Immune interventions to preserve β cell function in type 1 diabetes

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ABSTRACT

Type 1 diabetes (T1D) is a chronic autoimmune disease that leads to destruction of pancreatic β cells, lifelong dependence on insulin, and increased morbidity and mortality from diabetes-related complications. Preservation of residual β cells at diagnosis is a major goal because higher levels of endogenous insulin secretion are associated with better short- and long-term outcomes. For the past 3 decades, a variety of immune interventions have been evaluated in the setting of new-onset T1D, including nonspecific immunosuppression, pathway-specific immune modulation, antigen-specific therapies, and cellular therapies. To date, no single intervention has produced durable remission off therapy in most treated patients, but the field has gained valuable insights into disease mechanisms and potential immunologic correlates of success. In particular, T-cell–directed therapies, including therapies that lead to partial depletion or modulation of effector T cells and preservation or augmentation of regulatory T cells, have shown the most success and will likely form the backbone of future approaches. The next phase will see evaluation of rational combinations, comprising one or more of the following: an effector T-depleting or -modulating drug, a cytokine-based tolerogenic (regulatory T-cells–promoting) agent, and an antigen-specific component. The long term goal is to reestablish immunologic tolerance to β cells, thereby preserving residual β cells early after diagnosis or enabling restoration of β-cell mass from autologous stem cells or induced neogenesis in patients with established T1D.

Type 1 diabetes (T1D), one of the most prevalent chronic diseases of childhood that also presents in adults,[1 2] results from destruction of insulin-producing β cells by autoreactive T cells that have escaped central and peripheral immune tolerance.[3] Type 1 diabetes is considered to be an organ-specific autoimmune disease that occurs in the context of disease-specific genetic changes as well as one or more environmental triggers, but the precise etiology remains elusive.[3] For reasons that are not understood, the incidence of T1D has been growing worldwide, particularly in children.[4] Insulin therapy is lifesaving but is required daily, heightens risks for major hypoglycemia, and lessens but does not avert other serious complications, including microvascular and macrovascular disease and death.[5] Because disease onset frequently starts in early childhood, the burden of T1D is lifelong, with a significant economic impact on individuals, families, and society. Higher levels of endogenous insulin secretion correlate with lower rates of complications, and hence, there is a need for safe interventions to preserve or restore β-cell function, reduce hypoglycemia, and improve short- and long-term outcomes.[6]

Intensive diabetes management with a target HbA1c level less than 7.0% is generally recommended because of proven benefits in terms of reduced risks of microvascular complications and cardiovascular disease, but in several surveys, only 13% to 15% of T1D patients met this target (reviewed in Lind *et al.*[5]). It is clear that despite significant advances in insulin delivery technologies, continuous glucose monitoring, and closed-loop pump sensor systems,[7] tight metabolic control remains difficult and, even with excellent glycemic control (HbA1c ≤6.9%), mortality in those with T1D is twice that of matched controls.[5] Such considerations have prompted interest in preventing disease progression in at-risk individuals before the onset of hyperglycemia or preserving residual islet mass in patients newly diagnosed with T1D. A longer-term goal is restoration of functional β-cell mass in established T1D patients with little or no remaining islets, by transplantation of allogeneic islets from suitable donors, generating autologous neoislets from stem cells or stimulating β-cell proliferation in vivo.[8] Regardless of the approach, long-term preservation of functional islets will require an immune intervention that halts the autoimmune attack and, ideally, restores immunologic tolerance.

TYPE 1 DIABETES IMMUNOPATHOLOGY

A comprehensive overview of T1D immunopathology is beyond the scope of this report, and the reader is referred to recent excellent reviews.[3 9 10] There is consensus that T1D results from an autoimmune process with a strong genetic predisposition and likely environmental triggers. The strongest genetic influence comes from polymorphisms in HLA class II alleles. There is a weaker effect of various HLA class I alleles, followed by 40 or more other loci, although only a handful is associated with a relative risk greaterthan 1.5.[10] Importantly, however, the vast majority of susceptibility alleles are immune response genes, reinforcing the notion that T1D is a disease of

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immune dysregulation.[10] Human leukocyte antigen susceptibility alleles may lead to alterations in binding affinities of the major histocompatibility complex (MHC)-peptide complex to cognate T-cell receptors (TCRs), which enable thymic escape of autoreactive T cells (failure of central tolerance). However, autoreactive T cells are also found in the peripheral blood of healthy controls,[11] indicating that additional mechanisms are at play in disease pathogenesis.

