Current and Future Immunomodulation Strategies to Restore Tolerance in Autoimmune Diseases

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Autoimmune diseases reflect a breakdown in self-tolerance that results from defects in thymic deletion of potentially autoreactive T cells (central tolerance) and in T-cell intrinsic and extrinsic mechanisms that normally control potentially autoreactive T cells in the periphery (peripheral tolerance). The mechanisms leading to autoimmune diseases are multifactorial and depend on a complex combination of genetic, epigenetic, molecular, and cellular elements that result in pathogenic inflammatory responses in peripheral tissues driven by self-antigen-specific T cells. In this article, we describe the different checkpoints of tolerance that are defective in autoimmune diseases as well as specific events in the autoimmune response which represent therapeutic opportunities to restore long-term tolerance in autoimmune diseases. We present evidence for the role of different pathways in animal models and the therapeutic strategies targeting these pathways in clinical trials in autoimmune diseases.

Autoimmune diseases are debilitating conditions that affect a large and growing portion of the population (~3%–5% in the United States) (Jacobson et al. 1997). Autoimmune diseases take a devastating toll on affected families and have a considerable economic impact. Thus, improving the understanding of autoimmune diseases and developing novel therapies have been significant goals in public health. The development of autoimmune diseases reflects a loss of tolerance of the immune system for self-antigens. With the exception of a few rare monogenic diseases such as immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX), and autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) syndrome, the development of autoimmunity is a complex and multifactorial process. This process usually involves genetic predispositions and poorly defined environmental factors that result in slight alterations in many different checkpoints, which in turn tilts the balance toward autoreactivity and away from immunoregulation. Although clearly there are key roles for B cells, antigen-presenting cells (APCs), and the innate immune response in...
the development and progression of autoimmune diseases, this article will focus on autoreactive T cells and potential targets of tolerogenic treatments (Fig. 1). In addition, we will discuss selected strategies currently available or being developed in the clinic as well as future opportunities to prevent and treat these diseases. Finally, current clinical strategies available as the standard of care for autoimmune diseases rely on immunosuppressive and anti-inflammatory treatments that curtail the pathological events, alleviate symptoms, and provide short-term relief in some patients. Thus, we will focus for the most part on...
immunotherapies aimed at reestablishing long-term tolerance.

**PATHOGENESIS OF AUTOIMMUNE DISEASES AND POTENTIAL TARGETS FOR REESTABLISHING IMMUNE TOLERANCE**

Different checkpoints are in place to ensure immune tolerance to self-antigens and prevent damage to tissues (Goodnow et al. 2005). Many potentially autoreactive T-cell receptors (TCRs) are excluded in central lymphoid organs by V(D)J recombination and deletion/cell death in the thymus and periphery. These mechanisms generally aim at eliminating cells with high affinity for self-antigens, although thymocytes with a repertoire skewed toward autoreactivity may actually be selected into the CD4⁺Foxp3⁺ regulatory T-cell (Treg) lineage. Thymocytes are selected by recognition of peptide-major histocompatibility complex (MHC) complexes presented on specialized APCs. The predominant association of given MHC haplotypes with susceptibility or resistance to many autoimmune diseases in both mice and humans, including type 1 diabetes (T1D), multiple sclerosis (MS), and rheumatoid arthritis (RA) (Wellcome Trust Case Control 2007), illustrates the importance of this process. Additionally, the autoimmune regulator (Aire) protein is crucial for the expression and presentation of tissue-specific antigens by medullary thymic epithelial cells (mTECs) during negative selection of potentially autoreactive thymocytes (Anderson et al. 2002). Negative selection of autoreactive T cells in the thymus is governed by quantitative factors such as the level of expression of self-antigens and intensity of TCR signaling as well as qualitative parameters such as the molecular nature of selecting peptide/MHC complexes. Furthermore, the molecular intricacies of self-antigen presentation in the thymus and periphery greatly influence the fate of autoreactive T cells (Stadinski et al. 2010a).

Many T cells with potentially autoreactive receptors escape thymic selection and can be readily detected in healthy individuals, which requires the existence of powerful mechanisms to control these autoreactive T cells and maintain peripheral tolerance in the majority of the population. Autoreactive T cells can be controlled by intrinsic and extrinsic mechanisms. Intrinsic control of autoreactive T cells is regulated by a complex network of costimulatory and inhibitory molecules that have differential effects on T-cell activation, expansion, migration, and effector function (Bour-Jordan et al. 2011). To be efficiently activated, T cells need to receive a “signal 1” provided by the TCR on recognition of cognate peptide/MHC complexes and a “signal 2” provided by costimulatory molecules such as CD28. Defective costimulation prevents T-cell activation and can lead to unresponsiveness, making it an attractive therapeutic strategy that is actively pursued in autoimmune diseases through blockade of costimulatory pathways or administration of self-antigens in the context of suboptimal costimulation. Inhibitory receptors such as cytotoxic T-lymphocyte antigen-4 (CTLA-4 or CD152) and programmed death-1 (PD-1 or CD279) exert a nonredundant intrinsic control on autoreactive T cells and are critical for the maintenance of peripheral tolerance (Fife and Bluestone 2008). Another important aspect of the autoimmune response is the migration of autoreactive T cells and trafficking into their target tissues. These processes are regulated by cell-surface molecules including integrins, selectins, chemokine receptors, and sphingosine-1-phosphate receptors and numerous therapies already approved or in clinical trials for autoimmune diseases target these pathways (Yopp et al. 2004). Finally, both CD4⁺ and CD8⁺ T cells have been shown to mediate autoimmune diseases, and the tissue damage inherent to autoimmunity can be mediated by a range of proinflammatory cytokines produced by innate cells or T cells (McFarland and Martin 2007; Bluestone et al. 2010). Thus, immunotherapies currently in development are targeting proinflammatory cytokines and other mediators of inflammation. Besides improving clinical parameters on administration, some of these approaches may promote long-term tolerance by dampening the inflammatory milieu that is deleterious for immunoregulatory mechanisms (Koulmanda and Strom 2010).
In addition to intrinsic control of autoreactive T cells, specialized suppressor cell populations (including regulatory B cells, dendritic cell [DC] subsets, other innate cells, and T-cell subsets) are crucial to maintain peripheral tolerance and prevent autoimmunity. Tregs control autoreactive T cells in the periphery (Wing and Sakaguchi 2010). Thymically derived CD4⁺ CD25⁺ T cells, often referred to as natural Tregs (nTregs), express the lineage-specific transcription factor Foxp3. Fatal multiorgan autoimmunity develops in “scurfy” mice or IPEX patients that have deficiency or loss-of-function mutations in the Foxp3 gene (Fontenot et al. 2003). In humans, many autoimmune diseases are associated with a lower frequency of circulating Tregs and alterations in suppressive function or molecular pathways that regulate Treg biology (Brusko et al. 2008). Additionally, several immune genes that have been identified as susceptibility alleles for autoimmune diseases, such as CTLA-4, IL-2, CD25, or PTPN22, are involved in pathways important for Treg homeostasis and function (Maier and Hafler 2009). Thus, many molecules that control conventional T cells are also expressed on Tregs and have important biological relevance for this subset. Tregs have a unique and highly robust therapeutic profile: Although the requirement for specific TCR-mediated activation limits Treg suppressive activity to the site or lymph node (LN) of interest, their effector function appears to work by bystander suppression and infectious tolerance and results in the regulation of local inflammatory responses through a combination of cell–cell contact and suppressive cytokine production (Tang and Bluestone 2008). Among their mechanisms of action, immunoregulatory cytokines transforming growth factor-β (TGF-β), IL-10, and IL-35 have been shown to participate in Treg suppressive function and direct contacts of Tregs with DCs are also important for Treg-mediated regulation through pathways involving interactions of CTLA-4 on Tregs with B7 on APCs. When measurable, increases in these parameters may represent surrogate read-outs for the efficiency of immunomodulatory treatments in restoring immunoregulation and peripheral tolerance.

