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Type 1 diabetes pathogenesis and the role of inhibitory receptors in islet tolerance

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Abstract

Type 1 diabetes (T1D) affects over a million Americans, and disease incidence is on the rise. Despite decades of research, there is still no cure for this disease. Exciting beta cell replacement strategies are being developed, but in order for such approaches to work, targeted immunotherapies must be designed. To selectively halt the autoimmune response, researchers must first understand how this response is regulated and which tolerance checkpoints fail during T1D development. Herein, we discuss the current understanding of T1D pathogenesis in humans, genetic and environmental risk factors, presumed roles of CD4⁺ and CD8⁺ T cells as well as B cells, and implicated autoantigens. We also highlight studies in non-obese diabetic (NOD) mice that have demonstrated the requirement for CD4⁺ and CD8⁺ T cells and B cells in driving T1D pathology. We present an overview of central and peripheral tolerance mechanisms and comment on existing controversies in the field regarding central tolerance. Finally, we discuss T cell- and B cell-intrinsic tolerance mechanisms, with an emphasis on the roles of inhibitory receptors in maintaining islet tolerance in humans and in diabetes-prone mice, and strategies employed to date to harness inhibitory receptor signaling to prevent or reverse T1D.

Keywords

type 1 diabetes; tolerance; non-obese diabetic mice; cytotoxic T lymphocyte associated protein-4; programmed death-1; lymphocyte activation gene-3

Introduction

The immune system evolved to recognize, respond to, and resolve threats to homeostasis. Cells and secreted mediators of the immune system work in concert to eliminate hidden viral factories, nascent tumor cells, and even expel parasitic worms. Immune recognition is the critical first step, and it involves making a distinction between “self” or “innocuous” (e.g., heart muscle cells, pancreatic beta cells, and food particles) and “foreign” or “harmful” (e.g., infected or transformed cells, fungi, and parasites). While there are numerous tolerance mechanisms in place to ensure the body’s healthy cells and tissues are spared, these are not

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Competing interests

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infallible. In fact, one in every 15 people in the United States suffers from a systemic or organ-specific autoimmune disease ¹.

Autoimmune type 1 diabetes (T1D) is characterized by immune-mediated attack and destruction of insulin-producing pancreatic beta cells. As many as 1.25 million Americans currently live with T1D ², and >40,000 new cases are diagnosed each year ². Alarming, disease incidence has been on the rise, with a 21% increase between 2001 and 2009 among adolescents younger than 20 years of age ³. Insulin injections or insulin pumps are life-saving therapies, but they are not curative. To cure T1D, targeted immunotherapies must be developed to re-establish tolerance or eliminate beta cell-specific T and B cells, and to replenish the depleted beta cell mass. Developing selective immunotherapies will require knowing which beta cell-derived antigens drive T1D, as well as understanding how cognate T and B cells escape tolerance checkpoints.

Herein, we discuss the role of lymphocytes in orchestrating T1D, provide an overview of central and peripheral tolerance mechanisms, and discuss the existing evidence in support of and against tolerance defects in T1D patients or diabetes-prone mice. We also highlight the importance of T cell inhibitory receptor signaling in T1D, with emphasis on cytotoxic T-lymphocyte associated protein-4 (CTLA-4), programmed death-1 (PD-1), and lymphocyte activation gene-3 (LAG-3). as blocking these pathways in animal models and in the clinic has led to T1D occurrence. We also discuss ongoing efforts to harness CTLA-4, PD-1, and LAG-3 signaling pathways to prevent or treat T1D.

Type 1 diabetes pathogenesis

T1D is orchestrated by islet-specific CD4⁺ and CD8⁺ T cells, as well as B cells, and involves precise targeting and elimination of insulin-producing pancreatic beta cells ⁴. Islet-reactive lymphocytes encounter cognate antigen in the pancreatic lymph nodes, become activated, and traffic to the pancreas. Lymphocytes first surround the islet (peri-insulitis), and subsequently enter the islet core (destructive insulitis), causing beta cell demise and eventual T1D. T1D arises in genetically susceptible individuals, with median onset at 10–14 years of age ^{4,5}. Disease concordance in twins is ~60%, suggesting that environmental factors compound disease risk ⁶.

Genetic and environmental risk factors for T1D

Approximately 50% of T1D heritability is attributed to the human leukocyte antigen (HLA) region ^{7–9}. HLA Class II haplotype DRB1*0405-DQA1*0301-DQB1*0302 (DR4/DQ8) confers the highest genetic risk for T1D development with odds ratio (OR) as high as 11.37 ^{10–12}. Some haplotypes, such as DRB1*1501-DQA1*0102-DQB1*0602 (DR2/DQ6) are considered protective (OR = 0.03; see Refs. 10, 12). Specifically, amino acid identity at position 57 in the DQB-chain correlates with either T1D risk or protection ¹⁰. HLA II haplotypes with aspartate at position 57 (Asp-57) of the DQB-chain are more represented among non-diabetic individuals, while non-Asp-57 haplotypes are significantly increased in frequency among diabetic individuals ^{11, 13}.

Genome-wide association studies identified >50 other risk-compounding candidate genes, including those encoding insulin, PTPN22, PTPN2, and CTLA-4^{14–18}. The insulin gene, and specifically the number of tandem repeats preceding the transcription start site, is associated with a specific level of insulin mRNA expressed in the thymus during T cell development¹⁹. PTPN22 and PTPN2 are phosphatase molecules that regulate T and B cell signaling,^{16, 20} while CTLA-4 is a T cell inhibitory receptor, which will be discussed further below.

Despite the identified genetic factors, disease concordance between monozygotic twins is not 100%, which indicates that environmental factors also influence T1D development. The exact environmental trigger(s) remains unknown, but epidemiological studies^{21–23} and preclinical work with non-obese diabetic (NOD) mice suggest that enteroviral infections may increase disease risk through either molecular mimicry or bystander inflammation^{24, 25}. Specifically, enteroviral genetic material and enteroviral proteins or anti-viral autoantibodies have been detected in gut mucosa,²⁶ peripheral blood,²³ and beta cells of autoantibody positive– and type 1 diabetic patients around the time of diagnosis, but not in non-diabetic individuals,^{27–30} prompting many to speculate that viral infections trigger T1D onset in susceptible individuals.

Autoantibody production as a biomarker of T1D and implicated antigens

Autoantibody seropositivity against islet proteins precedes T1D development. Approximately 15% of individuals with autoantibodies against a single islet antigen develop T1D within 10 years. As many as 70% of individuals with autoantibodies against two or more different islet epitopes develop T1D within 10 years, and nearly all become diabetic over time, suggesting that epitope spreading is a biomarker of aggressive disease³¹. Autoantibody production likely results from B cell–CD4⁺ T cell interactions, following a breach in B and T cell tolerance to islet antigens. Many of the islet antigens recognized by B and T cells are therefore shared.³² Most common antigens include insulin, glutamic acid decarboxylase 65 (GAD65), zinc transporter 8 (ZnT8), and islet antigen-2 (I-A2). Notably, persistently high titers of insulin autoantibodies (IAA), but not anti-GAD65 or anti-I-A2, in children strongly correlate with rapid progression to T1D by 5 years of age^{33, 34}. Age at IAA onset and IAA titer are the most reliable predictors of age at disease onset, further suggesting that insulin and insulin-derived peptides constitute a critical target, at least in a subset of patients³¹. Understanding the dynamics of B cell–CD4⁺ T cell cross-communication in T1D might allow for earlier identification of at-risk patients and for intervention with immunotherapy prior to the extensive loss of beta cell mass.

Role of T cells in human T1D

Pancreas sections from pre-diabetic autoantibody positive and overtly diabetic individuals have shown CD4⁺ and CD8⁺ T cell infiltrates, suggesting that both cell types are involved in T1D^{35–38}. CD8⁺ T cells are thought to directly target and kill beta cells, while CD4⁺ T cells are thought to promote beta cell death via cytokine secretion and via help to CD8⁺ T and B cells³² (Fig. 1). HLA II associations with T1D risk and animal studies using CD4⁺-deficient mice support the role of CD4⁺ T cells as critical mediators of T1D in humans. Islet-reactive CD4⁺ and CD8⁺ T cell clones have been isolated from islets and from the pancreatic lymph

nodes of diabetic donors,^{39–43} as well as detected in peripheral blood of T1D patients (but not controls)^{44, 45}. Importantly, CD4⁺ and CD8⁺ T cells transductants or clones, respectively, isolated from patient islets have been shown to infiltrate the pancreas after transfer into humanized mice.^{43, 46}

With that in mind, T cell–targeting strategies have been attempted in the clinic, with some success. Treatment with the humanized antibodies anti-CD3 teplizumab and oteplizumab led to stabilization of C-peptide and reductions in exogenous insulin use and in hemoglobin A1c for 18–48 months in 40–75% of new onset patients^{47–53}. The mechanism of action is still unclear, but two possibilities have emerged based on pre-clinical data. Anti-CD3 may either promote an increase in regulatory T cell responses or promote apoptosis of T effector cells that infiltrate the pancreas^{54, 55}. It is important to note that patients showed a transient reduction in peripheral blood T cells, and some experienced viral reactivation⁴⁹. Anti-CD3 effect on disease stabilization correlated with age and with higher residual beta cell mass at treatment initiation, but long-term benefits were not seen^{49, 50}. Several trials tested the effect of low dose anti-CD3 on C-peptide stabilization and insulin use and found no difference between treatment and placebo groups^{56, 57}.