It is known that β cells undergo an increased rate of physiologic turnover at specific stages of growth and development (reviewed in Wang *et al.*[8]), with release and processing of β-cell antigens and subsequent presentation by antigen-presenting cells (APCs). Although hotly debated, this process may be enhanced by β-cell–trophic enteroviral infections (including widespread Coxsackie virus strains), which also lead to upregulation of MHC class I molecules and creation of an inflammatory milieu.[12] These events, combined with presumed genetic defects in peripheral tolerance checkpoints, lead to the activation of autoreactive T cells and the initiation and propagation of an islet-specific immune attack and the characteristic lesion in T1D, known as insulitis. A defect in peripheral tolerance is suggested by various lines of evidence, including the association between T1D and genes encoding interleukin 2 (IL-2) and the IL-2 receptor α subunit, CTLA4, and the FoxP3 transcription factor,[3 10] all of which are required for the development and maintenance of regulatory T cells (Tregs). At the same time, effector T cells (Teff) in T1D, which are thought to mediate β-cell death, seem to be unusually resistant to suppression by Tregs,[13] a process that likely involves inflammatory mediators.

Taken together, the evidence suggests that there are defects in both central and peripheral immune tolerance resulting in the emergence, activation, and persistence of autoreactive effector and memory T cells (both $CD4^+$ and $CD8^+$) that damage and eventually destroy most of the insulin-producing β cells in the pancreatic islets (figure 1). As noted, in addition to conventional T cells, there are also important contributions from Tregs, APCs (including B cells, monocyte/macrophages, and dendritic cells), and a variety of soluble mediators to the immunopathology of T1D, presenting a multitude of potential intervention targets, as discussed further later.

TARGETS FOR IMMUNE INTERVENTION

Once the autoimmune etiopathology of T1D became increasingly accepted,[14] attempts were made to induce remission with nonspecific immunosuppressive agents such as cyclosporine, azathioprine, and prednisone. Although these efforts were met with some success, the benefits were lost after discontinuation of therapy, leaving the prospect of lifelong immunosuppression and concomitant toxicity, which were unacceptable in this disease (reviewed in Rigby and Ehlers[15]). Fortunately, recent decades have witnessed enormous strides in our understanding of the immune system as well as the development of powerful therapeutic tools, notably fusion proteins and monoclonal antibodies that target various receptors expressed on B and T cells and a range of cytokines, which has ushered in an era of targeted immune interventions. In this report, I will highlight those interventions that have shown some success in the clinic or which have provided important mechanistic insights that will likely advance understanding in the field.

Targeting innate immunity

It is generally agreed that components of innate immunity (ie, nonantigen-specific responses) are involved in the immunopathology of T1D, including soluble inflammatory mediators and cells of the monocyte-macrophage lineage (figure 1). However, thus far, clinical studies with agents targeting innate immunity have been sparse and results have been mixed. In a pilot study evaluating the anti-tumor necrosis factor α (TNF-α) agent etanercept in new-onset T1D, there was a signal of efficacy at 6 months, but the study was too small (n=18) to draw firm conclusions.[16] Nevertheless, this was a potentially important result that deserves further study. Tumor necrosis factor α is a key inflammatory cytokine that drives inflammation and tissue injury in several autoimmune conditions, including rheumatoid arthritis (RA), psoriasis, and inflammatory bowel disease. Moreover, more recent reports have indicated wider roles for the cytokine, notably a direct effect on Tregs leading to impaired suppressive function,[17] making TNF blockade attractive in T1D.

Interleukin 1 is a central inflammatory cytokine, which had prompted interest in IL-1 blockers in autoimmunity. Interleukin 1 is also of special interest in T1D because of reports that it is directly toxic to β cells. However, 2 adequately powered, randomized trials of anti-IL-1 agents (the anti-IL-1ß mAb canakinumab and the IL-1 receptor antagonist anakinra) failed to show any benefit in new-onset T1D.[18] This result was unexpected and may require a reevaluation of the role of IL-1 in the pathogenesis of T1D.[19] Clinical trials evaluating the effects of the following 2 other anticytokine agents are planned or were recently launched: a trial of tocilizumab (anti-IL-6 receptor mAb) and a trial of ustekinumab (anti-IL-12/23 mAb), which should throw further light on the utility of blocking key inflammatory mediators that drive immune responses in autoimmunity.

