eliminating Acute Rejection Improved Graft Survival?"). In addition, long-term immunosuppression exacts a major toll by contributing to higher rates of cardiovascular disease, infection, and cancer (25, 26). These complications have motivated the development of new protocols to minimize maintenance immunosup-

pression and, in some cases, to induce transplant tolerance.

Mixed chimerism. In 1999, tolerance to a renal transplant was induced by creating mixed allogeneic chimerism in a patient with multiple myeloma and renal failure (27, 28). The patient was cured of multiple myeloma and later withdrawn from maintenance immunosuppression without subsequent rejection of the graft. In a study supported by the Immune Tolerance Network (ITN), Sykes and colleagues studied six patients with renal failure as a result of multiple myeloma to determine if a nonmyeloablative conditioning regimen followed by simultaneous bone marrow and kidney transplantation from HLA-identical sibling donors would successfully treat the myeloma and promote long-term allograft acceptance. Despite losing detectable chimerism, three of the patients accepted their kidney allografts and remained free of immunosuppressive therapy for 1.3 to >7 years (29). While in vitro studies detected antidonor alloresponses, cytotoxic T cells from these ostensibly tolerant patients were unresponsive to cultured renal tubular epithelial cells from the donor. At least two of these patients had complete operational tolerance to the kidney allograft; however, some developed chronic GVHD and suffered complications from the myeloma. More work is needed to reduce the incidence and severity of GVHD in this setting and improve our understanding of tolerance mechanisms.

Costimulation. Also in 1999, Kirk and colleagues reported that periodic administration of anti-CD154 monoclonal antibody was sufficient to maintain long-term kidney allograft survival in nonhuman primates (30). Initial enthusiasm led to numerous clinical studies of anti-CD154 antibodies for several transplant and autoimmune indications, but these studies were halted owing to an unexpectedly high incidence of thromboembolic complications, most likely related to the reactivity of the antibody with soluble or

ITN: Immune Tolerance Network

Costimulation: secondary signals that must accompany signals delivered through the T and B cell antigen receptor to achieve cellular activation

membrane-bound CD154 from activated platelets (31). Nonetheless, studies in small and large animal models continue to stress the importance of the CD40:CD154 pathway in tolerance mechanisms and its relevance as a target for intervention. Interestingly, in mice receiving an allogeneic heterotopic heart transplant, Ochando et al. (32) found that donor-specific transfusion combined with CD154 blockade (a well-established experimental tolerogenic protocol) induced the expansion of allospecific Tregs that act to control allograft rejection. Importantly, the induction of allospecific Tregs in this model depended on a direct interaction with alloantigen-bearing DCs in the lymph node of the grafted mice. This special relationship between Tregs and certain subsets of DCs provides a unique framework from which to develop clinical applications that capitalize on these regulatory populations.

The agent CTLA4Ig was among the earliest antagonists of the CD28 pathway developed for the autoimmune and transplant setting (33). A mutant form of CTLA4Ig, termed LEA29Y (or belatacept), which binds CD80 (twofold) and CD86 (fourfold) more strongly than CTLA4Ig, exhibits significant efficacy in the nonhuman primate transplant setting (34). A phase II trial was recently completed in which kidney transplant recipients were treated with anti-CD25 (basiliximab), mycophenolate mofetil and prednisone, and then randomly allocated to receive two different doses of either LEA29Y or cyclosporine (35). The incidence of acute rejection was similar among the three treatment groups; however, at 12 months, kidney function was better in both of the belatacept-treated groups than in the cyclosporine-treated patients. Chronic allograft nephropathy was also less common in the belatacept-treated groups. These results suggest this calcineurin-sparing regimen may prevent acute rejection as well as current therapies do, but with less long-term deterioration in renal function that may be related in part to diminished chronic rejection. These and other related studies are described in de-

tail elsewhere in this volume (F.G. Vincenti, M. Luggen, "T Cell Costimulation: A Rational Target in the Therapeutic Armamentarium for Autoimmune Diseases and Transplantation").