Anti-thymocyte globulin (ATG) treatment during early insulinitis or at disease onset effectively reduced T1D incidence in a pre-clinical setting by decreasing the number of effector T cells and preserving T regulatory cells^{58, 59}. The first trial in recently diagnosed human patients with high dose ATG (6.5 mg/kg) showed little to no stabilization of C-peptide or reduction in insulin use, and actually led to detrimental cytokine release and a decrease in regulatory T cells^{60, 61}. A subsequent trial utilized a low dose of ATG (2.5 mg/kg) and showed stabilization of C-peptide for up to a year⁶². Collectively, anti-CD3 and ATG clinical trials suggest that modulating T cell responses can delay disease progression. However, neither strategy reversed T1D, and both were associated with viral reactivation, as they were directed against all T cells.

Strategies that enhance the regulatory arm of the immune system have also been employed in the context of T1D, with exciting preliminary results. Expansion and reinfusion of polyclonal, patient-derived regulatory T cells led to C-peptide stabilization for more than 2 years in a phase I clinical trial, and it was well tolerated⁶³. Importantly, infused cells were detected long term, and appeared stable. While patients enrolled in this trial did not present with opportunistic infections, global immune suppression with polyclonal regulatory T cell therapy is still a concern. Efforts are underway to expand or design islet antigen–specific regulatory T cells, and to circumvent the non-specificity problem as well as potentially enhance therapeutic benefit⁶⁴.

Role of B cells in human T1D

Even though autoantibodies are a reliable marker of T1D development and are still used to stratify at-risk patient cohorts, the role of B cells in T1D has been a matter of debate. A case study reported T1D development in a patient with severe hereditary B cell deficiency⁶⁵. However, recent studies demonstrated a positive correlation between B cell presence in insulinitic lesions and fast beta cell destruction, early onset time, and more aggressive disease^{66–68}. Furthermore, B cell depletion with anti-CD20 (rituximab) within 100 days of

diagnosis demonstrated stabilization of C-peptide production and reduced the need for exogenous insulin among treated patients, compared with placebo controls, for up to a year⁶⁹.

Typically, antibody production results from cognate B cell–CD4⁺ T cell interactions in the germinal center region of the lymph node follicle^{70, 71}. Studying antigen-specific germinal center reactions in patients with autoimmunity is technically challenging given the small precursor populations of autoreactive B and T cells and limited access to lymph node samples. One study noted fewer germinal centers in the pancreatic lymph nodes of new-onset versus either control or long-standing diabetic patients⁷². However, others found an increase in peripheral blood CD4⁺ T cells with a T follicular helper (T_{FH})-like signature in T1D patients,^{73, 74} and reported a decrease in circulating T_{FH}-like cells in patients treated with rituximab.⁷⁵ Mechanistic studies performed in NOD mice shed more light on the involvement of B cells in T1D and are discussed below.

NOD mouse model of T1D

Non-obese diabetic (NOD) mice were originally derived as a substrain of Jcl:ICR mice at the Shionogi Research Laboratories in Japan for studying cataract development⁷⁶. NOD mice develop high fasting blood glucose levels and have been an invaluable tool for studying T1D ever since⁷⁷. T1D incidence in NOD mice is colony- and sex-dependent. Incidence in female mice ranges between 50 and 90% by 30 weeks of age, as compared to 20–30% in male mice^{32, 77}. Unless otherwise noted, all studies discussed below were performed in female mice.

Genetic factors accounting for T1D susceptibility in mice

As in humans, major histocompatibility complex (MHC) class II haplotype in mice confers the highest risk of T1D development. NOD haplotype H-2^{g7} expresses I-A but not I-E molecules. Expression of other I-A alleles, such as I-A^b, completely protects NOD mice from T1D⁷⁸, as does introducing a functional I-E locus⁷⁹. Interestingly, amino acid identity at position 57 in the beta chain is associated with T1D risk in NOD mice, as it is in humans. Swapping out the native serine at position 57 for aspartate in the I-A^{g7} beta chain prevents T1D⁸⁰, further substantiating clinical studies^{11, 81}. It is thought that aspartate in this position facilitates the formation of a salt bridge at the periphery of the peptide-binding groove with arginine at position 76 of the alpha chain. A non-aspartate amino acid would prevent salt bridge formation, likely altering the peptide binding repertoire and, by extension, influencing the T cell repertoire as well⁸². Therefore, I-A^{g7}, specifically non-aspartate residues at beta chain 57 position, is required for overt T1D in NOD mice. However, it is important to note that I-A^{g7} alone is not sufficient for disease. C57BL/6 (B6) mice expressing NOD I-A^{g7} (B6.g7 mice) do not develop diabetes,⁸³ indicating that other non-MHC loci account for disease susceptibility on the B6 background. To identify them, researchers have crossed NOD mice to diabetes-resistant inbred mice and identified >35 mono- or polygenic *Idd* (insulin dependent diabetes) loci. Gene identity and inter-loci interactions are still an area of active inquiry, but several loci have already been thoroughly mapped and include cytokines and immune receptors implicated in regulating T cell

responses such as *Il2*, *Il21* (*Idd3*), *Ctla4*, *Icos* (*Idd5*), *Lck*, and *Tnfrsf9* (*Idd9*), some of which are discussed further below.

Environmental factors associated with T1D onset in NOD mice

Increase in autoimmune disease incidence parallels the decline of infectious disease burden in Western countries⁸⁴. This observation led to the formulation of the hygiene hypothesis, which links the lack of early microbial exposures to an exaggerated immune response to future threats and innocuous antigens⁸⁴. As mentioned above, newly diagnosed T1D patients have been shown to have anti-Epstein Barr virus antibodies or evidence of prior exposure to other enteroviruses, such as coxsackie B3^{27–30}. While a causal relationship is difficult to establish, studies in NOD mice have shown context-dependent effects of viral infection on T1D development. Lymphocytic choriomeningitis virus (LCMV) or coxsackie virus B3 infection can prevent or delay T1D in <6-week-old NOD mice that lack insulinitis, supporting the hygiene hypothesis^{85–87}. However, LCMV or coxsackie B3 infection in NOD mice with pre-established insulinitis dramatically accelerates T1D, potentially explaining how some patients have evidence of prior viral exposure around the time of T1D diagnosis^{87, 88}.

CD8⁺ T cells directly kill beta cells in T1D

CD8⁺ T cell-deficient NOD mice are completely protected from insulinitis and T1D,^{89–92} indicating that CD8⁺ T cells have a critical role in disease initiation and propagation. CD8⁺ T cell clones isolated from patient islet lesions or from diabetic NOD mice cause T1D, demonstrating that these cells are directly pathogenic.^{43, 93, 94} It is thought that CD8⁺ T cells become activated in the pancreatic lymph node and traffic to the pancreas where they interact with pancreatic beta cells. CD8⁺ T cell action is TCR-peptide-MHC I dependent (Fig. 1) and involves the release of perforin and granzyme B from T cell cytolytic granules. Perforin then creates holes in the beta cell membrane, allowing granzyme B to enter the cytoplasm, cleave caspase-3, and activate the apoptosis pathway^{95, 96}. Interestingly, perforin, but not granzyme B, is required for T1D development,^{97, 98} suggesting that other secreted mediators are in play as well.

B cells present antigen and secrete autoantibodies

B cell presence⁹⁹ and B cell-intrinsic MHC I and II expression are necessary for insulinitis progression to overt T1D^{100, 101}. These findings indicate that B cells are critical antigen-presenting cells to both CD4⁺ and CD8⁺ T cells in the advanced stages of disease (Fig. 1).