There have been no clinical trials directly targeting proin-flammatory macrophages in T1D. Dendritic cells (DCs)—specialized APCs that reside in peripheral and lymphoid tissues and circulate in the blood—have attracted a great deal of interest because of their role in influencing T-cell lineage commitment (effector vs regulatory), and hence, there are efforts to generate "tolerogenic DCs" that can skew responses toward a regulatory profile.[20 21] First-in-man studies are underway in various indications, including T1D,[22] but to date, there have been no reports confirming proof-of-concept in autoimmune disease, such as preservation of C-peptide in T1D.

Targeting adaptive immunity

The adaptive immune system comprises antigen-specific responses, principally mediated by B and T lymphocytes. Type 1 diabetes is thought to be an autoantigen-driven inflammatory disease, and hence, autoreactive lymphocytes are considered to be the principal effector cells. There is wide consensus that T cells are the main culprits, but B cells may also play a role.

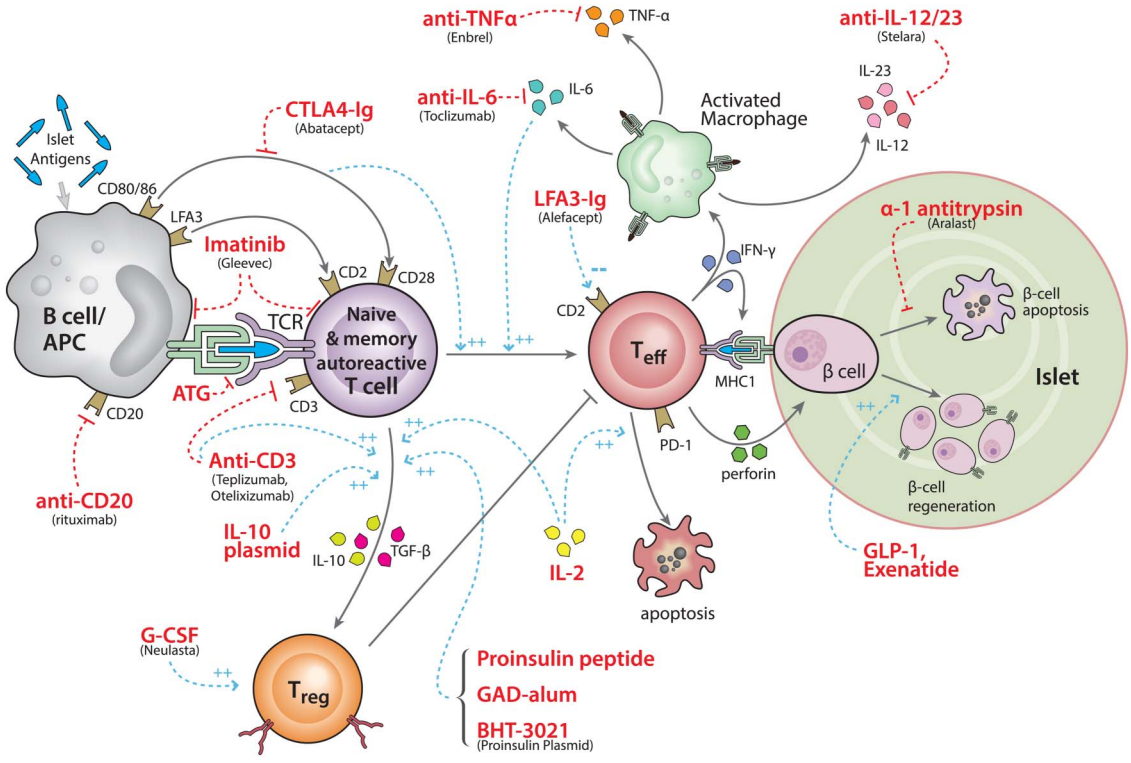

Figure 1 Type 1 diabetes immunopathology and targets for immune intervention. Type 1 diabetes is a T-cell–mediated autoimmune process in which islet antigens are presented to autoreactive T cells, which, in the context of appropriate costimulation, differentiate into activated memory and effector cells that damage β cells. Islet autoimmunity is driven by inflammation and innate immune cells such as macrophages and dendritic cells (APCs). Regulatory T cells have the capacity to downregulate autoreactive responses. There are numerous potential targets for intervention, and all of the drugs shown (in red) have been evaluated in new-onset T1D or are currently in clinical trials.