Interestingly, a series of studies has suggested that direct activation of the CD28 pathway in T cells using a superagonist anti-CD28 mAb could selectively expand Tregs, promoting tolerance in the autoimmune and transplant setting (35a). These studies led to the clinical development of an anti-human CD28 superagonist for the treatment of autoimmune disease. Unfortunately, an early phase I trial has raised significant safety concerns as the monoclonal antibody developed by TeGenero and tested in normal volunteers induced a strong cytokine storm that caused significant toxic-shock-like symptoms (35b). Thus, harnessing this complex and potent costimulatory pathway for tolerance induction has proven to be challenging (35c).

Targeting memory T cells. In the past several years, the challenges to transplant tolerance presented by memory T cells have been a focus of therapeutic attempts to induce tolerance. Memory T cells have less stringent activation requirements than do naive T cells, with reduced, and in some cases, no requirements for costimulation. It is difficult, if not impossible, to induce tolerance in mice with memory T cells (36); thus, a permissive tolerance model becomes a very stringent one. Lymphodepletion has been investigated as a primary tolerance strategy in the clinical setting. In one study, profound lymphodepletion using total lymphoid irradiation and parenteral administration of antithymocyte globulin allowed withdrawal of all immunosuppressive therapy in 3 of 28 patients who had received a cadaveric renal transplant (37). However, homeostatic proliferation of memory T cells following profound T cell depletion may represent a significant barrier to the induction of tolerance in the transplant setting (38–40). Thus, studies using Campath

1H, a monoclonal antibody directed at the cell surface molecule CD52, which is expressed on multiple cell types, have generated mixed results (41). The profound but selective depletion of certain cells with this drug is significantly immunosuppressive but may impede tolerance induction.

Autoimmune Diseases

Unlike the induction of transplant tolerance, treatment of autoimmune diseases can typically proceed only after clinical diagnosis. By the time of initial presentation, tissue inflammation and injury are already substantial. The immune response may already have expanded beyond the initial “triggering” antigen (or self-antigen) to encompass a diverse array of T and B cell clonal specificities, by a process termed epitope spreading. Extensive T cell memory may already be established, creating a more complex scenario for tolerance induction. Diverse clones of pathogenic T and B cells are generally considered a barrier to antigen-specific therapies. However, recent studies in animal models suggest that antigen-specific therapies may produce immunoregulatory effects that suppress responses to other antigens (42).

Nonmyeloablative stem cell transplants.

With a wealth of preclinical evidence that complete ablation of autoreactive T and B cells followed by autologous bone marrow transplantation can control autoimmune disease, a number of human clinical studies have been undertaken (43). High-dose immunosuppressive therapy with autologous stem cell transplantation is believed to offer a comparatively safe and flexible alternative to intensive immunosuppression in many autoimmune diseases (44). A variety of nonmyeloablative strategies have been employed, including agents such as cyclophosphamide, fludarabine, antibodies to T cells (antithymocyte globulin) and/or B cells (rituximab), and Campath 1H. In humans, bone marrow ablation followed by autologous stem cell trans-

plants has shown some success in inducing long-term remission in severe, refractory systemic lupus erythematosus (SLE), with mixed results in other indications such as multiple sclerosis, Crohn’s disease, and systemic sclerosis (43). There is general concern, however, that a genetic predisposition to autoimmunity may result in disease recurrence, despite full reconstitution of the immune system. In addition, allogeneic bone marrow transplantation has been tested in some cases; however, this approach leaves patients at risk for GVHD and other associated comorbidities.

T cell receptor complex targeting. Antibodies directed against the TCR or its coreceptors have been successfully used to induce a state of immunological tolerance in animals. However, initial studies with depleting or partially depleting anti-CD4 monoclonal antibodies failed to show treatment benefits in rheumatoid arthritis (RA) and led to persistent CD4 lymphopenia, raising significant safety concerns (45). Several groups are developing nondepleting anti-CD4 monoclonal antibodies that promise greater safety and efficacy in humans (46–48). In addition, there has been growing interest in the use of polyclonal anti-T cell antibodies (49). Initial clinical studies are now beginning in type 1 diabetes using Thymoglobulin™ (Genzyme), a polyclonal rabbit anti-T cell antibody preparation that affects immunity by clearing T cells from the body and modulating T cell activation, homing and cytotoxic activities.

