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Mechanistic Basis of Immunotherapies for Type 1 Diabetes Mellitus

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Abstract

Type 1 diabetes (T1D) is an autoimmune disease for which there is no cure. The pancreatic beta cells are the source of insulin that keeps blood glucose normal. When susceptible individuals develop T1D, their beta cells are destroyed by autoimmune T lymphocytes and no longer produce insulin. T1D patients therefore depend on daily insulin injections for survival. Gene therapy in T1D aims at the induction of new islets to replace those that have been destroyed by autoimmunity. A major goal of T1D research is to restore functional beta cell mass while eliminating diabetogenic T cells in the hope of achieving insulin independence. Multiple therapeutic strategies for the generation of new beta cells have been under intense investigations. However, newly formed beta cells would be immediately destroyed by diabetogenic T cells. Therefore, successful islet induction therapy must be supported by potent immunotherapy that will protect the newly formed beta cells. Herein, we will summarize the current information on immunotherapies that aim at modifying T cell response to beta cells. We will first outline the immune mechanisms that underlie T1D development and progression and review the scientific background and rationale for specific modes of immunotherapy. Numerous clinical trials using antigen-specific strategies and immune-modifying drugs have been published, though most have proved too toxic or have failed to provide long-term beta cell protection. In order to develop an effective immunotherapy, there must be a continued effort on defining the molecular basis that underlies T cell response to pancreatic islet antigens in T1D.

INTRODUCTION

Type 1 diabetes (T1D) is an autoimmune disease. T1D patients present with absolute insulin deficiency; hyperglycemia occurs when the vast majority of the pancreatic beta cells in the body are destroyed by autoimmunity. To date, insulin replacement remains the mainstay of treatment. In the last three decades, the introduction of multiple forms of insulin formulations that exhibit widely varying durations of action has significantly added to our armamentarium for the clinical management of diabetes. The continued development of better insulin pumps and glucose monitoring systems will further improve glycemic control among diabetics. These advances notwithstanding, insulin injection therapy is not a cure for diabetes, as exogenously administered insulin cannot mimic the natural glucose stimulated insulin secretion (GSIS) pattern from the normal pancreas.

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The induction of new beta cells, or beta-like cells, in T1D subjects could lead to a cure. Strategies for the generation of new beta cells include the directed differentiation/transdifferentiation of pancreatic non-beta cells, and the directed transdetermination of liver progenitor cells, into beta cells, and contrasting the two in vivo approaches with the ex vivo approach of directed differentiation of embryonic stem cells and induced pluripotent stem cells into beta cells in vitro (recently reviewed in *Translational Research* by Wagner et al., 2010¹).^{2,3} For instance, we showed that gene transfer of the lineage-defining transcription factor Neurogenin3 leads to reversal of diabetes and restoration of normal glucose and insulin dynamics in STZ-diabetic mice. Neurogenin3 gene therapy induces the reprogramming of hepatic progenitor cells (also known as oval cells) into neo-beta cells that closely mimic normal pancreatic beta cells functionally, biochemically, and in ultrastructure.^{4,5} Even if we assume that a gene therapy regimen succeeds in inducing new beta cell, the latter would be destroyed immediately by the ongoing autoimmunity. Any effective therapy that enables the acquisition of new beta cells must be supported by a potent immunotherapy to reverse T1D. Herein, we will review the mechanistic background information on the autoimmune process that underlies T1D and the current status of immunotherapy that addresses specific T1D autoimmune processes.

EFFECTIVE IMMUNE THERAPY/MODULATION: A PREREQUISITE FOR SUCCESSFUL GENE THERAPY OF TYPE 1 DIABETES

Over the last decade, there has been steady progress towards the therapeutic induction of neo-beta cells in vitro and in experimental animals in vivo. Overt T1D is preceded by autoimmune destruction of pancreatic beta cells such that, at presentation, T1D patients have lost 70-90% of their beta cell mass. Gene therapy-based beta cell neogenesis has the capacity to restore insulin production and reverse hyperglycemia. However, for a sustained therapeutic response, the neo-beta cells must be protected from the ongoing autoimmune process. Over the last decades, many laboratories have tested different immunomodulatory therapies with the objective of preventing, postponing, or reversing the onset of T1D. The same principle applies if and when diabetes gene therapy-mediated neo-islet regeneration becomes a reality. The islet neogenesis regimen must be supported by an immunotherapy that allows the new beta cell to survive the onslaught of autoimmunity so it can continue to do its job.

T1D occurs when the body's T cells attack and destroy insulin-producing beta cells in the pancreatic islets. If the T cell-mediated immune attack could be halted, residual beta cells (or neo-beta cells produced by gene therapy) in T1D patients would be preserved and perhaps be given a chance to proliferate and produce sufficient insulin to normalize blood glucose. The fact that T cells that directly attack beta cells are islet-antigen autoreactive^{6,7} forms the basis for the many antigen-based therapies for T1D that have been devised over the years. Unfortunately, clinical trials using such antigen-specific strategies have been largely unsuccessful in delaying human T1D progression.^{8,9} Numerous other clinical trials have depended on the use of immune-modifying drugs to treat T1D. Treatments using such drugs have often been borrowed from effective therapeutic regimens for transplant rejection or other T cell-mediated autoimmune diseases. However, use of these drugs in T1D patients only transiently delays (but does not abrogate) beta cell loss.^{8,9} Therefore, rather than assuming that T cell-mediated beta cell destruction can be halted by common immunosuppressive drugs, the rational development of innovative immunotherapy for T1D depends on a clear understanding of the molecular basis of T cell response against the T1D patient's own pancreatic islets.