B cells

The anti-CD20 mAb rituximab substantially depletes B cells and is widely used to treat B cell lymphomas. Approximately a decade ago, rituximab was shown to be effective in RA, a surprising finding because RA was considered to be a T-cell–mediated autoimmune disease. However, B cells are known to be important APCs and this is the presumed mechanism of action[3] (figure 1). Like RA, T1D is also characterized by the presence of autoantibodies, which, although considered to be nonpathogenic, highlight the participation of B cells in the autoimmune process. A trial of rituximab in new-onset T1D demonstrated significant preservation of C-peptide secretion at 12 months,[23] but this difference was no longer significant at 18 or 24 months[24] and was accompanied by a significant increase in viremias associated with BK virus reactivation.[25] One rituximab-treated patient had a self-limited JC virus viremia, which is of potential concern because of the link to rare but fatal progressive multifocal leukoencephalopathy.[26]

At this time, it is unclear how to move forward with rituximab or other B-cell–depleting agents in T1D. The regimen used in the T1D trial led to profound B-cell depletion, which only recovered completely at 18 months,[24] and yet efficacy was modest. Repeated courses seem ill-advised because of the risk of infection; the same would be true of combination with T-cell–depleting agents. Combination with a T1D-specific autoantigen with the hope of skewing the reconstituting B cell compartment toward a more tolerogenic profile may be appealing, but this strategy has had no or only modest success in the nonobese diabetic mouse model.[27]

T cells

Costimulatory blockade

Costimulation constitutes the key second signal required to activate T cells after a first encounter with antigen and is the bridge between innate and adaptive immunity. Antigen-presenting cells process and present antigen-derived peptides via MHC-peptide complexes to TCRs. However, T-cell activation requires a second signal via costimulatory receptors; in the absence of costimulation, the T cell becomes anergic (unresponsive) or may undergo apoptosis. The classic costimulatory ligand-receptor pair is comprised of the ligands CD80 and CD86 (also known as B7-1 and B7-2) on APCs and the receptor CD28 on naive $CD4^+$ and $CD8^+$ T cells[28] (figure 1). After T-cell activation, a second CD80/CD86 receptor is expressed on T cells, CTLA4, which, unlike CD28, imparts an inhibitory signal.[29] Further work has uncovered an array of both costimulatory and coinhibitory pathways,[30 31] many of which are amenable to pharmacologic intervention.[32] The first drug targeting costimulation was the fusion protein CTLA4-Ig,[33] later known as abatacept and approved for RA, which blocks costimulation by acting as a soluble decoy receptor binding to CD80/CD86 and preventing ligation of CD28.[34]

A trial of abatacept in new-onset T1D demonstrated significant preservation of C-peptide secretion at 24 months,

although the degree of C-peptide preservation was modest and after 6 months seemed to parallel the decline seen in the placebo group despite ongoing treatment for 2 years.[35] Analysis of peripheral T-cell subsets by flow cytometry revealed a modest but significant increase in naive CD4 T cells and a decrease in central memory CD4 T cells, which seemed to correlate with C-peptide preservation.[36] Of some concern was a parallel and significant decrease in Tregs,[36] which may have contributed to the observation that C-peptide responses began to decline soon after treatment began, albeit at an initially slower rate than in controls.[35] The effect on Tregs is not surprising, given that the development and peripheral survival of Tregs is CD28 dependent.[34] Also, because cell-surface CTLA4 may be a core mechanism through which Tregs control APC function,[37] there are unresolved questions about the effect of ongoing treatment with soluble CTLA4-Ig on Treg function. Nevertheless, the abatacept trial in T1D provided an important first insight into the potential for costimulatory blockade in T1D and warrants further study. A trial evaluating abatacept in the prevention of T1D in at-risk patients is currently ongoing.