Among the more promising TCR-directed strategies is targeting the CD3 coreceptor complex on T cells. In the early 1990s, work in the NOD mouse showed that treatment with antibodies against CD3 could prevent or reverse autoimmune disease and induce tolerance (50–52). Two humanized anti-CD3 monoclonal antibodies were initially developed for clinical use (53, 54) that had more reduced Fc receptor binding than their parent molecules. This design enhancement was intended to curb the severity of the cytokine

SLE: systemic lupus erythematosus

RA: rheumatoid arthritis

release reaction that typically occurs after the first dose of the unmodified version of these antibodies. A randomized phase I/II open-label trial of hOKT3 γ 1(Ala-Ala) for the treatment of patients with new-onset type 1 diabetes showed a significant reduction in the loss of insulin production, as measured by stimulated C-peptide responses, compared with an untreated control group (55). Improvement in the C-peptide responses was evident even two years after treatment, although these responses declined during the second year (56). The other antibody, anti-ChAglyCD3, showed similar efficacy in a randomized placebo-controlled trial involving 80 patients with type 1 diabetes (57). Consistent with observations in mice, patients treated with anti-CD3 monoclonal antibody [hOKT3 γ 1(Ala-Ala)] develop IL-10-producing CD4⁺ T cells, as well as CD8⁺ regulatory T cells (58, 59). Clinical efficacy of anti-CD3 monoclonal antibodies has also been observed in other chronic inflammatory states, including psoriatic arthritis and ulcerative colitis (60; S. Plevy, personal communication).

Costimulatory blockade. The first of the costimulatory blockers, CTLA4Ig (abatacept), has been shown to be effective for the treatment of RA (61, 62), but only a minority of patients in clinical trials improved to the point of clinical remission. Clinical trials of abatacept are also under way in SLE. Costimulatory blockade alone has yet to be proven tolerance-inducing in the autoimmune setting.

B cell targeting. The emergence of B cells as an important target in autoimmune diseases has been fueled primarily by the initial success of rituximab therapy for RA. Rituximab is an anti-CD20 chimeric monoclonal antibody that mediates depletion of mature B cells, while sparing pro-B cells, pre-B cells, and most immature B cells, as well as plasma cells (63). Rituximab therapy has been shown to reduce the signs and symptoms of RA, and

it achieves variable decreases in the serum levels of rheumatoid factor without significantly altering serum levels of IgM, IgG, and IgA (64). B cells typically return ~5–8 months after treatment and are generally accompanied by a disease relapse. Thus, in RA, anti-CD20-mediated B cell depletion alone does not induce a prolonged tolerant state.

In uncontrolled studies, rituximab therapy produced clinical remission of antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (e.g., Wegener's granulomatosis and microscopic polyangiitis), with elimination of serum ANCAs even after the return of B cells (65). Other studies have begun examining the clinical efficacy and safety of rituximab for a vast array of other autoimmune diseases, including SLE, primary Sjögren's syndrome, type 1 diabetes, multiple sclerosis, autoimmune hepatitis and bullous pemphigoid. As a possible alternative to anti-CD20, studies in mice have demonstrated that anti-CD19 antibodies can more effectively deplete B cells, eliminating nearly all of the pre-B cells in the bone marrow (66). Importantly, this approach results in the elimination of B cells before expression of their antigen receptors, and it may have implications for tolerance.