**SYSTEMIC IMMUNOTHERAPIES TO RESTORE THE BALANCE OF PATHOGENIC versus REGULATORY CELLS**

Autoimmunity stems from an imbalance in pathogenic versus regulatory cell populations that result from defects in both populations. Thus, therapies targeting the T-cell compartment will likely be most efficacious at restoring tolerance if they influence the balance by concomitantly reducing the pathogenic T-cell population and enhancing the numbers or function of suppressive pathways (Fig. 2). Several strategies that affect T cells in a systemic manner are being developed to achieve this goal and have shown promising results in clinical trials. Two related reagents known as antilymphocyte serum (ALS) and antithymocyte globulin (ATG) target polyclonal T cells nonspecifically and result in massive but transient T-cell deletion (van de Linde et al. 2006). In the nonobese diabetic (NOD) model, ATG efficiently reverted disease in new-onset diabetic mice, possibly owing to preferential reconstitution of the peripheral T-cell compartment by Tregs (Simon et al. 2008). In humans, in addition to depletion of conventional T cells, ATG has been shown to expand Treg populations both by conversion of CD4⁺CD25⁻ cells into Tregs and, to a lesser degree, proliferation of nTregs (Lopez et al. 2006; Feng et al. 2008), supporting a potential tolerogenic profile for ATG therapy in autoimmune diseases. A clinical trial of ATG in a small number of T1D patients was beneficial for preservation of islet function but accompanied by side effects associated with a “cytokine storm” (Saudek et al. 2004). A phase II trial supported by the immune tolerance network is currently underway to determine the effects of a different dosing protocol of ATG in T1D, with the hope of reducing side effects while preserving or maximizing the clinical benefits. The immune tolerance network (ITN—www.immunetolerance.org) is an international consortium created with the goal of achieving clinical tolerance in autoimmunity, transplantation, allergy, and asthma, and it has sponsored many clinical trials of various tolerogenic treatments in autoimmune diseases.
diseases, some of which are described in this article.

The anti-CD3 antibody OKT3 was the first FDA-approved mAb with initial applications in transplantation. In the NOD model, anti-CD3 mAbs restored normoglycemia in new-onset diabetic mice, generating interest in using this strategy in humans (Chatenoud et al. 1997). Second-generation FcR-nonbinding (FNB) reagents have been developed and FNB anti-CD3 treatment was efficacious at reversing diabetes in NOD mice and ameliorating disease in the MS model, experimental autoimmune encephalomyelitis (EAE) (Kohm et al. 2005). In humans, humanized FNB mAb OKT3γ1ala-ala (teplizumab) retained the beneficial effect of OKT3 on renal transplantation without the deleterious cytokine storm side effect (Woodle et al. 1999). Treatment of T1D patients with a short course (12–14 d) of teplizumab within 6 wk of diagnosis significantly reduced the loss of C-peptide for up to 2 yr but ultimately could not maintain the residual β-cell function (Herold et al. 2002, 2009). Similar results were obtained using another FNB anti-CD3 mAb called ChAglyCD3 (otelixizumab) (Keymeulen et al. 2005). Nevertheless, the observation of clinical benefits that lasted much longer than the short course of treatment suggested that FNB anti-CD3 mAbs may have tolerogenic properties, and an ITN-sponsored phase II clinical trial is underway in T1D patients to determine whether β-cell function will be prolonged by administration of a second course of teplizumab after 13 mo. Teplizumab is also being evaluated in clinical trials for psoriasis and psoriatic arthritis. Teplizumab does not significantly deplete T cells but may have tolerogenic properties owing to altered TCR signaling, although the mechanisms underlying the efficacy of teplizumab in humans are still not fully defined. In mice, FNB anti-CD3 mAbs preferentially induce deletion and anergy of effector T cells (T eff ), in particular, potentially pathogenic Th1 cells, whereas CD4\(^+\) Tregs are preserved, resulting in a shift in the T eff:Treg ratio (Belghith et al. 2003; Penaranda et al. 2011). FNB anti-CD3 mAbs also increased expression of the transcription factor Helios (originally described as a marker for more stable nTregs) on remaining Tregs, suggesting that treatment may alter the Treg transcriptome and possibly favor the stability of the Treg lineage, an important point given recent concerns about the instability of a subset of Tregs that may be associated with autoimmunity in mice and humans (Zhou et al. 2009;
In T1D patients, teplizumab treatment in T1D patients has been associated with the induction of a putative regulatory population of CD8\(^+\)Foxp3\(^+\) cells and an increase ratio of IL-10 to IFN-\(\gamma\) production, which is compatible with increased immunoregulation (Herold et al. 2003). Additionally, a recent study in humanized mice and T1D patients suggested that teplizumab induced gut tropic CD4\(^+\)Foxp3\(^+\)CCR6\(^+\) Tregs, which produced IL-10 on migration to the gut and subsequently returned to the circulation (Waldron-Lynch et al. 2012). Finally, oral administration of low doses of the “classical” form of anti-CD3 mAbs efficiently suppressed active forms of EAE, autoimmune diabetes, and other autoimmune diseases in mice without the toxicity associated with IV injection, possibly owing to the induction of CD4\(^+\)CD25\(^-\)LAP\(^+\)Th3 suppressor cells in gut-associated tissues (Ochi et al. 2006). These encouraging data have prompted the initiation of clinical trials of oral OKT3, currently for the treatment of active ulcerative colitis.