In trying to get us closer to the identification of optimal therapeutic targets, we review herein the literature on T1D immunotherapies in the context of a mechanistic framework for

T1D immune-pathogenesis, exploring in detail the following: (1) genetic factors and mechanisms responsible for the generation of islet antigen-specific T cells, to be followed by discussions on antigen-based therapies for T1D; (2) T cell mediated pathogenesis in T1D and its modulation and control by broad immunosuppressants; and (3) immunotherapies that selectively target the chain of events that enable and sustain the effector function of diabetogenic T cells.

TARGETED ISLET-ANTIGEN RECOGNITION AND ANTIGEN-BASED THERAPIES

Altered self-antigen recognition generates diabetogenic T cells

T cells are the master regulators that control the quality and quantity of adaptive immune responses. A majority of mature T cells express their own unique T cell receptor (TCR) consisting of an α and a β chain, which recognize specific “foreign” or “self” peptide antigens presented by major histocompatibility complex (MHC) molecule. To ensure immune tolerance to self antigens, the developing T cells in the thymus that have a TCR recognize a self-antigen/MHC with high affinity generally undergo apoptosis, a process called negative selection or central tolerance.^{10, 11} However, not all self-reactive developing T cells are eliminated during development. Some of them may leave the thymus and circulate in the periphery. To maintain peripheral tolerance to self antigens, the self-reactive T cells entering the periphery can be eliminated, become anergic (a lack of reactivity against specific antigen), or be actively suppressed by regulatory T (Treg) cells.¹² The development of autoimmune T1D is the consequence of impaired thymic deletion and peripheral tolerance of diabetogenic T cells.¹²

To efficiently delete self-reactive developing T cells, self-antigens need to be displayed in the thymus and presented to the developing T cells. The transcription factor Autoimmune Regulator (Aire) influences the ectopic expression of thousands of self-tissue specific antigens (including the pancreatic beta cell-restricted insulin) in the medullary thymic epithelial cells (mTECs).¹³ Disruption of Aire function eliminates the expression of insulin and other tissue specific antigens in the thymus, which leads to lethal autoimmunity that often includes T1D as part of the disease phenotype.^{14, 15} Hence, Aire mutations may cause a T1D phenotype by impairing negative selection of insulin-reactive T cells. Aire mutations are rare in the general population. The altered insulin expression levels in the human thymus are mainly attributed to the variable number of tandem repeat (VNTR) polymorphism that lies upstream of the INS promoter. While class III INS-VNTR genotype provides protection from T1D and associates with high expression of insulin in mTECs, class I INS-VNTR genotype predisposes to T1D associated with lower thymic insulin expression.¹⁶⁻¹⁸ Recently, Fan et al.¹⁹ have genetically inactivated insulin expression in the mTECs of C57BL/6 background mice while the animals were allowed to maintain pancreatic insulin production. These mice develop diabetes spontaneously around 3 weeks after birth. Therefore, defect in negative selection of insulin-reactive T cells in thymus indeed causes T1D.

Self-antigen is presented to both developing and mature T cells by MHC molecules. Therefore, polymorphisms of human MHC, the human leukocyte antigen (HLA), may affect the generation and effector function of diabetogenic T cells. Indeed, polymorphisms of HLA represent the primary etiological pathway of T1D.²⁰ For instance, the predisposing HLA class II haplotypes, DRB1*03, DRB1*04 and DQB1*0302, are present in 39% of patients who develop T1D before age 20 and confer the highest T1D risk. Up to 50% of the T1D risk will occur in children who have such HLA genotype and have two or more T1D relatives. By contrast, children with a T1D family history but with protective HLA-DQB alleles (e.g.

DQB1*0602) have only about 1% of T1D risk.²¹ Genome-wide association studies (GWAS) have verified that the major T1D susceptibility loci indeed map to the HLA-DQB1 and HLA-DRB1, and that several HLA class I regions are also associated with T1D.^{22, 23}

Knowledge of susceptibility MHC molecules in the generation and function of diabetogenic T cells is still in its infancy. It has been suggested that the particular polymorphic peptide-binding pockets of the susceptibility HLA molecules may afford T1D susceptibility by presenting a unique set of peptides.²⁴ Interestingly, non-obese diabetic (NOD) mice express only one MHC class II allele, I-A^{g7}, which has a substantially wider peptide-binding pocket accounting for distinct peptide preferences compared with other MHC class II alleles.²⁵ The unusual peptide-binding pocket of I-A^{g7} leads to its weak interaction with proinsulin peptides, which may in turn provide low binding affinity and avidity to autoreactive T cells.²⁶ Autoreactive T cells with low avidity are capable of escaping thymic negative selection and peripheral tolerance mechanisms.²⁷ To drive the chronic progression of T1D, polyclonal autoreactive T cells escaped into periphery with low-avidity may transit into a set of oligoclonal T cells with higher-avidity for cognate antigens.²⁸ Taken together, both susceptibility MHC molecules and altered thymic expression of self-antigens account for the generation of diabetogenic T cells.