Still, fine-tuning B cell functions may require other therapies that target the various pathways of B cell survival. Several companies are developing new drugs that block the binding of BAFF, a B cell activation factor of the TNF family, with its receptors. These reagents include anti-BAFF/BLySTM (belimumab, Lymphostat-BTM, Human Genome Sciences), BR3-Fc (Genentech), and TACI-Ig (Zymogenetics). Initial testing of belimumab in RA suggests only modest clinical efficacy but no major safety concerns (67, 68). Interestingly, Cambridge et al. (69) have shown that serum BAFF levels rise immediately after rituximab-mediated depletion of CD20⁺ B cells, with a subsequent decline to baseline following return of circulating B cells. This increase in serum BAFF levels following B cell depletion may represent a homeostatic mechanism, albeit undesirable, as it may provide

a fertile ground for the survival and expansion of self-reactive clones. Thus, it may be advantageous to combine a B cell-depleting antibody with an anti-B cell survival strategy, such as anti-BAFF, BR3-Fc, TACI-Ig, or anti-CD22 monoclonal antibody, to keep self-reactivity at bay during reconstitution of the B cell population.

Antigen-specific approaches. Tolerance induction using antigen-based therapy is appealing because of its relative safety compared with nonspecific immunomodulatory agents. Although the full array of self-antigens for a given disease may not be known, antigen-based therapies may tap into regulatory T cells that produce bystander suppression and more global downregulation of autoimmunity. However, there are at least three limitations to this approach. First, only a few autoimmune diseases, mostly organ-specific, have been convincingly linked to a stimulating self-antigen. Second, the antigen-specific approach must be designed to induce immunoregulatory effects that ameliorate clinical disease without risking exacerbation. Third, the timing of antigen-specific therapy with respect to disease onset is a key variable. In humans with type 1 diabetes, oral insulin administered at the time of diagnosis fails to prevent disease progression (70). However, in nondiabetic relatives with high titers of insulin autoantibodies (a subpopulation with a high rate of disease progression), there is preliminary evidence that oral insulin therapy reduces the incidence of diabetes development (71).

Several antigen-specific agents are currently under clinical investigation, including peptides derived from neural antigens such as myelin basic protein, islet antigens such as GAD and insulin, and heat shock proteins, including hsp60, which represent a class of self-proteins that often have molecular mimics whose cross recognition may lead to the development of autoimmune disease. Notably, a recent animal study showed that a combination of inhaled proinsulin peptide and

anti-CD3 antibody treatment profoundly decreased progression of type 1 diabetes (72).

Allergic Diseases

The incidence of atopic disorders such as asthma, rhinitis, atopic dermatitis, and food allergies is increasing in epidemic proportions across the developed and developing world (73). Although pharmacological interventions can effectively suppress the symptoms of many of these disorders, they do not provide sustained remission or cure and therefore require long-term use to maintain disease control. In contrast to autoimmunity, allergy can be defined as a state of immune responsiveness to an exogenous antigen(s) that is not otherwise harmful to the host. Allergic tolerance is not immune ignorance or anergy, but rather a balanced cellular and humoral immune response that occurs in the majority of humans exposed to potential inhalant, skin contact, and food allergens. Thus, the goal of tolerance induction in allergic disorders is the establishment (primary prevention) or re-establishment (treatment) of the functional state of a nonharmful immune response to exogenous allergens.

We now know that immune tolerance in allergic disease requires a balanced, protective response that has both Th1 and Th2 characteristics. As a result, the focus of tolerance induction strategies has shifted away from direct attempts to polarize T cell responses with cytokines and cytokine antagonists. Rather, efforts are under way to induce tolerance by regulating DCs (the key players for determining Th1/Th2 balance) that modulate regulatory T cell function and drive the profile of antibody isotypes for a given antigen exposure. Future efforts are likely to combine standard or novel forms of allergen exposure with immunomodulatory agents, such as CTLA4Ig, to manipulate the specific immune response.

Allergic inflammation is characterized by tissue eosinophilia, the recruitment and activation of mast cells and basophils, and heavy-chain switching of B cells in favor of

IT: immunotherapy

IgE—features reflecting exaggerated Th2 responses and increased production of Th2 cytokines, including IL-3, IL-5, IL-9, IL-4, and IL-13. More recently, APCs, particularly FcεRI-bearing DCs, have been recognized as key players in polarizing immune responses. This interplay of immunoregulatory effects provides a road map to “allergic tolerance.”