IL-2 plays a central role in the expansion and survival of CD4\(^+\) and CD8\(^+\) antigen-specific effector and memory T cells. However, IL-2 is also critical to support the development, survival, and suppressive function of Tregs (Cheng et al. 2011). Thus, the fatal multorgan autoimmune disease that develops in mice deficient in IL-2, and in mice or humans with deficient IL-2-receptor (IL-2R) chains, is primarily owing to a defective Treg compartment (Malek et al. 2002). In autoimmune diabetes, deficient IL-2 production by effector T cells locally in pancreatic islets and suboptimal IL-2 signaling in Tregs have been described in NOD mice and T1D patients, respectively, and may contribute to the loss of peripheral tolerance and development of autoimmunity (Tang et al. 2008; Long et al. 2010). Thus, the IL-2 pathway is an attractive target for immunomodulation of autoimmune diseases aiming at restoring effective Treg-mediated tolerance but proper dosing protocols will have to be carefully evaluated, particularly in view of preclinical murine studies showing that low-dose versus high-dose IL-2 therapy may preferentially induce a relative increase in Tregs versus Teff populations, respectively (Bozman et al. 2006; Tang et al. 2008). Alternatively, IL-2 may be combined with therapies targeting pathogenic T cells. Rapamycin (Sirolimus and its analog Temsirolimus) is an mTOR inhibitor that selectively inhibits Teff proliferation, notably in the Th1 and Th17 subsets (Delgoffe et al. 2011). Importantly, rapamycin has been shown to promote Treg survival and function and results in a relative enrichment in Tregs in mice and humans (Battaglia et al. 2005; Delgoffe et al. 2009). Rapamycin is being extensively developed in transplantation owing to its tolerogenic profile and clinical trials are also underway in MS and systemic lupus erythematosus (SLE). Treatment with rapamycin was previously shown to increase Treg function in a small number of T1D patients (Monti et al. 2008). In NOD mice, a combination of rapamycin and IL-2 afforded long-term protection against diabetes (Rabinovitch et al. 2002). A phase I trial sponsored by the ITN and designed to restore the balance of effector and regulatory T cells in T1D by administration of rapamycin and IL-2 recently concluded with encouraging results (Long et al. 2012). In this trial, new-onset diabetic patients treated with rapamycin plus IL-2 displayed a transient increase in Treg frequency and a stable restoration of STAT5 phosphorylation on IL-2 signaling in Tregs, which is defective in T1D patients (Long et al. 2010). Unfortunately, patients displayed a dramatic increase in natural killer (NK) cells and a transient decrease in \(\beta\)-cell function. Thus, although IL-2 therapy may have beneficial consequences on Tregs, its effects on NK cells and the relative inefficiency of rapamycin in this trial highlighted the necessity to better define the primary target of treatment and design alternative strategies to selectively expand Tregs.

Several systemic therapies that are not directly targeting T cells have shown efficiency and tolerogenic properties in T-cell-mediated autoimmune diseases. B cells are critical for the presentation of self-antigens and production of autoantibodies, and depleting B cells reduced disease in preclinical models of T1D and MS (Hu et al. 2007; Monson et al. 2011).
Many clinical trials of the B-cell-depleting drug rituximab are currently underway in a variety of autoimmune diseases, including prototypical T-cell-mediated diseases such as T1D and MS. Phase II and III clinical trials in RA, MS, and T1D have shown promising results and a potentially tolerogenic effect of rituximab as evidenced by improvement of disease for almost a year with a short-course treatment with rituximab (Edwards et al. 2004; Hauser et al. 2008; Pescovitz et al. 2009). Combination of rituximab with methotrexate or administration of two courses of rituximab showed improved efficacy in RA (Tak et al. 2011). The mechanisms underlying the efficacy and tolerogenic potential of rituximab in autoimmune diseases are not well defined. In mouse models expressing a human CD20 transgene, improvement of T1D and EAE was associated with the induction of regulatory cell populations, including myeloid-derived suppressor cells (MDSCs) and Tregs, and reduced responsiveness of self-antigen-specific Teff (Monson et al. 2011; Hu et al. 2012).

Finally, intravenous immunoglobulins (IVIG) may promote tolerance in autoimmune diseases. IVIG are therapeutic preparations of normal human polyclonal IgG purified from large donor plasma pools and they are widely used for the treatment of autoimmunity and inflammation. IVIG therapy has shown clinical benefits and is currently in phase II/III clinical trials in several autoimmune diseases, including relapsing–remitting MS (RRMS), myasthenia gravis, and T-cell-mediated peripheral neuropathies Guillain–Barré syndrome and chronic inflammatory demyelinating polyneuropathy (CIDP) (Arnson et al. 2009). Long-term IVIG therapy consists of recurrent high doses but the efficacy of maintenance therapy with lower doses or treatment discontinuation has not been evaluated. The modes of action of IVIG are not completely understood but they are complex and involve immunoregulation of various cell types and molecular pathways (Nimmerjahn and Ravetch 2008). IVIG contain natural antibodies with reactivity to T-cell-surface molecules such as TCR and CD4, and IVIG has been shown to inhibit T-cell activation and proliferation either directly or indirectly through modulation of DC function (Bayry et al. 2003; MacMillan et al. 2009). Importantly, recent reports have shown that IVIG could bind human Tregs and promoted Treg-mediated suppression via mechanisms that may include F(ab′)2-dependent direct stimulation of Tregs and activation of Tregs by Treg-specific MHC class II-restricted epitopes (“Tregitopes”) from the Fc fragment of IgG (Kessel et al. 2007; De Groot et al. 2008; Tha-In et al. 2010). In mice, IVIG treatment induced the expansion of nTregs in vivo and afforded protection from EAE or allograft rejection in a Treg-dependent manner (Ephrem et al. 2008; Tha-In et al. 2010). Thus, IVIG may have tolerogenic properties that are worth pursuing clinically in autoimmune diseases, notably in combination with B-cell-depletion strategies given the primary indication of IVIG as an immunoglobulin replacement therapy.