Similar to humans, autoantibody presence in NOD mice serum predicts time to T1D onset.¹⁰² However, the role of autoantibodies in disease has been a matter of debate. Serum transfer from diabetic mice to non-diabetic mice does not accelerate T1D¹⁰³, which is in stark contrast to other autoimmune diseases and autoimmune disease models such as rheumatoid arthritis and systemic lupus erythematosus (SLE).^{104–107} Because of this finding, some researchers have dismissed autoantibodies and B cells as critical players in T1D. Yet, in other work, if B cells are altered so they cannot secrete autoantibodies, T1D incidence and penetrance are significantly reduced¹⁰⁸. Subsequent studies have shown that serum autoantibody transfer in a TCR transgenic setting stimulates CD4⁺ T cell activation

through an Fc receptor–dependent mechanism¹⁰⁹. Similarly, in the context of viral infection, antibody-mediated antigen delivery to dendritic cells and macrophages via Fc receptors has been shown to be important for priming CD4⁺ and CD8⁺ T cell responses^{110, 111}. Therefore, it is possible that, instead of directly causing pathology, autoantibodies help capture limiting antigen and facilitate T cell priming, thus propagating the autoimmune response. B cell–CD4⁺ T cell interactions and the mechanism underlying autoantibody generation are further discussed below.

CD4⁺ T cells have multiple roles in T1D

CD4⁺ T cells are MHC class II-restricted; therefore, the strong association between MHC II haplotype and T1D in both mice and humans suggests that CD4⁺ T cells play a critical role in T1D. Indeed, genetic manipulation and antibody depletion studies have shown that CD4⁺ T cells are required for disease initiation. NOD mice lacking MHC II expression are protected from destructive insulinitis and overt T1D^{89, 112}. Additionally, NOD mice lacking the class II transcription activator (CIITA), a non-DNA binding transcription factor that promotes MHC II expression, are also protected from invasive insulinitis and overt T1D¹¹². Furthermore, CD4⁺ T cell depletion at 6 weeks of age and at disease onset prevents and reverses T1D, respectively^{113–115} suggesting that CD4⁺ T cells are required for T1D initiation and progression.

As mentioned above, CD4⁺ T cell transductants can infiltrate the pancreas and cause diabetes after transfer into humanized mice receiving low dose streptozotocin⁴⁶. Similarly, CD4⁺ T cell clones isolated from diabetic NOD pancreata, such as BDC2.5 CD4⁺ T cells, cause T1D after transfer, without the need for CD8⁺ T or B cells^{116, 117}. CD4⁺ T cells are thought to provide CD40 ligand co-stimulation to dendritic cells and enhance cross-presentation to islet-reactive CD8⁺ T cells³² (Fig. 1). In the pancreas, activated T_H1 CD4⁺ T cells are a key pathogenic cell population, as they promote beta cell death by secreting proinflammatory cytokines interferon-gamma (IFN- γ) and tumor necrosis factor alpha (TNF- α)¹¹⁸ and polarize islet-resident macrophages to an M1 phenotype¹¹⁹ (Fig. 1). M1 macrophages similarly secrete cytokines and amplify the beta cell death cycle¹²⁰. Recent studies have demonstrated MHC II expression on a subset of beta cells themselves; therefore, it is possible that CD4⁺ T cells exert their diabetogenic role in part through directly interacting with beta cells¹²¹ (Fig. 1). In addition, CD4⁺ T cells provide help to B cells in the germinal center (see below) and promote autoantibody affinity maturation and class switching^{70, 71, 122} (Fig. 1). Finally, a subset of CD4⁺ T cells with regulatory function (T_{reg} cells) limits immune responses and helps maintain islet tolerance. The role of regulatory T cells in T1D has been an extensive area of investigation both in animal models and in human patients. The role of T_{reg} cells in T1D will be further discussed below.

Germinal centers and T1D

CD4⁺ T cells and cognate B cells interact at the T:B zone border and in the GC region of lymph nodes to promote B cell differentiation into antibody-secreting plasmablasts and then maturation into germinal center B cells (Fig. 2).^{70, 71, 122, 123} Germinal center B cells are “auditioned” for reactivity to cognate antigen through their interactions with T_{FH} CD4⁺ T cells. B cell receptor (BCR) mediated antigen intake leads to antigen processing and

presentation in the context of MHC II. Cognate CD4⁺ T cells “perceive” the amount of presented antigen (which is proportional to BCR antigen affinity) and produce interleukin (IL)-4 and IL-21 and provide CD40 ligand to germinal center B cells¹²⁴ (Fig. 2). These signals turn on activation-induced cytidine deaminase in the B cells whose BCR then undergoes somatic hypermutation (SHM), a process that introduces random mutations into the V(D)J regions of the immunoglobulin. Cells with favorable mutations bind antigen with a higher affinity, receive more CD4⁺ T cell help, and are stimulated to proliferate and, ultimately, differentiate into memory B cells or long-lived plasma cells^{70, 71}. While CD4⁺ T_{FH} cells stimulate B cells, a subset of T_{reg} cells called T follicular regulatory (T_{FR}) cells oppose their function and limit germinal center reactions¹²⁵ (Fig. 2). Much of what is currently known about the germinal center dynamics stems from studies of vaccination or infection. The kinetics and dynamics of B cell–CD4⁺ T cell interactions in spontaneous autoimmunity have been more difficult to delineate. This is primarily due to self-antigen-specific CD4⁺ T and B cells being rare and difficult to track, and due to inherent stochasticity of infrequent antigen encounter(s). To begin to bridge this knowledge gap, Wan *et al.* used an adoptive transfer model of transgenic insulin-specific CD4⁺ T cells and transgenic insulin-specific B cells¹²⁶. In their study, NOD.*Rag*^{−/−} recipient mice developed high IAA titers and T1D after cell transfer, while a large population of CD4⁺ T cells and B cells in the pancreatic lymph node acquired the germinal center phenotype¹²⁶. Increases in bulk T_{FH}-like cells or increases in a T_{FH}-like gene signature have been documented in peripheral blood of patients with T1D^{73–75, 127} and in patients with other autoimmune diseases^{128–131}. An increase in T_{FH}/T_{FR} ratio has recently been documented in patients with rheumatoid arthritis, and further indicates that germinal center cells contribute to autoimmune pathology in humans as well¹³².

Major autoantigens in NOD T1D

Similarly to humans, IAA production in mice can be used to predict time to T1D onset¹⁰² and correlates with the extent of beta cell damage¹³³. Anti-insulin reactivity in both the T cell and the B cell compartment is required for T1D^{134, 135}. Insulin B10–23 peptide appears to be immunodominant, since 90% of insulin-specific CD4⁺ T cells recognize this epitope.¹³⁶ Mutating residue 16 in the B10–23 peptide (a TCR contact site) abrogates T1D, insulinitis, and IAA, suggesting that T cell reactivity to this epitope is critical for disease¹³⁴. Additionally, promoting antigen-specific tolerance using whole insulin-coupled apoptotic splenocytes reverses T1D in ~50% of NOD mice, further highlighting the importance of anti-insulin responses in T1D.¹³⁷ Treatment with a monoclonal antibody that blocks insulin B10–23–I-A^{g7} complex delays T1D, suggesting that preventing the activation of insulin-specific CD4⁺ T cells has a therapeutic benefit.¹³⁸

While most studies in the NOD mouse model focused on the role of anti-insulin responses, recent work has implicated GAD65, islet amyloid polypeptide (IAPP), islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), chromogranin A (ChgA), as well as a newly identified class of epitopes termed “hybrid insulin peptides”. Using adoptive cell transfer, antigen-deficient mice, and antigen-specific tolerance researchers have attempted to establish an epitope hierarchy model, or at least determine which T cell specificities are inciting T1D.

GAD65 is one of the major autoantigens in human T1D, but its role in NOD mice has been controversial. One study reported anti-GAD65 autoantibodies in NOD serum,¹³⁹ while others found no evidence of GAD-specific autoantibodies in diabetic mice.^{140–142} NOD T cells can be stimulated in response to GAD65 and appear to produce IFN- γ ,¹⁴³ suggesting that at least the T cell compartment breached tolerance to GAD. However, GAD65 likely is not the inciting antigen, as GAD65-deficient mice developed T1D at the same rate as NOD mice¹⁴⁴ and GAD65 expression under a MHC class I promoter did not prevent T1D¹⁴⁵.

CD4⁺ T cells that recognize IAPP become activated, and infiltrate the pancreas as NOD mice approach disease onset (>12 weeks of age), suggesting that they play a role in disease¹⁴⁶. However, IAPP-deficient NOD mice develop T1D at a similar rate as wild-type mice, indicating that IAPP is not the inciting antigen either¹⁴⁶. IGRP has mostly been studied as a target of CD8⁺ T cells^{147–149}. Indeed, as many as 30% of islet-infiltrating CD8⁺ T cells respond to IGRP peptide stimulation¹⁴⁹. Importantly, IGRP-specific 8.3 TCR transgenic CD8⁺ T cells can directly cause disease upon transfer into NOD recipients. Despite this evidence, anti-IGRP responses during spontaneous disease likely are not sufficient, as establishing tolerance to IGRP does not prevent anti-insulin responses or T1D¹⁵⁰.