Allergen immunotherapy. Immunologists began using allergen immunotherapy (IT) in humans nearly 100 years ago as a means of tolerizing specific human responses in allergic rhinitis and asthma. Allergen IT is effective in this regard, particularly for seasonal rhinitis, asthma and hymenoptera sting; 3–4 years of treatment can induce long-term disease remission for at least three years following treatment (74). In children, IT has prevented sensitization to new allergens and reduced progression from rhinitis to asthma (75, 76). Clinical allergic tolerance is accompanied by emergence of CD4⁺CD25⁺ IL-10 producing (77) and TGFβ-producing (78) T regulatory cells that are detectable in antigen-driven peripheral blood T cell cultures and within the nasal mucosa during natural pollen exposure. These altered T cell responses are accompanied by the emergence of protective IgG antibodies (79), particularly IgG4 subclass antibodies, at 3–6 months, followed by a very gradual reduction in allergen-specific IgE levels over 3–5 years (80). Conventional IT, which is associated with a 10%–20% incidence of systemic allergic reaction, requires several years to reach full effectiveness (81). In compressed “rush” protocols that circumvent some of the inconvenience of conventional IT, the speedy ramp-up in dose increases the risk for systemic allergic reactions.

A recent ITN study showed that the administration of subcutaneous monoclonal anti-IgE (omalizumab) prior to a rush regimen of whole ragweed allergen IT significantly improved the safety of IT. This controlled study reported an 80% reduction in systemic adverse reactions with omalizumab pretreatment compared with IT alone, as

well as a synergistic improvement in seasonal rhinitis symptoms (82). This approach could be extended to sublingual allergen therapy for high-risk infants to reduce allergen-specific IgE and thereby enable early prevention of the allergic state.

Allergen peptide-based approaches.

Creticos et al. (83) described a promising alternative to conventional IT in another ITN-supported study, where a series of six injections of allergen-linked “immunostimulatory sequences” (DNA-ISS, bacterial CpG-rich oligonucleotide DNA sequences conjugated to the major ragweed allergen Amb A1) reduced seasonal rhinitis. Symptomatic improvements lasted through the next allergy season with no additional treatments. A parallel study found that disease remission following DNA-ISS treatment was associated with a local switch from allergen-driven Th2 responses to Th1 responses within the nasal mucosa (84). Because the ligand for CpG, Toll-like receptor 9, is expressed by myeloid cells, including DCs, macrophages, and B cells, this novel adjuvant may ameliorate allergic responses through its effects on APCs. Another peptide-based approach has been described by Larché et al. (85), who showed that the intradermal injection of T cell peptides derived from the major cat allergen Fel d1 suppressed skin responses to whole cat allergen. These preliminary results establish proof of principle that peptide-specific modulation of allergic response can be a successful tolerance-inducing strategy in the near future.

Chimeric human IgG-allergen fusion proteins represent another unique mode of allergen IT. For example, human IgG Fc has been covalently linked to Fel d1 to create a reagent that can paralyze allergic cell reactivity during the treatment phase while inducing allergic tolerance (86). In humans, coaggregation of FcγRIIβ molecules with FcεRI molecules on mast cells and basophils leads to active inhibition of mediator release. By providing Fel d1 in the

context of IgG Fc, it may be possible to give large doses of IT in a rush protocol to rapidly produce a salutary effect on cat allergy with an acceptable safety margin. As most of the key food allergens have been cloned (87), such an approach holds significant potential for food allergy as well.

Regulatory cells. Regulatory cells have received increasing attention for the treatment of allergic diseases. For example, a DC-focused allergen T cell epitope vaccine for allergic airway diseases has been proposed as an alternative to protein-based IT. Targeting DEC-205, an integral membrane protein expressed at high levels on cortical thymic epithelium and DCs in humans as well as in mice (85, 88), results in transient proliferation of specific T cells, followed by tolerance (89, 90). This outcome is achieved through the conversion of naive CD4⁺ T cells to regulatory T cells expressing FoxP3 (91).