Insulin immunotherapy

Insulin was the first identified human T1D antigen. Insulin antibodies can be detected in T1D patients prior to exogenous insulin treatment.²⁹ Several preproinsulin (PPI)/insulin epitopes can also be recognized by human T cells.³⁰⁻³³ For instance, certain CD4⁺ T cell clones obtained from pancreatic lymph nodes recognize the insulin A 1-15 peptide restricted by HLA-DRB1*0401 allele,³⁰ and some circulating CD8⁺ T cells react to PPI15-24 bound to the HLA-A*0201 molecule.³¹ In NOD mice, insulin B chain 9-23 peptide (insulin B: 9-23) is a primary autoantigen that initiates T1D. Hence, autoimmune diabetes can be prevented in transgenic NOD mice that express in place of native insulin a functional insulin with a Tyr→Ala substitution in position 16 of its B chain.³⁴ Insulin B:9-23 reactive CD4 T cells and B:15-23 reactive CD8 T cells are the predominant infiltrating cells in the islets with initial insulinitis from 4-week old NOD mice. However, the proportion of those cells is dramatically reduced at or near disease onset (usually > 12-week old).^{35, 36} Pathogenic T cells recognizing other antigens further accumulate in the pancreas during the early progression of T1D. The progressive involvement of other PPI epitopes or other pancreatic beta cell molecules is called “antigen spreading”.³⁶

Mucosal (oral or nasal) administration of insulin has been used clinically in an attempt to restore immune tolerance to insulin antigens. In particular, antigens crossing from the intestine into the gut-associated lymphoid tissue will be embedded in an environment enriched with suppressive cytokines (e.g. TGF- β and IL-10) as well as unique CD103⁺ gut dendritic cells (DCs) that express indoleamine 2, 3-dioxygenase and retinoic acid. Such an environment favors the induction of tolerance to antigens by increasing Treg cells or deleting antigen-specific T cells.³⁷ Indeed, mucosal insulin inhibits insulin-specific T cell and antibody responses in humans.³⁸ Nevertheless, it has proven difficult to delay or prevent human T1D by mucosal insulin immunotherapy.³⁸⁻⁴⁰ The reason for the lack of success may be due to the occurrence of antigen spreading prior to oral insulin administration. Moreover, in contrast to inbred NOD mice, there is no conclusive evidence that PPI/insulin is “the initiating antigen” in human T1D. Further studies are required to define the role of PPI/insulin antigenicity in distinct human populations.

In NOD mice, intranasal administration of insulin peptides prevents the development of T1D.^{41, 42} When combined with a low dose of systemic anti-CD3 mAb treatment, oral or intranasal administration of proinsulin can even enhance remission from recent-onset

autoimmune diabetes in mice.^{43, 44} Other PPI/insulin delivery strategies have also been found to reduce the T1D incidence in NOD mice, such as intramuscular injection of a plasmid DNA vaccine encoding proinsulin II,⁴⁵ intravenous administration of syngeneic splenocytes ECDI cross-linked to intact insulin or specific peptides (B:9-23 or B:15-23),⁴⁶ and administration of an agonistic insulin mimetope (replacing the arginine residue [R22] in the natural peptide B:12-23 with glutamic acid).⁴⁷ The clinical utility of these strategies remains to be determined.

Other antigen-based therapies

Islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP) is a diabetic antigen that is expressed exclusively in pancreatic islets. Because IGRP is not expressed in the thymic mTECs, it regulates the response or tolerance of IGRP-reactive T cells mainly in the periphery.⁴⁸ TCR transgenic mice with CD8⁺ T cells specific for IGRP206-214 epitope (the NOD8.3 mice) develop accelerated diabetes, but the development of such phenotype requires the presence of autoimmunity against proinsulin.^{49, 50} Thus, in NOD mice, IGRP may be one of the many “spreading” antigens following pathogenic proinsulin-specific autoimmunity. In humans, several HLA-A2-restricted IGRP epitopes have been identified as diabetic antigen. Circulating CD8⁺ T cells from patients with recent-onset T1D but not from healthy controls respond to the identified IGRP epitopes,⁵¹ indicating that the presence/absence of IGRP-reactive T cells may be a marker for monitoring the progression of T1D.