In recent years, ChgA has emerged as a critical antigenic target in T1D. BDC2.5 TCR transgenic CD4⁺ T cells recognize ChgA-derived peptides, and induce T1D upon transfer into NOD recipients.¹⁵¹ Importantly, ChgA-deficient NOD mice are completely protected from T1D, placing this antigen high in the epitope hierarchy.¹⁵² Recent discovery of hybrid insulin peptides, likely formed through transpeptidation of insulin and ChgA, suggests that immune responses to these types of neopeptides could kick-start T1D, and explains why both insulin and ChgA are required for disease.^{40, 41, 153, 154} Less is known about initiating antigens in human T1D patients, but intense research is focused on defining the critical T and B cell targets.

Tolerance mechanisms in T1D

Autoreactive lymphocytes are normally pruned from the T and B cell repertoire during development (central tolerance) or held in check by various mechanisms in the periphery (peripheral tolerance). T1D, like other autoimmune diseases, is thought to arise due to defects in central and peripheral tolerance checkpoints, discussed below.

Central tolerance

T cells develop in the thymus, where they rearrange their TCR V(D)J regions and commit to the $\alpha\beta$ or the $\gamma\delta$ lineage. Developing thymocytes are selected for reactivity toward self-peptide–MHC molecules to ensure a repertoire capable of responding to MHC-presented peptides. This positive selection is facilitated by dendritic cells, B cells, and thymic epithelial cells (TECs), which display intracellular peptides.¹⁵⁵ TECs located in the medulla (mTECs) also express autoimmune regulator (*AIRE*), a transcription factor that induces expression of tissue-restricted self-antigens.^{156–161} TCR beta and gamma chain VDJ rearrangements occur simultaneously in developing T cells, and lineage commitment occurs following successful chain rearrangement and signaling through a pre-TCR¹⁶². Beta-lineage committed thymocytes then undergo alpha chain VJ rearrangement. The resulting alpha

chain pairs with beta to form a TCR, which is then tested for functionality. Weakly reactive or highly autoreactive thymocytes may undergo “receptor editing” and rearrange their alpha locus ¹⁶³. Receptor editing can therefore salvage a developing T cell or have an undesirable effect on its receptor affinity. Thymocytes with a low affinity for self-peptide–MHC receive weak survival signals and are deleted from the repertoire (“death by neglect”). This form of negative selection eliminates cells that are unlikely to participate in a future immune response to a foreign antigen. Thymocytes with a very high affinity for self-peptide–MHC receive too strong of a signal through their TCR and undergo “clonal deletion” ¹⁶⁴. This form of negative selection purges T cells which are likely to cause tissue damage in an effort to prevent autoimmunity. As many as 75% of developing T cells undergo deletion during thymic selection ^{165, 166}. The remaining thymocytes have TCRs with medium affinity, and these T cells proceed through development and differentiate into mature CD4⁺ or CD8⁺ T cells (positive selection). CD4⁺ single positive thymocytes with moderate-to-high affinity for self-antigen can also undergo agonist-driven differentiation into regulatory T cells ^{167–172}.

Self-antigen expression in the thymus is critical for self-tolerance. Humans lacking *AIRE* develop profound systemic autoimmunity known as APECED (autoimmune polyendocrinopathy, candidiasis, ectodermal dystrophy) and similar pathologies are observed in mice as well. ^{159, 173, 174} Insulin gene expression is *AIRE*-regulated, ¹⁵⁹ and it is thought that low expression of insulin in the thymus and/or poor binding of native insulin-derived peptides to MHC II molecules contribute to defective central tolerance in T1D. ^{175–177} Variable number of tandem repeats (VNTR) in the insulin gene promoter influence *AIRE* binding, and by extension, also influence the level of insulin mRNA in the thymus. VNTR can therefore have a strong association with T1D disease risk or protection ¹⁹. Specifically, 140–200 repeats are associated with a high expression of insulin, and account for T1D protection. Having 26–63 repeats is associated with a low expression of insulin in the thymus, and consequently with diabetes risk ^{175, 178–180}. Thymus-specific deletion of insulin promotes T1D ¹⁸¹, while transgenic expression of proinsulin under the MHCII promoter protects NOD mice from disease development ¹⁸². By extension, promoting low expression of insulin-derived peptides in the thymus of insulin-specific CD4⁺ TCR retrogenic mice allows the escape of insulin-specific CD4⁺ T cells, while high expression of insulin-derived peptides promotes the deletion of cognate T cells ¹⁷². Using transgenic mice that express enhanced green fluorescent protein (eGFP) under the control of different promoters, and tetramers to track GFP-specific CD4⁺ T cells, Malhotra and colleagues linked the level of antigen expression in the thymus to a specific mode of tolerance induction;¹⁸³ ubiquitin- or beta actin-driven GFP expression promoted efficient deletion of GFP-specific CD4⁺ T cells (tolerance cluster 3). Insulin 2, Foxp3, and CD207 gene promoters induced GFP expression in pancreatic beta cells and mTECs, regulatory T cells, and thymic dendritic cells, respectively. These expression patterns led to a partial deletion of GFP-specific CD4⁺ T cells in the thymus and development of GFP-specific regulatory T cells (tolerance cluster 2). Insulin 1 and FOXD1 gene promoters induced GFP expression exclusively in pancreatic beta cells and during kidney and eye development, respectively. Thus, in insulin 1 (*ins*)-eGFP and *Foxd1*-eGFP mice, GFP was excluded from the thymus; and while GFP-specific CD4⁺ T cells developed normally in these animals they remained ignorant of their antigen (either insulin or FOXD1) in the periphery (tolerance Cluster 1).

As mentioned above, CD4⁺ T cells with medium to high affinity for self antigens can differentiate into T_{reg} cells in the thymus^{183–186}. Thymic T_{reg} cells are characterized by stable expression of FOXP3 transcription factor, and have the ability to suppress CD4⁺ and CD8⁺ T cell activation and effector responses, reduce co-stimulatory ligand expression on antigen presenting cells, and secrete anti-inflammatory cytokines IL-10 and TGF- β to alter the cytokine milieu¹⁸⁷. T_{reg} cells are critical for tolerance maintenance, as T_{reg} cell deficiency or depletion leads to devastating autoimmunity^{188, 189}. In humans, FOXP3 deficiency leads to multi-organ autoimmunity manifested as IPEX (X-linked immune polyendocrinopathy syndrome)¹⁹⁰. Similarly, FOXP3-deficient (*Foxp3*^{-/-}) scurfy mice develop lethal systemic autoimmunity^{191–194}.

There is conflicting evidence regarding whether NOD mice have defects in autoreactive T cell deletion. NOD thymic epithelial cells, but not B6 or BALB/c, have been shown to support the development of diabetogenic CD4⁺ and CD8⁺ T cells in the context of a thymus transplant into B6 athymic recipients, suggesting that the tolerance defect may partially reside in the antigen presenting cell compartment¹⁹⁵. Other studies attempted to discern whether NOD thymocytes themselves have inherent properties that make them less susceptible to clonal deletion. Medullary semi-mature thymocytes were shown to express high levels of caspase inhibitors upon TCR stimulation¹⁹⁶ and thus acquire apoptosis resistance or fail to express the pro-apoptotic factor Bim¹⁹⁷. Using CD4⁺ or CD8⁺ TCR transgenic systems (BDC2.5, AI4, 3A9, or 8.3) and MHC-matched diabetes-resistant mice, several studies demonstrated a NOD T cell-intrinsic failure to delete highly autoreactive lymphocytes^{198–203} likely due to higher T cell signaling thresholds necessary to mediate deletion²⁰⁴. Using the BDC2.5 CD4⁺ T cell transgenic system and mixed bone marrow chimeras where B6.g7- and NOD-derived BDC2.5 thymocytes developed in the same thymus, others reported that clonal deletion in response to agonist stimulation was comparable between NOD and B6.g7 strains²⁰⁵. Since early TCR transgene expression has been shown to alter development fates,²⁰⁶ it is possible that the conclusions were confounded by the analysis of TCR transgenic systems. In order to truly discern whether defects in clonal deletion contribute to NOD autoimmunity, endogenous islet-reactive T lymphocytes should be assessed.