**ANTIGEN-SPECIFIC IMMUNOTHERAPY**

Antigen-specific tolerogenic therapies are appealing from a safety point of view because they are not expected to induce global immunosuppression like systemic approaches. Conversely, it is still not clear whether induction of tolerance to a small number of dominant self-antigens will efficiently control autoimmunity after epitope spreading has occurred. Encouraging results from animal models have shown that induction of tolerance to a single self-antigen can efficiently thwart a polyclonal autoreactive response owing to mechanisms of “bystander suppression” and dominant antigen nonspecific immunoregulation (Tang et al. 2004; Tarbell et al. 2004; Fife et al. 2006). Another potential caveat for antigen-specific therapy consists in the difficulty in choosing the appropriate protein or peptide in view of recent reports showing that the molecular intricacies of self-antigen presentation related to the unique flexibility of certain self-peptides to bind to certain MHC class II molecules could greatly affect the generation and activation of autoreactive T cells. As an example, I-Aβ, the only class II allele expressed in the nonobese diabetic (NOD) mouse, a spontaneous model for multiple autoimmune
diseases, is essential for the development of autoimmunity (Wicker et al. 1995). Determination of the crystal structure of peptide/MHC complexes uncovered that the binding groove of I-A\textsuperscript{B7}, the only class II allele and strongest susceptibility gene for diabetes in NOD mice, as well as of the highly disease-associated HLA-DQ8 in humans, is distinct from other MHC class II molecules at both terminal ends and is thus permissive for unique peptide-MHC structural interactions (Corper et al. 2000; Latek et al. 2000). Insulin is a key autoantigen in T1D in humans and NOD mice, and a peptide from the insulin β chain (B:9–23) is a major target of autoreactive CD4\textsuperscript{+} T cells (Zhang et al. 2008). This peptide can bind the peptide-binding groove of I-A\textsuperscript{B7} molecules in at least three overlapping adjacent positions (or “registers”), and B:9–23 peptide registers may be distinct in the thymus and pancreas (Levisetti et al. 2007; Stadinski et al. 2010c). Discrete T-cell populations have been identified that recognize the insulin peptide in each register (Mohan et al. 2010, 2011; Crawford et al. 2011). A subset of T cells recognizes a register generated by intracellular processing of insulin by APCs in the thymus and periphery, and these T cells are efficiently depleted in the thymus, whereas distinct peptide-binding registers may be generated from soluble peptides present in vesicles of APCs in the pancreas. This finding may help to explain how self-reactive T cells specific for insulin peptides can escape thymic negative selection. Similarly, dominant epitopes of self-antigens chromogranin A in T1D and myelin basic protein (MBP) in MS and EAE bind the peptide-binding groove in an unusual register that would have been predicted to result in very poor binding or no binding (He et al. 2002; Stadinski et al. 2010b). Importantly, TCRs from MBP-specific T cells in MS patients have been shown to interact with MBP peptides bound to MHC class II molecules in a very unusual position (Li et al. 2005), suggesting that self-antigenic peptides may be recognized in atypical registers in humans as well. Other mechanisms have been described for the generation of new epitopes specifically in peripheral tissues or conditions of local inflammation and may contribute to the escape of autoreactive T cells from thymic deletion and their activation in the periphery in RA, celiac disease, MS, and T1D (Anderton 2004; Goverman 2011). These recent advances have tremendous implications for the design of antigen-specific immunotherapies and may explain the disappointing results obtained in clinical trials to date.

Several approaches have been used to induce tolerance to self-antigens in preclinical models. Oral or nasal administration of soluble self-antigens efficiently prevented several autoimmune diseases in animal models, including EAE and colitis, through mucosal tolerance pathways of anergy, deletion, or induction of TGF-β- or IL-10-producing Tregs (Karpus et al. 1996). However, attenuation or reversal of ongoing disease has been less successful (Kennedy et al. 1997; Bai et al. 1998). High-dose tolerance achieved by intravenous injection of soluble peptides or DNA vaccination has shown some success in mouse models of T1D (Coon et al. 1999), but anaphylactic responses and exacerbation of disease have also been observed in the NOD mouse and EAE models (Weaver et al. 2001; Smith et al. 2005), raising concerns about the safety of this approach in patients. Altered peptide ligands (APLs) are derived from dominant peptides driving the autoimmune response and modified to alter interactions with autoreactive TCRs, thus functioning as antagonists or partial agonists of T-cell signaling (Nicholson and Kuchroo 1997). APLs of myelin epitopes and the clinically relevant APL glatiramer acetate prevent and reverse disease in EAE models, through immune deviation of Th1 or Th17 responses to Th2 responses and a role of immunosuppressive cytokines TGF-β and IL-10 (Nicholson et al. 1995; Maron et al. 2002). Finally, it was recently shown that nanoparticles coated with complexes of MHC class I molecules bound to peptides from self-antigens insulin or islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP) could reverse disease in NOD mice and in a humanized model of T1D through the expansion of autoreactive regulatory CD8\textsuperscript{+} T cells that are naturally generated during the autoimmune process (Tsai et al. 2010).
In humans, antigen-specific therapies have been evaluated in clinical trials in MS and T1D patients. In MS, antigen-specific approaches usually target myelin antigens MBP, proteolipid protein (PLP), and myelin oligodendrocyte glycoprotein (MOG), which are believed to be major targets of the T- and B-cell response. Results from a phase III study evaluating the efficacy of intravenous injection of MBP8298, an MBP peptide containing immunodominant T- and B-cell epitopes in HLA-DR2 patients, in secondary progressive MS showed no clinical benefit compared with placebo (Freedman et al. 2011). Phase I/II trials of DNA vaccination with full-length MBP in secondary progressive and relapsing–remitting MS showed decreased myelin-specific T-cell responses and autoantibodies but no significant improvement of clinical parameters (Bar-Or et al. 2007; Garren et al. 2008). Clinical trials using APLs of the immunodominant MBP epitope yielded mixed results. Adverse effects including exacerbation of disease and hypersensitivity reactions were observed with high doses of APLs and halted one of the trials out of safety concerns (Bielekova et al. 2000; Kappos et al. 2000). However, some improvement was observed in patients receiving the lower doses of APLs. The APL glatiramer acetate, a random mixture of amino acids glutamine, lysine, alanine, and tyrosine peptides of various lengths, did not provide any significant benefit in primary progressive MS but showed some efficacy in reducing the frequency of relapses and decreasing disability progression in RRMS, and it is FDA approved for this indication (Wolinsky et al. 2007; Ford et al. 2010).