Antigen-specific therapies

As noted in the section on tolerogenic DCs, therapies that can specifically skew immune responses to a tolerogenic profile are of interest, particularly if those responses are antigen specific. This has led to exploration of antigen-specific therapies in autoimmunity, meaning regimens that incorporate disease-specific antigens (autoantigens). The hope is that if autoantigens are presented in a tolerogenic context, for example, via a tolerogenic route (mucosal vs subcutaneous), in combination with tolerogenic APCs, or along with appropriate coinhibitory signals, there will be down modulation of autoreactive Teff cells with concomitant up-regulation of antigen-specific Tregs.[38] This concept is attractive because there is no requirement for broad immunosuppression and the antigen therapy can be repeated as needed.

However, despite efforts for several decades in multiple indications, progress has been slow and, in some cases, there has been disease exacerbation.[39] Principal stumbling blocks have included determination of the therapeutic dose (there is evidence that the dose response may be U shaped), formulation, inclusion of adjuvants (and if so, type of adjuvant), route of administration, and dosing frequency. Lack of success has also been the norm in T1D, in which results of antigen-specific approaches have been negative or equivocal.[40] Nevertheless, a recent pilot study of a DNA plasmid encoding proinsulin (BHT-3021, figure 1) revealed interesting results. At 1 of 4 doses tested, there was significant preservation of C-peptide at week 15 (3 weeks after the last of 12 weekly doses; thereafter, C-peptide declined similar to the placebo group) and there was a significant correlation between decreased proinsulin-specific $CD8^+$ T cells and C-peptide preservation.[41] Although this trial was too small to draw firm conclusions, it suggested that with appropriate dose optimization, it may be possible to achieve desirable antigen-specific responses in T1D. Considerable additional work will be required to assess the true potential of antigen-specific therapies, including combination with other agents.[42]

T-cell depletion

In one of the earliest immune intervention trials, a pilot study of the combination of antithymocyte globulin (ATG) plus prednisone in recent-onset T1D gave a signal of efficacy in terms of improved HbA1c and reduced insulin dose in some of the treated patients.[43] These data together with other promising pilot clinical and preclinical results led to the randomized, doubleblind Study of Thymoglobulin to ARrest Type 1 diabetes (START) trial comparing ATG with placebo in patients with new-onset T1D. Surprisingly, there was no benefit in the ATG group compared with placebo at 12 months, and there was even a suggestion that C-peptide responses in the ATG-treated subjects showed accelerated decline in the first 6 months before stabilizing in the second 6 months.[44] A clue to what may have happened with this intervention was the observation that virtually all treated patients experienced cytokine release syndrome during the drug administration period and serum sickness 7 to 10 days later, accompanied by brief but substantial increases in serum levels of IL-6 and acute-phase proteins, suggesting adverse immune activation early on. Unintended immune activation is also thought to be the mechanism for transient disease exacerbation seen in a pilot study of IL-2 plus rapamycin in T1D,[45] discussed further later. Also notable was the finding that despite profound $CD4^+$ and $CD8^+$ T-cell depletion, including naive and central memory subsets, effector memory T cells were resistant to depletion, which, together with strong depletion of Tregs, led to an unfavorable Treg/Teff ratio in the first 6 months of the trial.[44]

The results of the START trial have forced the community to reassess the value of broad T-cell–depleting therapies in T1D. It is useful to remember that a key feature of the Biobreeding rat, an accepted model for T1D susceptibility, is profound lym-phopenia,[14] and it has been suggested that lymphopenia or lymphodepletion predispose to autoimmunity, including T1D, because homeostatic expansion, driven by IL-7, enables low-affinity autoreactive T cells to survive and proliferate (reviewed in Smilek *et al.*[42]). It is interesting to contrast the START trial with the recent report of a pilot study of the combination of ATG with granulocyte colony-stimulating factor (GCSF) in T1D, which suggested clinical efficacy in association with relative preservation of Tregs.[46] It is unclear whether the effects seen in this trial reflect the lower dose of ATG that was used (one-third of the dose used in START) or the combination with GCSF (figure 1). A larger, fully powered trial comparing the combination of ATG/GCSF with ATG alone and placebo, now underway, should answer that question.