Interestingly, allergen IT using a subcutaneous depot aluminum hydroxide-adsorbed grass pollen extract (*Pleum pratense*, ALK Abello Denmark) is highly effective for severe seasonal allergic rhinitis and seasonal asthma (74). This preparation has been shown to induce long-term remission, prevent the onset of sensitization to new allergens, and reduce progression from rhinitis to asthma—the so-called “allergic march” (75, 76). The mechanisms responsible for its beneficial effects include the induction of peripheral CD4⁺CD25⁺ IL-10-producing T cells (77) and enhancement of protective IgG/IgG4 antibody responses, which suppress IgE-dependent basophil histamine release and IgE-facilitated antigen presentation (79). Recently, the same grass extract administered as rapidly dissolving sublingual tablets has been shown to be effective and safe for home administration (93). Although effective, the sublingual route, which employs aqueous extracts, may be less effective than the subcutaneous route employing alum-based extracts. Alum-potentiated sublingual IT may

be a suitable alternative because of alum’s immunomodulatory effects when given with an antigen. These alum formulations may modulate DCs, resulting in a Th1-biased response. Indeed, the addition of alum to allergen-stimulated human peripheral blood mononuclear cell cultures has been shown to profoundly suppress Th2 cytokine production (94).

SUMMARY

The explosion of new knowledge in immunology has illuminated new fundamental mechanisms regulating the immune system, to the point that tolerance induction by deliberate medical intervention is now foreseeable. A major shift has occurred in the paradigm of therapeutic tolerance induction. No longer is it believed that wholesale T or B cell depletion or cytokine targeting alone will bring about a tolerant state. Rather, tolerance induction will require restoration of a fine balance in the function of naive, memory and regulatory cells. The discovery and characterization of the role of CD4⁺CD25⁺FoxP3⁺ Tregs has perhaps done the most to motivate the development of clinical strategies based on this new concept. Equally important are DCs, which are strategically equipped at the interface between innate and adaptive immunity to produce broad-ranging immunoregulatory effects. Exciting opportunities lie ahead for clinical trials of novel tolerance-inducing therapies with the burgeoning arsenal of immunomodulatory drugs. The insights gained from these trials are being enhanced by new technologies that directly identify antigen-reactive cells, as well as by genomic and multiplex proteomic assays that can begin to elucidate phenotypic and functional “signatures” of immune tolerance. Therapeutic tolerance is indeed reaching the clinic, where it promises to dramatically improve the outcomes of patients with organ transplants, autoimmune diseases and allergic disorders.

SUMMARY POINTS

1. The discovery of CD4⁺CD25⁺FoxP3⁺ T regulatory cells has shifted the paradigm in tolerance research toward the clinical testing of therapies that induce active regulation.
2. Costimulatory blockade has finally translated into an approved drug, but as a therapeutic for tolerance induction, it will likely be most useful in combination with other strategies.
3. Memory T cells represent a major barrier to tolerance induction in the transplant setting and may make tolerance induction more difficult in ongoing immunological diseases such as autoimmunity and allergy.
4. Nonmyeloablative stem cell transplantation holds promise for the treatment of autoimmune diseases.
5. T cell receptor-directed antibodies that induce tolerance in animal models of autoimmunity appear to reduce pathogenic T cell responses and provoke regulatory T cells in humans.
6. B cell targeting remains a promising approach for promoting tolerance in autoimmune diseases and may be effective in certain transplant settings.
7. Significant efforts are under way to determine if CpG-rich oligonucleotides linked to allergens can ameliorate allergic responses.

FUTURE ISSUES

The avenues of investigation likely to influence tolerogenic therapies in the coming years include:

1. Therapeutic agonists for inhibitory receptors of T and B cell activation
2. Modulation of individual T cell subset pathways including: Th1, Th2, Th17 and Treg
3. Mixed chimerism as a means to induce tolerance in the transplant setting under nonmyeloablative conditions in conjunction with drugs that effect peripheral tolerance
4. Methods to exploit the use of regulatory T and dendritic cells as cellular therapies for transplantation, autoimmunity and allergic diseases
5. Evaluation of new biomarkers that improve our understanding of disease and treatment mechanisms

DISCLOSURE STATEMENT

JB has a financial interest in OKT3 γ 1(Ala-Ala). JB and GSE are consultants for MacroGenics and Bayhill Therapeutics, which are developing immunotherapies for autoimmune disease and type 1 diabetes. AS, through the University of California, has a patent interest in the use of chimeric proteins to produce allergic tolerance. (The University of California holds the patent to this approach and has licensed this intellectual property to Biogen-IDEC Inc.) MHS is a consultant for Genzyme Corp.