A number of autoantigens have been characterized in T1D patients, including insulin, GAD65, and IGRP (Bluestone et al. 2010). Insulin has been used for immunotherapy in newly diagnosed patients and prevention studies. Several phase I and II trials were performed in new-onset diabetic patients using oral insulin or NBI-6024, an APL for the dominant 9–23 insulin B chain epitope, with no clinical efficacy (Chaillous 2000; Walter et al. 2009). In the Diabetes Prevention Trial-Type 1 Diabetes (DPT-1) study, relatives of T1D patients positive for islet-cell autoantibodies and deemed at relatively high risk for developing disease were treated with IV plus subcutaneous (sc) or oral insulin therapy and followed for the diagnosis of diabetes (DPT-1-Diabetes-Study-Group 2002). Insulin therapy did not delay or prevent diabetes in the DPT-1 study, with the exception of some clinical benefits in a subgroup with high levels of anti-insulin autoantibodies. A new trial is ongoing to specifically test insulin therapy in this subpopulation. A large prevention trial of intranasal insulin in at-risk individuals also showed no effect on the onset of diabetes (Nanto-Salonen et al. 2008). Antigen therapy using alum-formulated GAD65 (GAD-alum) in newly diagnosed T1D patients was recently shown in a phase III trial to have no effect on the decline in stimulated C-peptide levels or any other secondary outcome despite some encouraging results in previous phase II trials (Wherrett et al. 2011; Ludvigsson et al. 2012). Finally, the most promising results for antigen therapy in T1D thus far have been obtained in recently diagnosed patients after SC administration of DiaPep277, a peptide from the heat shock protein HSP60, which injection preserves islet-cell function in NOD mice (Elías and Cohen 1996). Phase I/II trials showed that DiaPep277 therapy was safe but had no clinical benefit in children (Lazar et al. 2007). In adults, DiaPep277 was shown in phase II trials to have some effects on preservation of C-peptide levels but no accompanying benefits on HbA1c levels or insulin requirements (Raz et al. 2007; Schloot et al. 2007). Two multicenter phase III trials are currently underway.

Overall, antigen-specific immunotherapy for the prevention or treatment of autoimmune diseases has been largely disappointing and failed to reproduce the efficacy observed in preclinical animal models and small human clinical trials. This may reflect the fact that antigen therapy in humans occurs in the context of a more complex immunological environment and higher exposure to proinflammatory signals compared with laboratory animals. Additionally, it has been shown in T1D patients receiving pancreas transplantation that effector and memory autoreactive T cells may be resistant to deletion.
or suppression, suggesting that antigen therapy may need to be combined with systemic approaches to eliminate memory populations before antigen administration (Laughlin et al. 2008; Vendrame et al. 2010). Finally, the presence of unique peptide registers specifically in tissues suggests that the cognate peptides involved in activation of autoreactive T cells in the periphery may have to be identified to develop efficacious antigen therapy.

IMMUNOMODULATION OF T-CELL COSTIMULATION, MIGRATION, AND INFLAMMATION

Immunotherapies targeting costimulatory and migration pathways have been developed with the rationale that they may preferentially target pathogenic T cells during an active autoimmune disease. The costimulatory molecule CD28 is expressed on T cells and its interaction with B7-1 and B7-2 is critical for many aspects of T-cell activation and effector function (Salomon and Bluestone 2001). Importantly, blocking CD28/B7 interactions during TCR signaling can result in tolerance mediated by T-cell deletion and/or anergy, which is particularly relevant from a therapeutic point of view to restore tolerance in autoimmune diseases (Bluestone et al. 2006). In mouse models, blockade of CD28 signaling using a CTLA4Ig fusion protein was extremely effective at controlling many autoimmune diseases including MS and SLE (Scalapino and Daikh 2008). The central role of CD28 in Treg homeostasis also led to the unexpected observation that treatment with CTLA4Ig significantly depleted Tregs and exacerbated diabetes in NOD mice (Salomon et al. 2000), emphasizing that immunomodulation of costimulatory pathways can adversely affect the balance of effector versus regulatory T cells in the context of a given immune response and local environment. CTLA4Ig (abatacept or the higher-affinity second-generation variant belatacept) has shown an >50% response rate in psoriasis and RA patients resistant to TNF-α-blocking therapy and is FDA approved for RA and (JIA) (Abrams et al. 1999; Genovese et al. 2005). In contrast, abatacept failed to reduce disease flares in a phase II clinical trial in SLE patients also receiving oral corticosteroids, and a phase III trial in Crohn’s disease was terminated early owing to a lack of efficacy (Merrill et al. 2010). In a recent multicenter trial in newly diagnosed T1D patients, treatment with abatacept for 2 years was well tolerated and delayed the reduction in β-cell function compared with placebo (Orban et al. 2011). However, the decrease in C-peptide levels with abatacept was parallel to that with placebo after 6 months of treatment, suggesting that the progression of disease became independent of CD28-dependent T-cell activation a few months after diagnosis and before the complete loss of β-cell function. Thus, the efficacy of CTLA4Ig in autoimmune diseases may be limited to CD28-dependent phases of disease, which could reflect the fact that some T cells are not as dependent on CD28 costimulation as naïve CD4+ T cells, notably memory T cells and CD8+ T cells. Clinical trials of abatacept and belatacept are presently underway in MS, SLE, and T1D. Current regimen of CTLA4Ig did not result in widespread immunosuppression or increased rates of infection, a clear benefit in the treatment of autoimmune diseases. Additionally, analysis of kidney allograft recipients treated with belatacept showed no long-term difference in the number of Tregs (Bluestone et al. 2008). These results contrast with murine studies, perhaps owing to a lower dosing regimen in humans that may preferentially affect T eff over Tregs. Should concerns arise about Treg homeostasis after CTLA-4-Ig treatment in future trials, single blockade of B7-2 may be considered as an alternative because it was most efficient at reducing the activation of autoreactive T cells while minimally affecting the Treg compartment (Bour-Jordan et al. 2004; Bour-Jordan and Bluestone 2009).