T-cell modulation

When attempting to restore the balance between regulatory and Teff cells in T1D or autoimmunity in general, depletion of relevant autoreactive Teff cells is only one strategy. Another is to modulate Teff-cell function by inducing a state of hyporesponsiveness, such as by promoting T-cell anergy, exhaustion, or senescence.[47] Induction of T-cell anergy by costimulatory blockade has already been noted, previously. Ideally, an intervention should combine Teff depletion or induction of unresponsiveness with enhancement or expansion of Tregs.[42]

The first demonstration that a T-cell–targeted therapy can preserve C-peptide secretion in new-onset T1D was with monoclonal antibodies against CD3, a component of the TCR complex (figure 1). In a landmark study, Herold *et al.*[48] showed that the Fc receptor-nonbinding hOKT3γ1 (Ala-Ala) anti-CD3 antibody (later called teplizumab) preserved C-peptide and reduced HbA1c levels and insulin use in patients with new-onset T1D. These results were confirmed by an independent European group using another anti-CD3 mAb (otelixizumab),[49] and both groups showed that these benefits were maintained for up to 3 years in a subset of patients.[50 51] However, this efficacy came at the cost of cytokine release syndrome during drug administration and Epstein-Barr virus (EBV) reactivation or EBV-related disease in a significant proportion of treated patients.[51] Subsequent larger trials failed to meet their primary endpoints, in part because the endpoints selected were too stringent[52] or because the selected dose was too low.[53 54] This is unfortunate but should not detract from further exploration of this potentially valuable immune intervention.

In a phase 2 trial of teplizumab, in which subject received a second course of the drug at 12 months, C-peptide was significantly preserved at 24 months in the treated patients versus controls. An important finding in this trial (the Autoimmunity-blocking Antibody for Tolerance in Early type 1 diabetes trial) was that a subgroup of responders could be identified that had excellent C-peptide preservation, whereas the nonresponders were almost indistinguishable from the controls.[55] Response was predicted by better glycemic control and lower insulin use at baseline as well as subtle immunologic differences. However, the basis for drug response and the true mechanism of action of teplizumab remain unresolved. Earlier studies, particularly in the nonobese diabetic model, had suggested that anti-CD3 treatment leads to selective depletion of pathogenic T cells with preservation of Tregs, but this mechanism has not been confirmed in the human studies.[56] A clue to the mechanism may come from the original observation that responders show a decrease in the CD4/CD8 ratio,[48 50] which is consistent with a more recent finding of an increase in $CD8^+$ central memory T cells in responders in the Delay trial, in which T1D patients beyond the new-onset period were treated with teplizumab.[57] Although expansion of a $CD8^+$ T-cell population would seem to be counterintuitive, this may suggest that anti-CD3 has a partial agonist effect and is inducing some form of Teff modulation. This could include induction of a $CD8^+$ Treg population, as suggested by studies with teplizumab-treated human peripheral blood mononuclear cells and samples from T1D patients treated with the drug.[58]

A second T-cell–modulating agent that has recently been evaluated in new-onset T1D is alefacept, an LFA3-Ig fusion protein that targets the CD2 costimulation pathway on T cells. CD2 is expressed on most lymphocyte subsets and is upregulated on effector and memory T cells (figure 1). Earlier work in psoriasis had demonstrated that alefacept selectively targets effector memory T cells, and the mechanism of action was presumed to be a combination of partial depletion of these subsets and modulation of CD2-mediated costimulation.[59] In psoriasis, 2 or more courses of alefacept result in prolonged off-therapy remission, which may be a form of tolerance.[60] These results prompted the Immune Tolerance Network to conduct the inducing remission in new- onset Type 1 Diabetes with Alefacept trial, which showed that two 12-week courses of alefacept in new-onset T1D result in preservation of C-peptide (as measured by the meal stimulated 4-hour area under the curve), a significant reduction in insulin use, and a remarkable 50% decrease in rates of major hypoglycemia in drug-versus placebo-treated patients at 12 months.[61]

Analysis of T-cell subsets by flow cytometry revealed that alefacept treatment led to significant reductions in central and effector memory CD4 and CD8 T cells, preservation of Tregs, and favorable increases in Treg/Teff ratios, providing a plausible mechanism for the clinical effects of this drug.[61] Analysis of the 24-month clinical and mechanistic results is ongoing. Of particular interest will be evidence of T-cell modulation, including induction of T-cell unresponsiveness and/or partial agonist effects, as has previously been suggested based on in vitro effects of alefacept on human PBMCs.[62 63] Alefacept seems to be a very promising agent because it is well tolerated with no reports of dug-associated serious adverse events and no between-group (alefacept vs placebo) differences in overall rates of adverse events,[61] suggesting that it may be an appropriate intervention in T1D, even in children.