There has also been a lack of consensus on whether T_{reg} cell development is compromised in NOD mice. T_{reg} cell diversity, as assessed by TCR alpha- and beta-chain usage, is lower in NOD compared to B6 mice, and appears to result from MHCII as well as other non-MHC genes, particularly those in *Idd9*^{207, 208}. Compared to B6 and BALB/c mice, NOD mice were shown to have fewer T_{reg} cells in the thymus²⁰⁹. However, T_{reg} cells can recirculate back to the thymus²¹⁰; therefore, it is possible that lower T_{reg} cell frequency in NOD thymii is due to inefficient T_{reg} cell generation or a lower degree of T_{reg} cell recirculation. Feuerer and colleagues used NOD and B6 fetal thymic organ cultures and found that optimal T_{reg} cell induction on the NOD background required at least 3-fold more antigen than on the B6 background.²¹¹ However, at high antigen doses on the NOD background more T_{reg} cells were selected.²¹¹ The authors interpreted these findings as evidence for enhanced selection of NOD T_{reg} cells in the thymus. Studies involving physiological levels of selecting antigen and markers that distinguish developing from recirculating T_{reg} cells are needed to fully address whether NOD mice have a defect in T_{reg} cell development.

B cells develop in the bone marrow, and similarly to developing T cells, B cells undergo V(D)J recombination to rearrange their immunoglobulin heavy and light chains.^{212, 213–216} Pre-B cells also “audition” their rearranged BCRs for reactivity to antigens found in the bone marrow. Weakly or overly reactive BCRs have an opportunity to undergo light chain rearrangement (also known as receptor editing) and to test a newly edited BCR for successful signal transduction or against autoreactivity^{214, 215}. An estimated 20% of developing B cells undergo receptor editing to reduce autoreactivity²¹⁷. Cells with a weak BCR do not receive survival signals and die by neglect, while B cells with high affinity BCRs that recognize membrane-bound or soluble antigens present in the bone marrow undergo deletion^{212, 213, 218}. B cells that receive sufficient tonic signal through their BCR in the bone marrow successfully leave and complete their development in the spleen.

Despite receptor editing, as many as 10% of B cells in healthy individuals are self-reactive, and these cells are held in check by peripheral tolerance mechanisms, including anergy²¹⁹. Anergic B cells have been shown to mutate away from self-reactivity in the germinal center and gain the ability to participate in protective immune responses, explaining in part why leaky B cell central tolerance might be beneficial²²⁰. Another potential explanation for incomplete central tolerance in the B cell compartment lies in B cell reliance on CD4⁺ T cell help for high affinity (auto)antibody production²²¹. Thus, although existent in the periphery, self-reactive B cells would be held in check due to a lack of CD4⁺ T cell help.

B cell central tolerance has been studied in the context of T1D. Using transgenic mice with fixed anti-insulin immunoglobulin heavy chain, Henry-Bonami and colleagues demonstrated that insulin-specific immature B cells were detected at a higher frequency in the bone marrow sinusoids of NOD compared to B6 mice, demonstrating impaired clonal deletion²²². Others have shown that light chains isolated from bulk NOD B cells show a lower degree of receptor editing compared to light chains isolated from B6 B cells²²³. When examining BCR transgenic insulin-specific B cells, Henry-Bonami *et al.* also observed significantly fewer edited insulin-specific BCRs in NOD compared to B6 mice, thus providing another line of evidence in support of faulty B cell central tolerance in T1D²²⁴.

Peripheral tolerance

Since central tolerance is not infallible, multiple checkpoints exist in the periphery to ensure that self-reactive T and B cells are held at bay. These include ignorance, clonal deletion, peripheral T or B regulatory cell induction and suppression, anergy, inhibitory receptor upregulation, and exhaustion. Each mode of peripheral tolerance is discussed further below.

Ignorance refers to the lack of cognate antigen recognition in the periphery either due to antigen exclusion from lymphoid organs or due to low antigen concentrations^{225–228}. As mentioned above, ignorance is the primary mode of tolerance that restrains CD4⁺ T cells specific for tissue-restricted antigens¹⁸³. If self-specific CD4⁺ T cells encounter a cognate antigen in the periphery, other modes of peripheral tolerance discussed below can act as a fail-safe (Fig. 3).

Self-antigen encounter in the secondary lymphoid organs may result in T cell deletion, which is likely mediated by Fas-ligand-expressing antigen presenting cells^{229–231} or by

extra-thymic AIRE-expressing cells (eTAC) in tissue draining lymph nodes²³². Transgenic NOD mice expressing IGRP or p31 (a peptide mimotope for the BDC2.5 CD4⁺ T cell) under the *Aire* promoter are resistant to diabetes induction by adoptive transfer of cognate 8.3 CD8⁺ or BDC2.5 CD4⁺ TCR transgenic T cells, further demonstrating the ability of eTACs to mediate peripheral deletion^{232, 233}.

T cell fate upon antigen encounter is context-dependent. Optimal T effector (T_{eff}) cell differentiation requires peptide–MHC recognition (signal 1), CD28/CD80 co-stimulation (signal 2), and cytokines (signal 3)²³⁴. IL-2, IL-12, IFN- α , and IL-21 are potent signal 3 inducers for CD8⁺ T effector differentiation²³⁴. Cytokines and TCR signal strength also dictate various CD4⁺ T cell differentiation programs: IFN- γ and IL-12 induce T_H1 polarization, IL-4 induces T_H2, TGF- β and IL-6 induce T_H17 cells, and IL-6 and IL-21 promote T_{FH} cell polarization^{70, 235}. Importantly, if CD4⁺ T cells encounter their antigen in the presence of TGF- β alone, they can differentiate into peripherally-induced T_{reg} cells that express Foxp3, IL-10, and TGF- β and participate in dampening immune responses, thus adding an additional layer of protection from autoimmunity^{236, 237} (Fig. 3).

T_{reg} cell-mediated immunosuppression is necessary for tolerance maintenance in NOD mice, but it is ultimately insufficient. In TCR transgenic systems, BDC12–4.1 or BDC2.5 CD4⁺ T cells differentiate into both T_{eff} and T_{reg} cell lineages in the periphery, and mice remain diabetes-free^{238, 239}. However, if these TCR transgenic mice are crossed to NOD.*Foxp3*^{-/-} animals, diabetes develops rapidly^{238, 239}. There has been considerable debate in the field regarding whether T_{reg} cells lose their suppressive function as diabetes develops, or whether T_{eff} cells become resistant to T_{reg} cell-mediated suppression. T_{reg} cells from 8-week-old, but not 16-week-old mice, delayed T1D onset induced by adoptive transfer of effector cells, and produced more IL-10, suggesting an age-dependent decline in T_{reg} cell function²⁴⁰. Tang and colleagues showed that the number of T_{reg} cells paradoxically increased in the pancreatic lymph nodes as mice approached disease onset, but that the T_{reg}:T_{eff} ratio in the islets decreased, possibly due to lower levels of CD25 and pro-survival factor Bcl-2 in islet-infiltrating T_{reg} cells²⁴¹. Others argued that the number of T_{reg} cells in NOD mice is not different compared to non-autoimmune prone mice, and that NOD T_{reg} cells had a similar suppressive capacity *in vitro* as did B6 T_{reg} cells at a high T_{reg}:T_{eff} ratio²⁴². This study further demonstrated that NOD T_{eff} were more resistant to T_{reg} cell-mediated suppression²⁴². A similar finding was also reported by others^{240, 243}. Similar T_{reg} and T_{eff} cell defects have been reported in T1D patients^{244, 245}, which prompted the Bluestone group to investigate the therapeutic efficacy of T_{reg} cell infusion. *Ex vivo* T_{reg} cell expansion and re-infusion into diabetic NOD mice effectively reversed spontaneous and adoptively transferred T1D²⁴⁶. In patients, *ex vivo* T_{reg} cell expansion and subsequent reinfusion led to C-peptide stabilization for 2⁺ years in a phase I clinical trial and was well tolerated⁶³. A phase II clinical trial is in progress and will inform future use of T_{reg} cell adoptive cell therapy.