Glutamate decarboxylase 65 (Gad65) is predominantly expressed in the pancreas and brain,⁵² and is an essential autoantigen in both T1D and stiff-person syndrome.⁵³ Autoimmune diabetes in NOD mice can be prevented not only by intrathymic injection of GAD65 at 3 weeks of age,⁵⁴ but also by intravenous injection of large amounts of GAD65 during later stages when autoimmunity has been initiated.⁵⁵ Hence, GAD65 antigen that has arisen very early in life could continuously mediate disease progression.^{56, 57} In humans, immunization with GAD65 formulated with alum was initially shown to delay the progressive loss of c-peptide (a measure of beta-cell function) in new onset diabetic individuals,⁵⁸ but a recent phase 3 trial of GAD65-alum did not show any clinical benefits.⁵⁹

The list of diabetic antigens is constantly growing, and approximately 20 antigens have now been identified to be targeted by diabetogenic T cells.⁶⁰ For instance, an extensively studied diabetogenic CD4⁺ T cell clone, BDC2.5, has recently been shown to recognize the peptide epitope from chromogranin A.⁶¹ Many antigen-based T1D therapies are under active clinical trial. For example, trials using heat shock protein 60 DiaPep277 peptide have shown some promise in preserving residual beta cell function.⁸ Moreover, innovative methods of antigen administration (e.g. injection of nanoparticles coated with peptide-MHC) are under investigation for the treatment of T1D.⁶² Overall, antigen-based therapy for T1D remains attractive because it offers the advantage of affecting only diabetogenic T cells. The success of this approach relies not only on the identification of primary antigen targets that initiate or aggravate T1D in distinct “outbred” human subjects, but also on the development of innovative and efficient antigen delivery systems.

BROAD IMMUNOSUPPRESSIVE THERAPIES

Pathogenetic role of T cells in T1D

A recent study of pancreas autopsy samples removed from 29 patients with recent-onset T1D showed that CD8⁺ cytotoxic T cells were the most abundant cell population identified in insulitis specimens. Macrophages and CD4⁺ cells were also prominent in the islet infiltrate of T1D patients.⁶³ However, it remains difficult to define the pathogenetic role of T cells in human T1D because of the difficulty in accessing pancreatic tissues from patients. By contrast, in NOD mice, there is overwhelming evidence that T1D is a T cell-mediated

autoimmune disease. Priming of islet-reactive T cells occurs in the pancreatic lymph nodes,^{64, 65} where islet antigens are present initially associated with a ripple of physiological beta cell death that occurs at 2 weeks of age.⁶⁶ Primed T cells then infiltrate into islets and cause further islet damage, which in turn release different peptides from islet proteins that may contribute to early antigen spreading.³⁶ Amplified T cell responses, together with the reaction of other immune cells, eventually induce T1D onset at around 12 wk of age or later. It is noteworthy that T cell entry to the pancreas is an antigen-specific process, and the majority of T cells recruited to the pancreatic islets in the course of T1D are islet autoreactive.⁷ Destruction of islet beta cells by diabetogenic T cells is mediated via perforin and granzyme, the Fas/Fas ligand pathway, and TNF- α -dependent pathway.⁶

A central step to start a T cell response is the activation of antigen-specific T cells. Naïve T cell activation requires at least TCR/CD3 and costimulatory signals, which are delivered by antigen-MHC and other molecules expressed on the surface of the antigen-presenting cell (APC). TCR/CD3 signaling activates various intracellular pathways (e.g., PKC/NF- κ B, AP-1, and calcium/calcineurin/NFAT) to fulfill the needs of T cell proliferation and effector function.⁶⁷ Thus, in theory, decreased TCR/CD3 signal strength may prevent T1D autoimmunity. Nevertheless, *PTPN22* gene encodes a negative regulator of TCR/CD3 signaling, lymphoid tyrosine phosphatase (LYP). The T1D susceptibility *PTPN22* increases LYP activity and suppresses TCR/CD3 signaling,⁶⁸ which results in a profound deficit in T cell responsiveness to antigen stimulation.⁶⁹ Therefore, it is not clear how changes in TCR/CD3 signal strength affects T1D autoimmunity, because TCR/CD3 signaling controls multiple processes involved in thymic T cell development, Treg cell generation, and naïve T cell activation and function.

Small-molecule immunosuppressants

Immunosuppressive drugs have been used to treat T1D, a T cell-mediated autoimmune disease. Cyclosporin A (CsA) is a calcineurin inhibitor that prevents T cell activation and function.⁷⁰ Continuous CsA treatment in patients with new-onset T1D was shown to eliminate the need for exogenous insulin, but the effect was not lasting.^{71, 72} Moreover, it also caused renal toxicity which considerably dampened enthusiasm for its use in T1D patients.

Rapamycin (also known as sirolimus) inhibits the mammalian target of rapamycin (mTOR) pathway, which is critical for cell growth, proliferation, motility, and survival.⁷³ A recent trial examined islet beta cell function in patients with long-term T1D, and showed that rapamycin monotherapy leads to an increase in serum c-peptide and a reduction in exogenous insulin requirements.⁷⁴ By contrast, another trial showed that rapamycin/IL-2 combination therapy in T1D patients actually impairs beta cell function.⁷⁵ The clinical benefit of rapamycin therapy, if any, is debatable.