Alefacept is an LFA-3-Ig fusion protein that blocks the interaction of LFA-3 on APCs with costimulatory molecule CD2 on T cells. Alefacept has shown efficacy in reducing lesions in a phase III trial of patients with psoriasis and is currently FDA approved for this indication (Sugiyama et al. 2008). Alefacept is being evaluated in an ITN-sponsored phase II trial in patients...
with new-onset T1D. Basiliximab and daclizumab are two similar mAbs targeting the high-affinity α chain of the IL-2 receptor, CD25. Daclizumab reduced the number of lesions and improved the clinical scores in RMS either as an adjunct therapy to IFN-β or in patients’ refractory to IFN-β treatment (Bielekova et al. 2009; Wynn et al. 2010). Importantly, CD25 blockade prevents expansion of alloreactive T cells in transplantation but also leads to Treg depletion and widespread autoimmunity in mice. Reduction in both frequency and function of Tregs has been observed after basiliximab and daclizumab treatment (Bluestone et al. 2008; Oh et al. 2009), raising concerns about the use of these reagents in immunotherapies designed to restore immune tolerance. Finally, interactions of CD154 (aka CD40L) on T cells and CD40 on APCs are important for the stimulation of autoreactive T cells, activation of APCs, and production of autoantibodies (Grewal and Flavell 1996). Therapeutic blockade of this pathway held tremendous promise in the 1990s owing to its high efficacy and tolerogenic potential in preclinical models of autoimmune diseases and transplantation (Law and Grewal 2009). Clinical trials of anti-CD154 monoclonal antibodies (mAbs) were initiated in several autoimmune diseases including SLE, Crohn’s disease, psoriasis, and MS but had to be halted owing to an unexpectedly high number of thromboembolic events. Novel antagonistic reagents are being developed against CD40 and CD154 to avoid these adverse effects and if their safety profile is adequate, they will undoubtedly be tested as part of the therapeutic arsenal to restore tolerance in autoimmune diseases.

Another class of immunomodulatory agents targets molecular pathways involved in leukocyte migration to the peripheral tissues affected by the autoimmune response. Importantly, although some of these therapies are primarily preventing the migration of pathogenic T cells to the site of inflammation, others, alone or in combination with other immunosuppressive drugs, have tolerogenic properties that may be related to the role of anatomic localization of antigen presentation in the outcome of T-cell priming versus unresponsiveness (Yopp et al. 2004). Indeed, T-cell homing to the LN is required for the generation of antigen-specific Tregs and induction of peripheral tolerance after immunotherapy in murine models of transplantation (Bai et al. 2002; Ochando et al. 2005). Thus, T-cell trafficking and LN occupancy during systemic immunosuppression are crucial determinants for the induction and maintenance of tolerance. As a consequence, immunotherapies targeting T-cell migration in autoimmune diseases could be key components of combinatorial approaches to promote homing of autoreactive T cells to LNs and their tolerization during immunosuppression. For example, in stringent mouse models of transplantation, anti-LFA-1 monotherapy was not tolerogenic but combination with anti-CD154 or anti-ICAM-1 mAbs synergistically induced donor-specific tolerance (Nicolls and Gill 2006). Anti-integrin mAbs natalizumab (against the α4 subunit of α4β1 and α4β7 integrins) and efalizumab (anti-LFA-1) are among the most successful drugs designed for cell-trafficking blockade, with indications in RRMS, Crohn’s disease, and psoriasis (Dubertret et al. 2006; Ransohoff 2007; Targan et al. 2007). Natalizumab reduced the rate of relapses and progression of disability in RRMS and increased rates of remission in Crohn’s disease. Unfortunately, cases of progressive multifocal leukoencephalopathy (PML) were associated with treatment, resulting in additional label warning for natalizumab and voluntary withdrawal of efalizumab by the manufacturer (Hartung 2009). Natalizumab is FDA approved in RRMS and Crohn’s disease for patients with severe disease or disease refractory to other standard treatments, but concerns remain about the safety profile of this drug.

Remarkable clinical results have been obtained after treatment with fingolimod (FTY720), a small compound affecting T-cell trafficking by interfering with the sphingosine pathway, which is critical for lymphocyte egress from the thymus and peripheral lymphoid organs (Mallolbien et al. 2004). Indeed, fingolimod results in down-regulation of S1P receptors and lymphocyte sequestration in lymphoid organs (Mandala et al. 2002), and it efficiently prevented or
attenuated disease in NOD mice, EAE, collagen-induced arthritis, and colitis (Webb et al. 2004; Maki et al. 2005). In phase III trials in RRMS patients, fingolimod improved the relapse rate and disability progression after 1 to 2 years as compared with placebo and even standard therapy, IFN-β (Cohen et al. 2010; Kappos et al. 2010). Fingolimod thus became the first oral therapy approved for the treatment of MS. However, adverse effects associated with fingolimod therapy were significant and reflected its systemic immunosuppressive properties. Second-generation reagents targeting the sphingosine pathway are being developed with possibly reduced side effects (Bagdanoff et al. 2010). Clinical trials of fingolimod and second-generation drugs are being conducted for several autoimmune diseases. The mechanisms of action of FTY720 are still unclear. FTY720 has been suggested to induce T-cell apoptosis and to increase Treg numbers and function (Sawicka et al. 2005; Daniel et al. 2007). In NOD mice, treatment with FTY720 at a time of significant insulitis prevented diabetes by sequestering T cells into tertiary lymphoid organs (TLOs) in the pancreas, but treatment withdrawal resulted in a rapid loss of TLO integrity and development of diabetes (Penaranda et al. 2010). Thus, despite its clinical benefits, fingolimod monotherapy may not be efficacious beyond the duration of treatment, and combination of fingolimod with immunosuppressive therapies that promote immunoregulation within the LNs may be optimal to restore long-term tolerance. In this regard, FTY720 plus antigen therapy, but not FTY720 alone, efficiently suppressed disease on discontinuation of treatment in an EAE model (Yoshida et al. 2011).