In recent years, populations of suppressive B cells have been described, and collectively termed B regulatory cells (B_{reg} cells)^{247–254}. B_{reg} cells have been shown to dampen a variety of immune responses, and several different subsets of B_{reg} cells have been identified^{247–249}. It appears that B_{reg} cells are induced in the periphery, likely in a T cell-independent

manner, and in response to proinflammatory cytokines IL-1 β , IL-6, GM-CSF and IL-15^{255, 256} as well as following TLR stimulation with lipopolysaccharide (LPS) and CpG^{257, 258}. Populations of B_{reg} cells have been identified among marginal zone and follicular B cell populations, as well as among peritoneal B1 B cells, and include CD5^{hi}CD1d⁺, CD5^{hi}CD1d⁺ Tim-1⁺, CD5^{neg}CD1d^{hi}B220^{low}CD11b⁺ IgM⁺, or CD200⁺ PD-L1⁺PD-L2⁺LAG-3⁺ cells^{250, 259}. B_{reg} cells secrete IL-10, IL-35 and/or TGF β to dampen DC priming²⁶⁰, CD4⁺, CD8⁺ T cell and B cell responses^{247–249, 261, 262}, induce CD4⁺ T cell apoptosis²⁶³ or CD8⁺ T cell anergy²⁶⁴, and promote peripheral T_{reg} cell differentiation^{261, 265, 266}. Because a universal set of phenotypic markers or a lineage-defining transcription factors have not yet been identified for B_{reg} cells, some investigators speculated that B_{reg} cell populations represent short-lived effector cells induced by ongoing inflammation²⁵⁰. Regardless, B_{reg} cells can modulate multiple autoimmune pathologies, including experimental autoimmune encephalomyelitis²⁴⁸ and T1D. NOD mice treated with LPS-activated B cells had reduced and delayed spontaneous and adoptively transferred T1D onset, through FasL-mediated apoptosis of diabetogenic CD4⁺ T cells²⁶³. Emerging evidence from clinical studies suggests that B_{reg} cells can help induce human peripheral T_{reg} cells^{267–269}, thus providing another way to expand patient's own T_{reg} cells prior to adoptive cell therapy.

If CD4⁺ or CD8⁺ T cells “see” their cognate antigen in the absence of signal 2 or signal 3, they become anergic (functionally silenced)^{270–279}. T_{reg} cell presence and interaction with dendritic cells is required for anergy induction in both the CD4⁺ and the CD8⁺ T cell compartment^{280, 281}. Anergic CD4⁺ T cells have a low proliferative capacity, low cytokine producing capability, and are largely confined to the secondary lymphoid organs^{274–276}. Similarly, anergic CD8⁺ T cells have low proliferative potential, impaired cytokine production, and low cytotoxic function^{278, 280, 282}. While anergic CD8⁺ T cells have been shown to express high CTLA-4 and low levels of pro-survival factor Bcl-2²⁸⁰, anergic CD4⁺ T cells can express folate receptor 4 (FR4) and CD73, an ectoenzyme involved in the breakdown of ATP to adenosine (Fig. 3)^{281, 283, 284}. Adenosine has an immunosuppressive function; therefore, it is possible that CD73 expressed by anergic cells participates in dampening nearby immune activation or by reinforcing a cell's own anergic phenotype. Recent work from Kalekar *et al.* has shown that neuropilin-1⁺ FR4⁺ CD73⁺ anergic cells have an ability to differentiate into T_{reg} cells in the context of lymphopenia (Fig. 3)²⁸⁵. These cells had a DNA demethylation pattern more similar to T_{reg} cells than T_{eff} cells at key T_{reg} lineage-specifying loci, including *Foxp3*, *Ctla4*, *Ikzf4*, and *Tnfrsf18*²⁸⁵. With a potential to suppress immune responses via CD73 and a propensity to differentiate into pT_{reg} cells in a lymphopenic niche, anergic CD4⁺ T cells might help keep other autoimmune cells at bay. This in turn may provide an evolutionary benefit to keeping anergic CD4⁺ T cells around instead of eliminating them through clonal deletion. Using peptide–MHCII tetramers to track endogenous insulin-specific CD4⁺ T cells, we have shown that ~10% of these cells express Foxp3, while ~40% express the anergy markers FR4 and CD73 and do not produce IFN- γ upon TCR stimulation²⁸⁴. Whether anergic insulin-specific CD4⁺ T cells eventually give rise to T_{reg} cells or lose their anergic state as diabetes progresses remains to be determined.

Like CD4⁺ and CD8⁺ T cells, anergic B cells respond poorly to antigen receptor stimulation. Anergic B cells express low levels of IgM while maintaining IgD, and they are detected in nonautoimmune mice and in healthy individuals. Elegant studies by the Goodnow and Jenkins groups have shown that B cell anergy is effectively induced among low affinity B cells reactive to ubiquitous membrane-bound self-proteins^{219,212, 221}. A subset of insulin-specific B cells in T1D patients was shown to be anergic; interestingly, the frequency of these cells decreased at onset, suggesting that a loss of anergy might account for disease activity²⁸⁶. As mentioned above, anergic self-specific B cells can mutate away from self-reactivity in the germinal center and participate in protective anti-microbial responses²²⁰. However, the reverse scenario is also possible: previously non-self-reactive B cells could undergo somatic hypermutation (SHM) in the germinal center and, due to the stochastic nature of this process, acquire self-reactivity²⁸⁷. To test this idea, Chan and colleagues tracked HyHEL10 BCR transgenic B cells capable of undergoing class switching and SHM in transgenic mice expressing either membrane-bound HEL^{4x} (mHEL^{4x}) ubiquitously or under tissue-specific promoters²⁸⁸. The native HyHEL10 BCR does not bind to HEL^{4x}, therefore naive HyHEL10 B cells are not self-specific in this model. HyHEL10 BCR instead binds to HEL^{3x}, which was used in the immunization regimen. As HyHEL10 B cells entered the GC and received T cell help, they underwent SHM to enhance affinity to HEL^{3x}, and in cases where mHEL^{4x} was not expressed in close proximity to the GC itself, HyHEL10 B cells acquired the ability to bind HEL^{4x} and produce high affinity autoantibodies²⁸⁸. This study therefore showed that B cell responses to foreign antigens may promote SHM toward self-reactivity and autoantibody production if the self-antigen is not abundant in either the GC or the lymph node, providing an explanation for cross-reactive autoantibody onset following infection²⁸⁷.

If self-specific CD4⁺ or CD8⁺ T cells successfully differentiate into T_{eff} cells and avoid T_{reg}/B_{reg} cell-mediated suppression, their function can be limited by several cell-intrinsic checkpoints. Following activation, T cells express a multitude of co-stimulatory and inhibitory receptors that upon ligand binding converge to modulate TCR and cytokine signaling, which thereby shape T cell function. The balance of co-stimulatory and co-inhibitory signals is critical for maximizing productive immune responses and minimizing immunopathology. Notable co-stimulatory receptors include CD28, CD40, inducible co-stimulator (ICOS), and CD137 (also known as 4–1BB), while critical co-inhibitory receptors include CTLA-4, PD-1, and LAG-3²⁸⁹.

As previously mentioned, CD28 interactions with CD80/CD86 are critical during T cell priming, as CD28 ligation leads to activation of Akt and mTOR. The latter influence transcription factor expression and promote T_{eff} cell function. Because CD28 is required for T_{reg} cell development, CD28-deficient NOD mice develop accelerated T1D due to a T_{reg} cell deficiency^{290, 291}. CD40 interactions with CD40-ligand are required during T cell priming and are also important for CD4⁺ T cell–B cell interactions in the GC reaction leading to productive antibody production. CD40-ligand deficiency or blockade prevents T1D in NOD mice and renders T cells unable to transfer T1D^{292, 293}. ICOS binding to ICOS-ligand promotes cytokine production and is critical for imprinting a T_{FH} cell program in CD4⁺ T cells. ICOS deficiency or blockade prevents T1D²⁹⁴, pointing to an important role in diabetogenic T cell costimulation. 4–1BB is thought to promote T_{eff} cell survival through

increasing Bcl-XL expression and promotes IL-2 and IFN- γ secretion. Interestingly, the NOD 4-1BB variant, part of the *Idd9* locus, renders T cells more responsive to 4-1BB-ligand stimulation compared with the B6 4-1BB variant, suggesting that this costimulatory molecule is important in T1D pathogenesis²⁹⁵.

Three diabetes-relevant co-inhibitory receptors expressed on T cells are CTLA-4, PD-1 and LAG-3. CTLA-4 and PD-1 are expressed shortly after antigen recognition, while LAG-3 is expressed on activated T cells several days after antigen encounter. Deficiency in, or blockade of, CTLA-4, PD-1, or LAG-3 accelerates T1D in NOD mice, suggesting that these receptors are critical tolerance checkpoints^{296–299}. Their roles in regulating CD4⁺ and CD8⁺ T cell function are discussed below.