Anti-CD3 mAbs

Binding of cell surface receptors by mAbs in vivo could result in the depletion of cells or in the agonistic/antagonistic effects mimicking/blocking the action of the receptor's natural ligands. Therefore, selecting mAbs against distinct receptors on immune cells is a promising approach to develop new immunotherapies. The first mAb approved for clinical use was a mouse anti-human CD3e (OKT3) mAb, which is a potent mitogenic agent for T cells by providing agonist effects via TCR/CD3 complex.^{76, 77} OKT3 mAb treatment elicits almost immediate massive cytokine release leading to mild to life threatening symptoms.⁷⁸ To reduce such side effects, FcR-nonbinding humanized CD3 mAbs (teplizumab and oteplizumab) were developed to reduce the quantity and quality of TCR signals and

attenuate the toxic reactions,^{79, 80}. FcR-nonbinding CD3 mAb treatment marginally and transiently depletes T cells, and exerts long-lasting immune regulatory effects.^{81, 82}

In the initial clinical trials, brief administration of FcR-nonbinding CD3 mAbs in new-onset T1D patients exhibited substantial therapeutic benefits. The cytokine release syndrome was largely eliminated, and the circulating T cell number recovered to pretreatment levels by one month of treatment. Most importantly, transient therapy with FcR-nonbinding CD3 mAbs preserved residual beta cell function in patients for more than two years.⁸³⁻⁸⁵ Depending on patient age and baseline residual beta cell mass, transient treatment with oteplixumab even preserved insulin production for up to 3-4 years.⁸⁶ In a small group of patients, brief teplizumab treatment preserved residual beta cell function as long as 5 years.⁸⁷ Nevertheless, in a recent phase 3 trial using teplizumab in 516 T1D patients within 12 weeks of diagnosis, the composite endpoint (glycated haemoglobin A1c [HbA1c] <6.5% and insulin dose <0.5 U/kg per day at 1 year) did not differ significantly between teplizumab treated groups and the placebo group.⁸⁸ A phase 3 trial of oteplixumab also did not meet the primary efficacy endpoint of change in C-peptide at month 12 in T1D patients.⁹ It is unclear if further optimization of therapeutic antibody concentration and timing of treatment would lead to better outcome.

Antithymocyte globulin

Antithymocyte globulin (ATG) is a rabbit polyclonal gamma-immunoglobulin against human thymocytes, and has multiple specificities for various molecules displayed on T cells and other immune cells. ATG is a potent immune-depleting agent. Transient ATG therapy delays the loss of beta cell function in T1D patients,⁸⁹ but does not induce long-lasting remission.⁹ Recently, a group of new-onset T1D patients have been transplanted with autologous nonmyeloablative hematopoietic stem cells. The majority of these patients achieved insulin independence after a mean follow-up of 29.8 months.⁹⁰ Importantly, these patients were also treated with cyclophosphamide and granulocyte colony-stimulating factor (G-CSF) for hematopoietic stem cell isolation, as well as with ATG and cyclophosphamide before cell transplantation.⁹¹ Thus, combination potent immune depletion and suppression may fallstall beta cell loss; however, potential adverse side effects may complicate such an aggressive approach.

IMMUNOTHERAPIES THAT TARGET EVENTS IN T CELL RESPONSE

Environmental triggers of T1D

An effective T cell response requires not only the presence of antigens and antigen-specific T cells, but also a proinflammatory environment. In the context of autoimmune T1D, diabetogenic T cell response may be mainly triggered by an undefined “sterile” inflammation. On the other hand, T1D onset could also be influenced by microbial infections and the intestinal microbial environment. For instance, inoculation of encephalomyocarditis virus induces diabetes in wild type SJL mice by triggering immune attack on beta cells.⁹² In NOD mice, interaction of the intestinal microbes with the innate immune system was found to be a critical factor modifying T1D predisposition.⁹³ In humans, rare variants of IFIH1 (interferon induced with helicase C domain 1) have been shown to provide protection against T1D. These variants are predicted to alter the expression and structure of IFIH1, and disrupt the normal function of IFIH1 to respond to viral RNA. Hence, viral stimuli through IFIH1 may be an environmental trigger of human T1D.⁹⁴ Over the last 50 years, a several-fold increase was observed in the incidence of human T1D, indicating an urgent need to identify possible diabetogenic environmental triggers.

Antigen-presenting cells

Local inflammation induces maturation of APCs, which in turn migrate to lymphoid tissues and activate antigen-specific T cells. DCs are a type of professional APC and can be divided into several subsets. For instance, conventional DCs are specialized for antigen processing and presentation, whereas plasmacytoid DCs express CD45RA and have poor antigen-presenting capacity. Conventional DCs can be further classified into CD4⁺ DCs, CD8α⁺ DCs, and CD4⁺CD8α⁺ DCs. Interestingly, NOD mice exhibit a defect in CD8α⁺ conventional DCs.^{95, 96} Some studies indicate that enhancing DC development or function predisposes to autoimmune disease.^{97, 98} By contrast, others showed that Fms-like tyrosine kinase-3 ligand (Flt3L) treatment prevents diabetes in NOD mice by upregulating the frequency of CD8α⁺ conventional DCs.^{95, 96} G-CSF treatment also prevents diabetes by recruiting plasmacytoid DCs.⁹⁹ To date, the exact role of DCs in T1D remains elusive, and the therapeutic use of DCs needs to be further investigated.¹⁰⁰