Finally, there is a renewed interest in immunotherapies targeting proinflammatory cytokines or other mediators of inflammation in autoimmune diseases, not only to improve clinical parameters but also to restore tolerance. Indeed, in addition to inducing tissue damage, proinflammatory mediators may be a barrier to the restoration of tolerance by altering the balance of pathogenic versus immunoregulatory cell populations, in particular at the site of inflammation, owing to their role in the differentiation and stability of T-cell subsets (Hanidzjar and Koulmanda 2010). For example, several TNF-α antagonists are currently approved in autoimmune diseases, including RA, psoriasis, and Crohn’s disease and they may have tolerogenic properties (Sfikakis 2010). Increased spontaneous apoptosis and defective suppressive function have been described in Tregs isolated from RA patients. Anti–TNF-α therapy has been shown to increase Treg numbers, reduce Treg apoptosis to normal levels, and restore suppressive function (Ehrenstein et al. 2004; Toubi et al. 2005). Both Treg defects and their reversal by TNF antagonists correlated with increased or decreased disease activity, respectively. Of note, this tolerogenic effect of anti-TNF therapy was related to the TGF-β-dependent induction of peripheral Tregs, which compensated for the defective nTreg population in RA patients (Nadkarni et al. 2007).

CELLULAR THERAPIES TO RESTORE TOLERANCE IN AUTOIMMUNE DISEASES

Several strategies for cellular therapy effectively prevented disease and restored tolerance in preclinical models of autoimmune diseases and are now being developed for clinical applications. Following the observation that allogeneic hematopoietic stem cell transplantation (HSCT) implemented for malignancies could have beneficial effects on coincidental autoimmune diseases, HSCT has been performed in hundreds of patients suffering from severe autoimmune conditions refractory to conventional treatments (Burt et al. 2008). ATG is often used as a conditioning reagent in conjunction with cyclophosphamide in nonmyeloablative autologous HSCT in autoimmune diseases. In mice, syngeneic bone-marrow transplantation induced remission from EAE and new-onset diabetes (Karussis et al. 1993; Wén et al. 2008). In humans, autologous nonmyeloablative HSCT has shown spectacular benefits in many patients, with long-term stabilization, amelioration or remission from disease in >50% of immunotherapy-refractory patients with MS, SLE, systemic sclerosis, and small cohorts of patients with T1D and Crohn’s disease (Craig et al. 2003;
Oyama et al. 2007; Couri et al. 2009; Illei et al. 2011). In contrast, autologous HSCT resulted in increased relapse rates in RA (Verburg et al. 2005). Currently, conditioning treatment toxicity and transplant-related mortality preclude the generalization of autologous HSCT to the general population of autoimmune patients. However, improvements in treatment regimens and completion of more rigorous clinical trials may result in a better definition of the most relevant clinical applications with the highest benefit to risk ratio. Mechanistically, studies in mouse models and patients with autoimmune diseases have led to the hypothesis that autologous HSCT restores tolerance to self-antigens. The initial conditioning regimen of cyclophosphamide and ATG eliminates autoreactive T cells as well as long-lived autoantibody-producing plasma cells (Zand et al. 2005; Alexander et al. 2009). The subsequent autologous HSCT increases thymic output and results in the generation of a novel T-cell repertoire and an increased number of Tregs (Farge et al. 2005; Muraro et al. 2005; Roord et al. 2008). Thus, autologous HSCT may allow the development of a novel immune system, perhaps in the context of different environmental and immunological conditions, in which immunoregulation may again be dominant in responses to self-antigens except in patients in which the genetic propensity to autoimmunity is too high. Finally, there is a growing interest in mesenchymal stem cells (MSCs), also called multipotent mesenchymal stromal cells, for the treatment of autoimmune diseases (Tyndall 2011). MSCs are stromally derived adult progenitor cells that can be obtained from various tissues, including bone marrow (BM), placenta, umbilical cord, and fat. The original interest in MSCs stemmed from their ability to transdifferentiate into other tissues and potentially induce tissue “regeneration,” but their primary clinical application in autoimmune diseases is now related to their immunomodulatory properties (Kode et al. 2009). MSCs have effectively restored tolerance in animal models of autoimmunity, and a large number of clinical trials in autoimmune diseases, including MS, Crohn’s disease, and SLE, have been initiated and are currently underway (Tyndall and Uccelli 2009). The mechanisms underlying their tolerogenic effects are not clear but may be mediated by both cell–cell contacts and soluble factors such as TGF-β, 2,3-indoleamine dioxygenase (IDO), and soluble HLA-G. Although MSCs represent a promising tolerogenic therapy for autoimmune diseases, many challenges remain for their clinical applications, in particular, in the standardization of the source and preparation of MSCs, which may affect their potency in patients.

Several therapeutic approaches take advantage of the long-term T-cell unresponsiveness induced by various forms of tolerogenic APCs. Induction of tolerance using antigen-coupled ethylene carbodiimide (ECDI)-fixed cells is a potent strategy that has been used successfully to prevent and reverse disease using insulin-coupled cells in NOD mice and MBP- and PLP-coupled cells in EAE (Vandenbark et al. 1996; Fife et al. 2006). In EAE, ECDI-fixed cells coupled to myelin peptides were significantly safer and more effective at curbing disease than soluble peptides (Smith et al. 2005). ECDI-fixed cells coupled to IGRP prevented the development of disease in a humanized model of diabetes (Niens et al. 2011). The mechanisms are not fully elucidated, but altered interactions of tolerated T cells with APCs, TCR signaling defects, intrinsic control by CTLA-4 and PD-1, and extrinsic regulation by Tregs may all be involved (Eagar et al. 2002; Fife et al. 2006, 2009). A clinical trial sponsored by the ITN is in final stages of development to evaluate the safety and efficacy of autologous peripheral blood leukocytes (PBLs) ECDI-coupled with a mixture of immunodominant epitopes from MBP, PLP, and MOG in patients with RRMS. Additionally, a clinical trial of insulin-coupled autologous PBMCs is currently being developed by the ITN for T1D patients. Other valuable approaches rely on the injection or targeting of tolerogenic DCs in autoimmune diseases (Hilkens et al. 2010). Several different populations of tolerogenic DCs have been described, including immature DCs in steady state that can become stimulatory after maturation and specialized populations of DCs that specifically promote regulatory cell populations even in conditions...
of inflammation, such as plasmacytoid DCs (pDCs) (Steinman et al. 2003). Subsets of DCs express distinct cell-surface markers that have been exploited to target self-antigens selectively to tolerogenic DCs using complexes of self-antigen and mAbs specific for appropriate markers such as DEC-205 and Siglec-H, resulting in antigen-specific T-cell unresponsiveness and reduction of autoimmunity in mouse models (Bonifaz et al. 2002; Petzold et al. 2010; Loschko et al. 2011). Additionally, DCs can be manipulated ex vivo to retain an immature phenotype and induce tolerance in vivo. Administration of immature DCs has shown efficacy in murine models, and phase I studies to determine the safety of autologous tolerogenic DC therapy have been initiated in T1D and RA (Machen et al. 2004; Harry et al. 2010).