High expression of co-inhibitory receptors PD-1, CTLA-4, and LAG-3 is associated with T cell exhaustion, a hyporesponsive state that arises after chronic antigen stimulation (Fig. 3). T cell exhaustion has been most extensively studied in CD8⁺ T cells in the context of persistent viral infections and cancer. Chronically-stimulated CD8⁺ T cells transition from a T-bet^{hi} Eomes^{lo} PD-1^{int} state to T-bet^{lo} Eomes^{hi} PD-1^{hi} state, lose the ability to make IL-2 and proliferate, and subsequently also lose the potential to produce TNF- α , IFN- γ , and release cytotoxic granules^{300–303}. Compared with T_{eff} cells and memory CD8⁺ T cells, exhausted cells have an altered metabolic profile and a different epigenetic landscape, suggesting that these cells are truly distinct^{304, 305}. Exhausted virus-specific CD4⁺ T cells have a unique transcriptional profile, different from CD4⁺ T_{eff} cell subsets, and partially overlapping with exhausted virus-specific CD8⁺ T cells³⁰⁶. CD4⁺ T cell exhaustion is also characterized by high expression of PD-1, CTLA-4, LAG-3, and BTLA (B- and T-cell attenuator), but, surprisingly, involves high expression of co-stimulatory molecules like ICOS and OX-40, but not CD28. Transcription factors Bcl6, Blimp-1 and Eomes are expressed in different subsets of exhausted CD4⁺ T cells, perhaps reflecting their prior effector state. Given that self-antigens are also chronically present, it is conceivable to predict that T cell exhaustion plays a role in containing autoimmunity. In fact, an increase in CD8⁺ T cell exhaustion signatures in T1D and SLE patients is associated with disease amelioration, while a decrease in exhaustion signatures correlates with poor disease outcomes³⁰⁷. Understanding how exhaustion is programmed in CD4⁺ and CD8⁺ T cells will bring closer the reality of designing therapeutic strategies that exploit this pathway for treating autoimmunity or cancer. Additionally, understanding how inhibitory receptors regulate self-specific CD4⁺ and CD8⁺ T cells will allow enhancement of these pathways in autoimmunity, and safely and selectively turning them off in cancer immunotherapy.

The role of CTLA-4 in T1D

Human CTLA-4 (CD152) is a 24.6 kDa receptor belonging to the immunoglobulin superfamily. CTLA-4 is constitutively expressed on T_{reg} cells and upregulated 24 hours after antigen encounter on conventional T cells. CTLA-4 is highly related to CD28 and binds to CD80 and CD86 on antigen-presenting cells (APCs), albeit with higher affinity and avidity than CD28 (see Ref. 308). This difference in ligand affinity allows CTLA-4 to compete with CD28 for ligand binding and regulate T_{eff} cell function. CTLA-4 homodimers on the T cell surface bind to two CD80/86 homodimers on the surface of an APC promoting their

transendocytosis, thus indirectly influencing T cell costimulation. Despite two decades of research, there is no consensus on CTLA-4–intrinsic signaling in T_{eff} cells. Its cytoplasmic domain contains no immunoreceptor tyrosine-based inhibitory motif (ITIM) or enzymatic activity, yet numerous studies have reported an association between CTLA-4 and SHP2 or PPA2 phosphatases, while others found no evidence of such interactions³⁰⁹. In T_{reg} cells, recent studies identified protein kinase C isoform PKC- η as a CTLA-4 binding partner and mediator of T_{reg} cell suppressive function³¹⁰. It remains to be seen whether the CTLA-4–PKC- η interaction modulates T_{eff} cell signaling, or whether CTLA-4 signals at all in T_{eff} cells.

In all strains of mice CTLA-4 deficiency leads to widespread lethal autoimmunity, placing CTLA-4 at the top of the inhibitory receptor hierarchy^{311, 312}. In NOD mice, CTLA-4 deficiency dramatically accelerates T1D²⁹⁶. However, CTLA-4 blockade appears to have age-dependent effects. In wild-type or BDC2.5 TCR transgenic NOD mice younger than 5 weeks of age, anti-CTLA-4 rapidly accelerates T1D^{313, 314}. In older mice with pre-existing insulinitis, anti-CTLA-4 does not accelerate disease progression^{313, 314}. Additionally, treating NOD mice with CTLA-4–Ig to block CD28 binding to CD80/CD86 was beneficial in young, (<5 week old) but not in older, mice (>10 week old)³¹⁵. Collectively, these studies imply that CTLA-4 regulates diabetogenic T cells following early activation and is dispensable once T cells acquire effector potential. This is in line with other reports showing that CTLA-4 signaling early during T cell priming determines T cell fate, and it may promote anergy induction after *in vivo* peptide injection^{316, 317} or suppress T_{FH} differentiation³¹⁸.

As mentioned above, single-nucleotide polymorphisms (SNPs) in the CTLA-4 gene (*Ctla4*) are associated with T1D risk. It is thought that these reduce CTLA-4 mRNA stability or reduce the production of ligand-independent CTLA-4, thus contributing to poor T cell regulation³¹⁹. Ligand-independent CTLA-4 (li-CTLA-4) is a naturally occurring isoform that acts in a CD80/86 ligand-independent fashion to reduce T cell proliferation and cytokine production. Interestingly, the NOD *Ctla4* allele produces 70% less li-CTLA-4 isoform compared with the B6 *Ctla4* allele forms. Forcing li-CTLA-4 constitutive expression or promoting its expression at a level similar to B6 mice in NOD T cells significantly reduce T1D incidence, thus providing a mechanism for epidemiological observations^{320, 321}. CTLA-4Ig as well as B cells expressing agonistic anti-CTLA-4 scFv reduce NOD T1D incidence^{315, 322}, and have prompted clinical trials with CTLA-4Ig (Abatacept). Recent-onset T1D patients treated with Abatacept experienced a slowed reduction in β -cell function over three years, compared with the placebo-treated patients, and had a similar rate of infections during the two-year treatment period^{323, 324}.

While forcing CTLA-4 signaling is desirable in T1D, blocking CTLA-4 function has been shown to lead to tumor clearance in multiple different mouse models^{325–328}. Success of anti-CTLA-4 treatment in research settings spurred the development of the fully human anti-CTLA-4 antibodies ipilimumab and tremelimumab, and their use in the clinic. In patients with advanced metastatic melanoma, ipilimumab has shown a 15% response rate that was durable for >10 years in some individuals^{329–331}. Not surprisingly, as many as 30% of treated patients experienced grade 3–4 immune related adverse events (irAE), including thyroiditis and hypophysitis³³². Still, ipilimumab outperformed standard chemotherapy in

treating advanced melanoma, and it has been FDA-approved since 2011. Several different mechanisms might account for the anti-tumor efficacy of CTLA-4 blockade and the occurrence of irAE. These include increased T_{eff} cell access to CD80/CD86 costimulatory molecules and, by extension, improved function³³³, reduction in T_{reg} cell numbers or frequency in the tumor³³⁴, or altered T_{reg} cell suppressive capacity³³⁵. The latter has been a matter of controversy, as one study found that T_{reg} cell-intrinsic CTLA-4 was required for optimal T_{reg} cell suppression in BALB/c mice and critical for keeping autoimmunity at bay³³⁵. However, this was recently challenged when T_{reg} cell-specific *Ctla4* deletion in adult B6 mice led to protection from experimental autoimmune encephalomyelitis³³⁶. Going forward, resolving this and other issues pertaining to CTLA-4 biology will allow for safer administration of CTLA-4 blockade in cancer, and more effective exploitation of this pathway in T1D and other autoimmune diseases.

The role of PD-1/PD-L1 in T1D

Like CTLA-4, PD-1 (CD279) is a 55 kDa receptor and a member of the immunoglobulin superfamily. PD-1 was originally described as an inducer of activated T cell death³³⁷, but further inquiry demonstrated that PD-1 is an immune checkpoint. PD-1 is expressed on activated T and B cells 24–48 hours after antigen encounter and binds to PD-ligand 1 (PD-L1; also known as CD274 and B7-H1) or PD-ligand 2 (PD-L2; also known as CD273 and B7-DC). PD-L2 is expressed mainly on professional APCs³³⁸, while PD-L1 is constitutively expressed on resting T cells, B cells, dendritic cells, and macrophages, and it is further upregulated upon cellular activation or in response to cytokines³³⁹. Importantly, non-hematopoietic cells can express PD-L1 upon cytokine stimulation, including tumor cells³³⁸ and, as we have recently shown, is expressed on pancreatic beta cells³⁴⁰.

PD-1 ligation leads to ITIM and immunoreceptor tyrosine-based switch motif (ITSM) phosphorylation and recruitment of phosphatases SHP1, SHP2, and SHIP2. The latter dephosphorylate kinases and signaling molecules downstream of the TCR and CD28, leading to decreased T cell proliferation, effector function, survival, and reduced contact with APCs^{338, 341–343}. SNPs in the *PDCD1* and *PDCD1LG1* loci have been associated with the development of T1D^{344–346}. Additionally, low levels of soluble PD-L1 are associated with disease onset³⁴⁶, suggesting that the PD-1–PD-L1 axis plays a role in islet tolerance maintenance in humans.