B cells are crucial APCs in the pathogenesis of T1D. Although B cell-derived autoantibodies against islet antigens are useful markers for predicting T1D disease activity, these autoantibodies may not be pathogenic.^{101, 102} By contrast, B cells themselves infiltrate the pancreatic islets⁶³ and present autoantigens to T cells.^{103, 104} NOD mice deficient in B cells are insulinitis resistant and protected from developing diabetes.¹⁰⁵ Moreover, in a transgenic NOD mouse expressing human CD20 (hCD20) on their B cells, transient depletion of B cells by an anti-hCD20 mAb delays the onset of diabetes and even reverses overt diabetes.¹⁰⁶ Therefore, a clinical trial with a course of an anti-hCD20 mAb (rituximab) showed that, at 1 year of therapy, the rituximab group had a significant higher level of C-peptide and required less exogenous insulin for treatment than the placebo group. However, B cell depletion persists for months and is associated with some adverse events.¹⁰⁷ A shortcoming of anti-CD20 mAb therapy is the risk of lowered systemic immunity.

Positive and negative costimulatory molecules

APCs express various costimulatory molecules to regulate T cell response. For instance, CD28 expressed on T cells is the receptor for B7.1 (CD80) and B7.2 (CD86) expressed on APCs. TCR/CD3 stimulation plus CD28 signaling are sufficient to activate naïve T cells. In the absence of CD28 costimulatory signal, TCR/CD3 stimulation induces functional anergy rather than activation of naïve T cells. T cell activation induces surface expression of cytotoxic T-lymphocyte antigen 4 (CTLA-4), which also binds to CD80 and CD86 on APCs. However, CTLA-4 transmits an inhibitory signal to “turn off” T cell activation and serves as a physiologic antagonist of CD28. Mice deficient in CTLA-4 die within the first month of life of severe pancreatitis and myocarditis, demonstrating the critical regulatory function of this negative costimulatory receptor.¹⁰⁸ In the context of murine T1D, specific CTLA-4 deletion in autoreactive T cells facilitates the induction of autoimmunity,¹⁰⁹ whereas artificial expression of a CTLA-4 agonist on B cells protects NOD mice from spontaneous autoimmune diabetes.¹¹⁰ In humans, an association of the CTLA-4 locus with susceptibility to T1D has been reported.¹¹¹ Moreover, a clinical trial of abatacept (CTLA4-Ig; a fusion protein of the CTLA4 and an IgG1 Fc domain) was conducted in T1D patients to block CD28 pathway. Continued administration of abatacept resulted in an estimated 9.6 months delay in C-peptide reduction. However, beta-cell mass and function continued to deteriorate.¹¹²

Inducible costimulator (ICOS) is a CD28-superfamily costimulatory molecule expressed on activated T cells. ICOS serves as a positive mediator for activated T cells by up-regulating cell proliferation and cytokine secretion. NOD mice deficient in ICOS or ICOSL (ICOS ligand) are protected from developing diabetes by limiting the activation of diabetogenic T cells.¹¹³ Combined treatment of mAbs blocking ICOS and CD40 ligand signaling also

prevents T1D in adult NOD mice.¹¹⁴ By contrast, abrogation of the ICOS pathway in BDC2.5 NOD mice exacerbates T1D, which may be due to the critical role of ICOS in homeostasis and functional stability of Treg cells in prediabetic islets.¹¹⁵ Therefore, caution should be exercised in immunotherapeutic use of ICOS blockade.

Activated T cells express programmed death 1 (PD-1). Binding of PD-1 with its two ligands, PD-L1 and PD-L2, maintains peripheral T cell tolerance by not only inhibiting primary T cell activation but also triggering exhaustion of activated T cells.^{116, 117} Thus, PD-1–PD-L1 pathway blockade with mAbs rapidly precipitates diabetes in prediabetic NOD mice.¹¹⁸ PD-1 deficiency in NOD mice also accelerates the onset and frequency of T1D development.¹¹⁹ It is worth noting that PD-L1 is expressed broadly on hematopoietic and parenchymal cells. PD-L1 expression on parenchymal cells rather than hematopoietic cells protects against T-cell mediated autoimmune diabetes.¹²⁰ PD-L2 is predominantly expressed on hematopoietic cells, and is less potent in controlling T1D in NOD mice.^{118, 120} Lymphocyte activation gene 3 (LAG-3) is another inhibitory co-receptor expressed on activated T cells. LAG3 acts synergistically with PD-1 to prevent autoimmunity in mice,¹²¹ possibly by inducing exhaustion of activated T cells.¹¹⁷ Moreover, CD137 (4-1BB), a member of the tumor necrosis factor receptor superfamily, is primarily expressed on activated T cells and may prevent their exhaustion.¹²² However, potential risks may associate with anti-4-1BB–based immunotherapy for autoimmune diseases,¹²³ which requires further study.