Altering the T reg compartment, by depleting T regs or interfering with molecular pathways necessary for their homeostasis or function, uniformly exacerbated autoimmune responses, whereas promoting Treg-mediated suppression has successfully prevented and reversed autoimmune diseases in models of T1D, MS, SLE, IBD, and others (Kohm et al. 2002; Mottet et al. 2003; Tang et al. 2004; Scalapino et al. 2006). Despite the therapeutic efficacy of Tregs in animal models, clinical studies of adoptive immunotherapy with Tregs in autoimmune diseases have been lacking. We have initiated the first U.S. clinical trial of Treg cellular therapy in patients with T1D (ClinicalTrials.gov Identifier NCT01210664). The study is a phase I safety trial of adult T1D patients within 2 years of diagnosis and is sponsored by the Juvenile Diabetes Research Foundation (JDRF). CD4<sup>+</sup>CD25<sup>+</sup>CD127<sup>lo/-</sup> Tregs will be purified from T1D patients (Liu et al. 2006) and expanded in vitro using an optimized protocol (Putnam et al. 2009) to increase the number of Tregs to therapeutically relevant levels and possibly improve their suppressive function (Tang et al. 2004; Chai et al. 2008). Escalating cell doses will then be infused back into each patient in an autologous manner to assess the safety and feasibility of Treg therapy. Possible risks associated with any polyclonal Treg therapy include adverse events related to immunosuppression, such as increased infections and malignancies, and to the possible instability of a fraction of Tregs that may result in acceleration of disease (McClymont et al. 2011). However, these events are unlikely considering the relatively low number of Tregs administered as compared with the endogenous population. Importantly, it was recently shown in the first clinical trial using Treg therapy in humans that infusion of expanded Tregs with the goal of improving graft versus host disease (GVHD) had no deleterious side effects and reduced the incidence of moderate to severe GVHD without affecting the graft-versus-leukemia effect (Brunstein et al. 2011). Thus, if safe and successful, Treg cellular therapy may revolutionize the approach to immunomodulation of autoimmune diseases.

The future use of Treg therapy in autoimmune diseases may be determined by the ability to develop antigen-specific Treg immunotherapy. Indeed, antigen-specific Tregs derived from either TCR transgenic (Tg) mice or endogenous antigen-specific T cells were more potent than polyclonal Tregs at suppressing autoimmunity (Tang et al. 2004; Masteller et al. 2005). The challenge in translating these findings to the treatment of autoimmune diseases lies in the isolation of sufficient quantities of antigen-specific T cells—a challenge that is not easily overcome in human patients (Brusko et al. 2008). Even when reagents are available to specifically label antigen-specific T cells (for example, via peptide-MHC multimer staining), efforts to expand endogenous self-antigen-reactive Tregs have been mostly unsuccessful. Novel strategies are being developed to circumvent these limitations and engineer Tregs with self-antigen specificity in vitro by introducing TCR genes of given specificity into polyclonal Treg populations using lentiviral or retroviral vectors. Human polyclonal Tregs engineered to express a TCR specific for the melanoma antigen tyrosinase could be successfully expanded in vitro and retained their ability to suppress tyrosinase-specific and bystander T-cell responses (Brusko et al. 2010). Similarly engineered murine Tregs could suppress antitumor responses in vivo. Additionally, murine polyclonal Tregs transduced with a TCR specific for a single model self-
antigen could efficiently suppress disease in murine models of colitis and arthritis (Elinav et al. 2009; Wright et al. 2009). Nevertheless, the potential of viral vectors to induce cellular transformation leading to leukemia have remained major concerns for the public and regulatory bodies. Additional safety concerns raised by the introduction of TCR chains include the generation of adverse specificities by mispairing of the transduced and endogenous TCR chains, although novel molecular strategies are specifically designed to avoid this complication (Govers et al. 2010). The expectation is that infused Tregs do not need to be long lived and will result in the induction of endogenous Tregs capable of maintaining long-term suppression thanks to mechanisms of “infectious tolerance.” Thus, engineered Tregs could include the cotransduction of “suicide genes” together with TCR chains, to allow the deletion of the injected cells and their progeny if needed (Guillot-Delost et al. 2008).

CONCLUDING REMARKS

Considerable progress has been made in the immunomodulation of autoimmune diseases and the understanding of the complex molecular and cellular pathways targeted by novel treatments. Despite the realization that the translation of effective therapies from animal models to patients is often difficult to achieve, a number of immunotherapies have become the standard of care and greatly improved the clinical outcome and quality of life of patients with some autoimmune diseases. Potential new targets are constantly being identified in animal models of autoimmune diseases and the generation of humanized models may be critical to better predict if results of preclinical models will translate to humans. Among the novel approaches being pursued, clinical trials address the “hygiene hypothesis” by testing whether stimulation of the immune system with parasitic worms or bacterial extracts could ameliorate autoimmune diseases (Alyanakian et al. 2006; Fleming et al. 2011). Small molecules targeting intracellular mediators of signaling in T cells or other cell populations have shown efficacy in preclinical models of autoimmune diseases and several are being developed for clinical trials in T1D, Crohn’s disease, RA, and MS (Louvet et al. 2008; Burli et al. 2010). Encouraging results have been obtained with inhibitors of JAK tyrosine kinases (Coombs et al. 2010; Fridman et al. 2010; Punwani et al. 2012). Therapies targeting the epigenetic regulation of immune responses may gain prominence in the future. Drugs targeting DNA methylation and chromatin remodeling (notably histone deacetylase [HDAC] inhibitors) are already in use in cancer and have shown tolerogenic properties in murine models of transplantation (Tao et al. 2007), and RNA silencing agents (siRNAs, miRNAs, antagonirs, etc.) are generating a lot of interest (Rigby and Vinuesa 2008). Finally, it is likely that immunomodulation of autoimmune diseases will require combinatorial therapy to truly restore long-term tolerance and abrogate disease, either by combining immunotherapies with approaches for tissue regeneration or by targeting two or more pathways to synergistically repair the different components of the autoimmune imbalance.

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