Treg augmentation

As noted earlier, restoring immunologic balance in autoimmunity can, in principle, be achieved by decreasing effector cells, increasing regulatory cells, or both (figure 1). Until recently, there seemed to be few practical approaches to directly augmenting Treg frequency or function. A pilot study in established T1D patients was the first to show that a combination of IL-2 and rapamycin can produce robust increases in peripheral Treg frequencies and restore pSTAT5 signaling toward levels seen in healthy controls.[45] However, despite these favorable changes, the treatment resulted in transient disease exacerbation (accelerated C-peptide decline), which might have been related to significant increases in peripheral natural killer cells and eosinophils and possibly other forms of immune activation.[45] Rapamycin, which was added to the combination to restrain Teff-cell activation, is suspected to be β-cell toxic and has been shown to be antitolerogenic in some situations (reviewed in Hartemann *et al.*[64]). There are ongoing trials to evaluate low-dose IL-2 monotherapy in T1D[64 65] with the aim of identifying a dose and regimen that can safely expand Tregs without activating effector cells.

Another approach to Treg augmentation is adoptive transfer of ex vivo expanded autologous Tregs. This approach has attracted considerable attention in recent years and is becoming a reality after development of a protocol for efficient ex vivo expansion of Tregs from patients with T1D.[66] There were recent reports of an open-label pilot study of infusion of autologous Tregs in new-onset T1D patients (n=20), which seemed to show that the regimen was safe and led to preservation of C-peptide in some subjects at 1 year.[67 68] Although encouraging, firm conclusions about the safety and efficacy of this intervention cannot be drawn at this stage. Among potential concerns is a debate about Treg plasticity and stability[69] as well

as the potential for pathogenic conversion of Tregs into Teff cells in the autoimmune milieu.[70] It may be necessary to support Treg stability after infusion by, for example, coadministration with IL-2, which promotes Treg stability and suppressive function.[71]

CONCLUSIONS

The past 3 decades have seen substantial progress in our understanding of the immunopathology of T1D, identification of targets for immune intervention, and the conduct of clinical trials involving nonspecific immunosuppressive drugs, targeted biologic agents, and even cellular therapies. Some of these interventions have shown promise, although no single intervention to date is able to induce lasting remission in a majority of treated patients. Much work needs to be done to better understand the phenomenon of responders versus nonresponders, and we need robust immunologic correlates of clinical efficacy.

We are now entering the "next phase" of immune intervention trials in T1D, with a greater emphasis on patient stratification prerandomization, broader use of predictive biomarkers, and, importantly, implementation of rational combinations of therapeutics based on a sound mechanistic hypothesis for induction of immunologic tolerance. For example, a "dream" combination might include an induction agent that can deplete or modulate key subsets of Teff cells (such as alefacept or teplizumab), followed by a protolerogenic agent that promotes deviation toward Tregs (such as tocilizumab or IL-2) combined with 1 or more T1D autoantigens (such as proinsulin peptides or DNA) that facilitate expansion of antigen-specific Tregs. Many other potential combinations can be envisaged, and each will be predicated on navigating complex issues, including building a scientific rationale (with, as appropriate, animal model data), gaining access to the investigational agents, developing risk mitigation strategies to protect patient safety, and obtaining regulatory approval.[72]

Only once we have reestablished immunologic tolerance can we realistically entertain the prospect of long-term preservation of residual β cells or maintenance of near-normal levels of β-cell mass after restoration by transplantation, differentiation from stem cells, or drug-induced neogenesis. For these reasons, continued efforts to optimize immune interventions in T1D are critical if we are to defeat this disease.

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