The development of immunotherapies targeting costimulatory pathways is an attractive but challenging undertaking. Activated CD4⁺ T helper (Th) cells express high levels of CD154 (CD40L) to control the activation and function of various CD40-expressing immune cell subsets. Blocking CD40-CD154 interaction by anti-CD154 mAb proved effective in inducing peripheral T cell tolerance in various animal models.¹²⁴ However, clinical trials of anti-CD154 mAb had to be prematurely terminated, because the mAb also binds to CD154 expressed on platelets and stimulates coagulation.¹²⁵ It is important to note that attempts to target costimulatory pathways (e.g., the use of CD28 superagonist antibody TGN1412) in humans have led to a life-threatening syndrome.¹²⁶ Thorough preclinical investigations are required to understand the therapeutic potential and possible toxicity of targeting costimulatory molecules.

Cytokines in Treg and effector T cell function

Activated T cells produce many cytokines that have a variety of actions on themselves and other immune cells. In particular, recent studies highlight that impairment of IL-2 pathway plays a central role in the pathogenesis of T1D.¹²⁷ IL-2 is predominantly produced by activated T cells and supports the expansion of activated T cells. However, IL-2 is also a critical cytokine for the development and homeostasis of Treg cells. In murine models, insulin dependent diabetes susceptibility 3 (Idd3) on NOD mouse chromosome 3 is the strongest single non-MHC T1D susceptibility locus, which contains *Il2* gene and transcribes much less IL-2 mRNA when compared to protective Idd3 alleles. This genetic deficiency in IL-2 production correlates with reduced Treg cell function in NOD mice.^{128, 129} Moreover, transient low-dose IL-2 administration in diabetic NOD mice can reverse the T1D progression by a local effect on pancreatic Treg cells.¹³⁰ Therefore, in NOD mice, the defect in IL-2–Treg pathway is critical for T1D development, and such defect can be corrected by IL-2 therapy.

IL-2–Treg pathway in “outbred” human T1D subjects should be broadly interrogated. First, the Foxp3 transcription factor is the master regulator for maintaining Treg cell phenotype and function. Humans with a rare Foxp3 mutation develop severe T1D and other lethal autoimmunity, demonstrating that defect in Treg cell function triggers human T1D.¹⁴

Second, Treg cells isolated from patients with recent-onset T1D exhibit markedly reduced suppressor function in vitro.¹³¹ Finally, polymorphisms in genes encoding IL-2 receptor alpha chain (IL-2R α or CD25) and IL-2 are strongly associated with human T1D.²⁰ In particular, individuals with *IL2RA* susceptibility genotypes is associated with lower IL-2 secretion by T cells as well as lower expression of CD25 on Treg cells, both of which may impair Foxp3 expression and Treg cell function.^{132, 133} Taken together, IL-2–Treg pathway could be a regulator controlling the development of human T1D. Nevertheless, effector T cells isolated from T1D patients are also resistant to suppression by Treg cells,^{134, 135} which may be a barrier for Treg cell-based therapy (i.e., low dose IL-2 administration). Indeed, in a recent clinical trial, IL-2 therapy combined with rapamycin in T1D patients cause transient beta-cell dysfunction rather than protection, though the treated patients exhibit increased Treg cell frequency.⁷⁵

Recent studies demonstrated that IL-21 plays an essential role in T1D onset of NOD mice.¹³⁶⁻¹³⁹ IL-21 and IL-2 are closely related cytokines, both of which are predominantly produced by activated Th cells. The genes encoding IL-21 and IL-2 are adjacent to each other, and receptors for IL-21 and IL-2 share a common γ chain. However, IL-21 and IL-2 play distinct roles in regulating the immune system. While both cytokines can support expansion and survival of antigen-specific T cells during acute viral infection, IL-21 is the major cytokine required for sustaining effector T cell function in chronic viral infection.¹⁴⁰⁻¹⁴² Moreover, IL-21 is not required for Treg cell homeostasis, but provides pleiotropic effects on many other immune cell types. Therefore, in contrast to IL-2–deficient mice that suffer from lethal autoimmunity due to Treg cell defect, IL-21 or IL-21 receptor (IL-21R) deficiency in mice prevents the induction of several autoimmune diseases.¹⁴³ This is proved to be true in NOD mice. Deficiency in IL-21 or IL-21R renders NOD mice resistant to the onset of T1D.^{136, 137} Recent studies further showed that CCR9⁺ Th cells in the pancreas produce IL-21,¹³⁸ and that IL-21R signal controls autoreactive T cell infiltration in pancreatic islets.¹³⁹ Such thorough investigations of IL-21 actions will facilitate the design of immunotherapy by targeting IL-21 signal.

IL-21R is expressed on CD4⁺ Th cells, CD8⁺ T cells, B cells, NK cells, DCs, macrophages, and keratinocytes.¹⁴³ Thus, IL-21 produced by activated Th cells has a broad range of actions on various immune cells.¹⁴³ For instance, Th17 cells produce IL-21, but IL-21 also serves as an autocrine cytokine for Th17 cell development and up-regulates IL-23R expression to maintain Th17 cell phenotype.¹⁴⁴⁻¹⁴⁶ Interestingly, enhanced Th17 immunity is observed in T1D patients.¹⁴⁷⁻¹⁴⁹ Inhibition of Th17 cell function even prevents diabetes in NOD mice.¹⁵⁰ Therefore, enhancing Th17 cell response may be one of many actions by which IL-21 underlies the pathogenesis of T1D.