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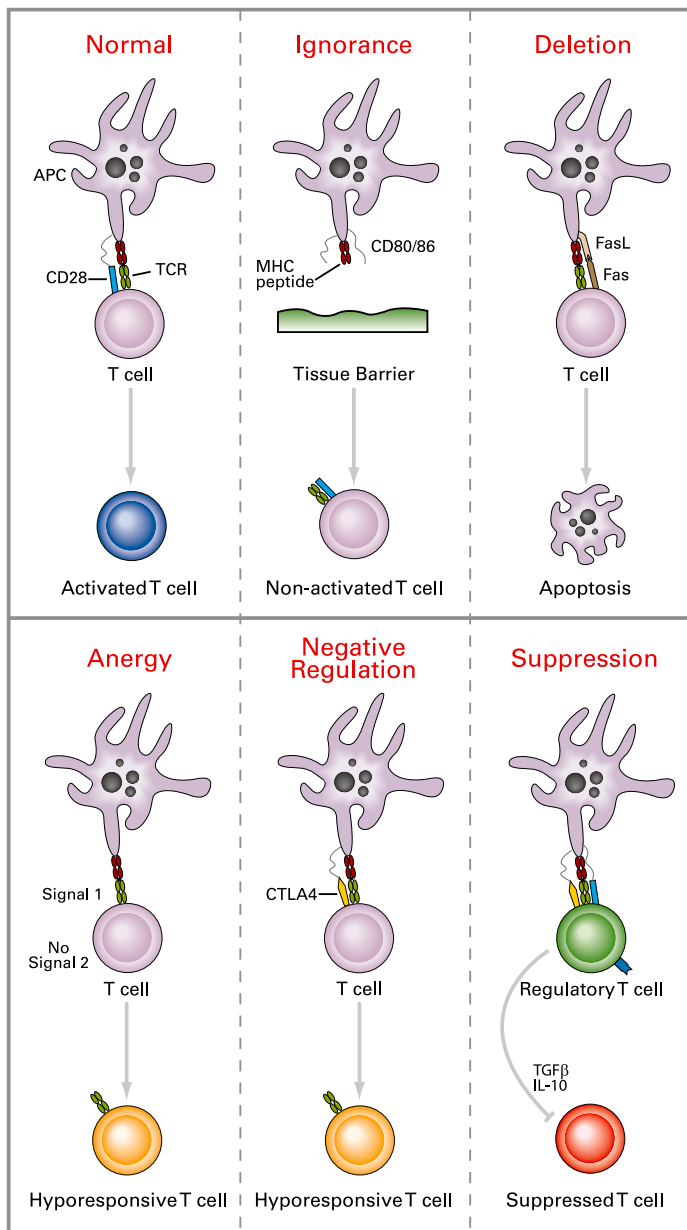


Figure 1

Peripheral mechanisms of tolerance. Normal T cell activation requires the interaction of MHC/peptide on APCs with the T cell receptor (Signal 1) and the interaction of costimulatory molecules, most prominently CD80/86 on APCs with CD28 on T cells (Signal 2). The peripheral mechanisms of tolerance that control effective T cell activation include: (a) ignorance, in which the APC is sequestered from the T cell through physical barriers; (b) deletion (e.g., Fas:Fas ligand binding); (c) anergy (e.g., Signal 1 in the absence of Signal 2); (d) negative regulatory pathways (e.g., CTLA-4 and PD-1); and (e) active suppression, in which regulatory T cells inhibit the functions of T cells through cell:cell contact-dependent mechanisms or secretion of cytokines (IL-10 and TGFβ). MHC, major histocompatibility complex; APC, antigen-presenting cell.

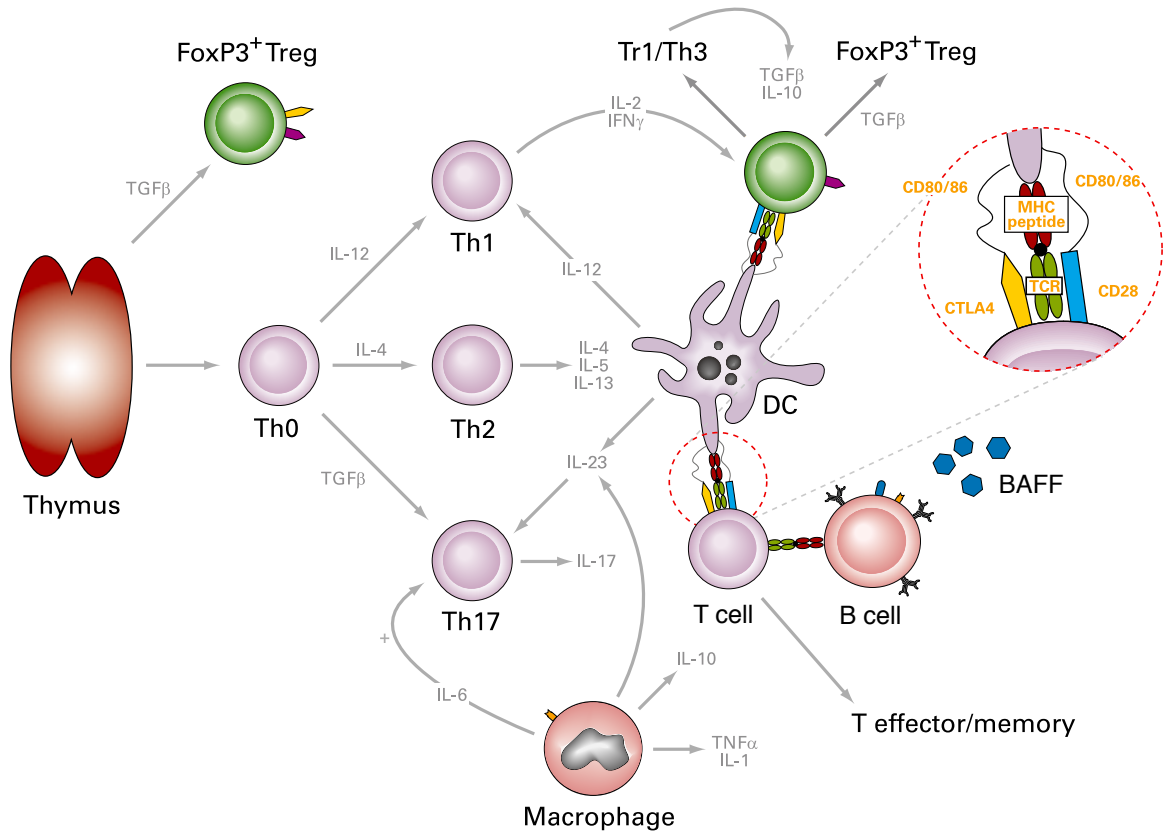


Figure 2

Potential targets for tolerance-inducing therapies. T cells and subsets of T cells (Th1, Th2, and Th17) are potential targets for intervention in tolerance-inducing strategies. Importantly, many different receptor:ligand costimulatory pathways can regulate T cell activation and may be modulated by specific agonists and antagonists. The activated dendritic cell (DC) and macrophage are the predominant professional antigen-presenting cells (APCs) and may be the focus of a tolerance intervention because of their central role in innate and adaptive immunity. The extent of T cell activation is counterbalanced by regulatory cells. Therapies that boost the function of regulatory cells may restore an imbalance of the immune system and downregulate a T cell-driven inflammatory response. Expansion of self-reactive B cells may stimulate autoimmunity, a process that may be potentially inhibited by blockade of BAFF or BAFF-R. In addition, TLR antagonists and cytokine inhibitors may downregulate certain types of inflammatory responses and promote a tolerant state. See text for further details.



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Errata

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