Inflamed tissues contain many types of immune cytokines, all of which play distinct roles in regulating immune responses. In the context of T1D, polymorphisms in genes encoding IL-7 receptor alpha, IL-10, and IL-27 also predispose to islet autoimmunity.²⁰ The biology of these cytokines in T1D should be investigated to evaluate their therapeutic potential. Moreover, proinflammatory cytokines (e.g., IL-1) can directly cause beta-cell destruction.¹⁵¹ Results from a recent clinical trial indicated that use of the IL-1 blocker anakinra lowers the requirement for exogenous insulin for months in children with new-onset T1D.¹⁵² Although IL-1 blocker alone may be insufficient to induce long-term remission of T1D, it should synergize with other immunotherapies in reversing diabetes.¹⁵³

PROSPECTS FOR IMMUNOTHERAPY IN PROTECTING NEO-BETA CELLS

In the previous sections, we have reviewed many of the approaches that had been tested either in laboratory animals, or in T1D patients in clinical trials. Here we offer additional

thoughts on possible strategies for immunotherapy. We note that similar immune mechanisms that have led to beta cell destruction in a T1D subject will also destroy neo-beta cells that are produced in response to novel gene and cell therapy. Hence, a safe and effective immunotherapy is not available for the long-term protection of neo-beta cells. A possible approach is to halt the autoimmune attack first with potent short-term general immunosuppression (e.g., FcR-nonbinding anti-CD3 mAb, ATG, or anti-CD20 mAb), and prevent or ameliorate late recurrence of autoimmunity by instituting prolonged or intermittent immunotherapies that target costimulatory signals or cytokines (e.g., CTLA4-Ig or IL-1 blocker). Each of those therapies delays beta cell loss for months. It would be interesting to determine the safety and efficacy of such combined therapies as a treatment strategy.

As immunologists continue their research on the mechanistic basis of the cause and progression of T1D, they should pay attention also to the precise pathogenic roles of diabetogenic T cells. Undoubtedly they will uncover new treatment targets which will help refine future immunotherapies. For example, it has been noted recently that IL-21 produced by activated Th cells is required to sustain the chronic immune response in different autoimmune diseases.¹⁵⁴ IL-21 and its related signaling molecules are thus attractive targets for T1D immunotherapy. Moreover, other innovative technology for immunotherapy should be explored. A plasmid DNA vaccine encoding mouse proinsulin II has been shown to reduce the incidence of diabetes in NOD mice,⁴⁵ which demonstrates the potential of DNA-based immunotherapy for T1D.

Immune response against neo-beta cells is only one of the challenges for the clinical application of beta cell regeneration. Efficient gene therapy to induce neo-beta cell formation *in vivo* still relies mostly on viral vectors, which themselves exert potent immunogenicity as well as toxic and other adverse effects. The generation of mature beta cells *in vitro* from pluripotent stem cells remains inefficient for obtaining clinically relevant cell numbers for therapy. The tumorigenicity of pluripotent cells also poses a potential threat that must be neutralized or circumvented before clinical application. Extensive preclinical investigations are required to resolve those challenges.

SUMMARY

The last decade has witnessed steady progress in the experimental *in vivo* reprogramming of nonbeta cells or adult stem cells *in situ*, and *ex vivo* targeted differentiation of embryonic and adult stem cells, to mature beta cells as a therapy for diabetes. To prepare for the clinical translation of the beta cell creation strategies in T1D patients in the future, we must add to our armamentarium an effective treatment method that counters the autoimmunity that underlies T1D. T1D patients harbor activated diabetogenic T cells that specifically attack beta cells. None of the current immunosuppressive agents can selectively eliminate diabetogenic T cells and protect the neo-beta cells in the long term. A better understanding of the T cell response in T1D would greatly improve our chances of developing a safe and effective immunotherapy that protects the *de novo* generated beta cells, and enables a cure of this devastating disease.

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Abbreviations

Ad	adenovirusy
Aire	autoimmune regulator
ATG	antithymocyte globulin
CsA	cyclosporine A
CTLA-4	cytotoxic T-lymphocyte antigen 4
DCs	dendritic cells
Flt3L	Fms-like tyrosine kinase-3 ligand
Gad65	glutamate decarboxylase 65
G-CSF	granulocyte colony-stimulating factor
GSIS	glucose stimulated insulin secretion
GWAS	genome-wide association studies
HLA	human leukocyte antigen
ICOS	inducible costimulator
Idd3	insulin dependent diabetes susceptibility 3
IFIH1	interferon induced with helicase C domain 1
IGRP	islet-specific glucose-6-phosphatase catalytic subunit-related protein
MHC	major histocompatibility complex
mTECs	medullary thymic epithelial cells
mTOR	mammalian target of rapamycin
NOD	non-obese diabetic
PD-1	programmed death 1
PPI	preproinsulin
STZ	streptozotocin
TCR	T cell receptor
Th	T helper
Treg	regulatory T
T1D	type 1 diabetes
VNTR	variable number tandem repeat

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