Unlike CTLA-4 deficiency, which is uniformly lethal, PD-1 deficiency in mice has strain-dependent effects. PD-1-deficient B6 mice develop anti-dsDNA autoantibodies and a lupus-like disease over time, while BALB/c PD-1-deficient mice develop anti-cardiac troponin I autoantibodies and autoimmune cardiomyopathy^{347, 348}. NOD mice lacking either PD-1 or PD-L1 develop fully penetrant accelerated T1D^{298, 299}. While PD-1/PD-L1 blocking antibodies accelerate T1D in 5 week old and 15 week old NOD mice, disease onset is quicker in older animals, likely because they have a larger pool of activated islet-specific lymphocytes³¹⁴. Dependence on PD-1, but not CTLA-4, signaling at all stages of disease progression points to there being non-redundant roles of CTLA-4 and PD-1 in restraining lymphocyte function. It is important to note that while PD-1 can bind to both PD-L1 and PD-L2, PD-L2 does not seem to regulate islet-reactive lymphocytes. Specifically, anti-PD-

L2 treatment had no effect on T1D progression in younger or in older NOD mice ³¹⁴. Additionally, PD-L2-deficient NOD mice had a similar T1D incidence compared to wild-type littermates ²⁹⁹. Besides binding to PD-1, PD-L1 can bind to CD80. Using an antibody that specifically blocks PD-L1-CD80 interactions, Paterson *et al.* showed accelerated T1D in 6–7 week-old and 13 week-old mice, albeit disease was quicker and more penetrant in older animals ³⁴⁹. Treatment with a monoclonal antibody that blocks both PD-L1-CD80 and PD-1-PD-L1 interactions dramatically accelerated T1D in young and in older NOD mice ³⁴⁹, confirming that PD-1-PD-L1 axis is important during both disease initiation and progression. Pancreatic lymph node removal prevented T1D development after anti-PD-L1 in 3-week-old, but not in 10-week old, mice ³⁵⁰. Therefore, early on during disease process, PD-1-PD-L1 interactions in the lymph node control T1D, while later, PD-1-PD-L1 signaling in the pancreas is important.

PD-1-deficient TCR transgenic CD4⁺ or CD8⁺ T cells acquire effector function and infiltrate the islet core more effectively than wild type or PD-L1-deficient T cells, demonstrating that PD-1 on the T cells is playing a critical role in regulating T1D pathogenesis ^{351, 352}. To understand the cellular location of PD-L1 expression, Keir and colleagues used elegant mixed bone marrow chimeras and demonstrated that PD-L1 expression by nonlymphoid hematopoietic cells was not sufficient to keep diabetogenic cells at bay ²⁹⁹. Additionally, in a syngeneic transplant setting, islet grafts lacking PD-L1 had reduced survival compared to wild-type islets ²⁹⁹. Collectively, these lines of evidence implied that PD-L1 expression by the non-hematopoietic compartment, and likely by beta cells themselves, was a gate-keeper of islet tolerance. In support of that idea, we recently showed that mouse and human beta cells upregulate PD-L1 in response to T cell infiltration and IFN- γ ³⁴⁰.

There have been several attempts to increase PD-1-PD-L1 signaling in autoimmune diseases or transplantation. Embryonic stem cell-derived dendritic cells transduced to express PD-L1 and myelin oligodendrocyte peptide reduced the severity of experimental autoimmune encephalomyelitis ³⁵³. Additionally, treatment with adenovirus encoding PD-L1 prolonged islet graft survival ³⁵⁴, as did PD-L1 overexpression in an ectopic islet neogenesis model ³⁵⁵. Treatment with PD-L1Ig extended graft survival when used as adjuvant therapy ³⁵⁶, suggesting that a similar strategy could be employed in T1D.

As mentioned above, chronically-stimulated T cells express high levels of PD-1 and have an exhausted, poorly functional phenotype. In the context of persistent viral infection or tumor models, PD-1 blockade has an ability to reinvigorate a subset of these cells and to re-boot virus or tumor control ^{357–361}. These exciting pre-clinical studies led to the development of the fully human anti-PD-1 monoclonal antibody nivolumab for the treatment of cancer. Nivolumab had an outstanding 37.5% response rate in a phase 1 clinical trial ³⁶² and was accompanied with a manageable toxicity profile. As a result, PD-1-PD-L1 blocking antibodies received rapid FDA approval and are currently used to treat 11 different malignancies, including melanoma (with 35–40% response rates) and Merkel cell carcinoma (with a >80% response rate), with irAE occurrence in 15% of treated patients ³⁶³.

The most common irAEs associated with anti-PD-1 treatment include skin rash, colitis, or thyroid issues. In addition, there have been numerous reports of T1D development following anti-PD-1 treatment. In some instances, disease onset took place less than a month after treatment initiation. T1D is estimated to arise in 1–3% of anti-PD-1-treated patients, and a recent study demonstrated that >70% of patients who went on to develop T1D after treatment with anti-PD-1/PD-L1 blocking antibodies expressed the HLA *DR4* allele, which is highly associated with T1D risk³⁶⁴. These observations suggest that the PD-1–PD-L1 pathway restrains islet tolerance in a subset of genetically predisposed individuals. To understand how PD-1 blockade affected self-specific CD4⁺ T cells, we tested the effects of PD-1 inhibition on insulin B10–23–specific CD4⁺ T cells in NOD and B6.g7 mice³⁶⁵. At baseline, NOD, but not B6.g7, mice had detectable antigen-experienced insulin-specific CD4⁺ T cells. Following PD-1 blockade, we observed a significant increase in the number of insulin-specific CD4⁺ T cells in the pancreatic lymph nodes and pancreas of NOD mice, and rapid T1D induction. Anti-PD-1–treated B6.g7 mice remained diabetes free and had few antigen-experienced insulin-specific CD4⁺ T cells³⁶⁵. We then addressed whether PD-1 blockade in NOD mice would either restore function to previously tolerized CD4⁺ T cells or expand the existing CD4⁺ T_{eff} cell population and found that PD-1 blockade did not reverse CD4⁺ T cell anergy, but it did enhance the function of effector cells³⁶⁵. Collectively, these findings have implications for cancer immunotherapy and autoimmunity development, and they suggest that T cell differentiation state pre-determines its susceptibility to PD-1 blockade–mediated function restoration.

It is important to note that more than 50% of patients who developed T1D after PD-1 checkpoint blockade presented with islet autoantibodies, which, in some cases, arose *de novo* after treatment. As mentioned above, PD-1–deficient B6 and BALB/c mice develop autoantibodies, yet the exact mechanisms remain unclear. Several studies have shown that PD-1 deficiency or blockade impairs the outcome of GC activity, resulting in fewer long-lived plasma cells³⁶⁶ and lower affinity antibodies^{367, 368}. Others have demonstrated that PD-1 blockade enhances antibody production in the context of malaria³⁶⁹ and fetal allojection³⁷⁰. Sage *et al.* revisited this problem and reported that PD-1 blockade enhanced both T_{FH} and T_{FR} CD4⁺ T cells, and that the relative ratio of these two cell subsets dictated the final outcome of GC activity^{371, 372}. Since auto-antibody production is a hallmark of spontaneous and PD-1 blockade–induced T1D, studies evaluating the role of PD-1 in regulating the anti-islet germinal center are warranted.

The role of LAG-3 in T1D

LAG-3 (CD223) is a 70 kDa inhibitory receptor, and another member of the immunoglobulin superfamily^{373, 374}. It is constitutively expressed on T_{reg} cells and upregulated on effector CD4⁺ and CD8⁺ T cells 48–72 hours after antigen encounter³⁷⁵. NK and NK T cells, a subset of B cells, as well as plasmacytoid dendritic cells have also been shown to express LAG-3³⁷⁶. LAG-3 shares structural homology with CD4 and also binds to MHC II, albeit with a greater affinity, and with a preference for stable peptide–MHC II complexes^{374, 377, 378}. Importantly, LAG-3 binding does not interfere with CD4 binding to MHC II, nor does it block TCR recognition of MHCII³⁷⁸. Instead, it appears that LAG-3 mediates suppressive function in T cells through its cytoplasmic tail^{378, 379}. While several

distinct motifs have been identified in the mouse and human LAG-3 cytoplasmic tail, there is no consensus in the field regarding which motifs are required, nor which downstream proteins are recruited to mediate signaling^{379–382}. Furthermore, LAG-3 appears to be regulated by ectoenzymes that facilitate its shedding, but the exact roles of soluble LAG-3 (sLAG-3) are unclear. The LAG-3 cytoplasmic domain lacks canonical ITIM and ITSM regions, and instead includes glutamic acid-proline dipeptide (EP) repeats, a unique KIEELE peptide motif, and an FxxL motif in the membrane-proximal region. The KIEELE motif in general and the lysine residue in particular were shown to be essential for LAG-3 function;^{380, 381} but in another study they were dispensable³⁷⁹. LAG-3-associated protein (LAP) has been shown to bind the EP domain and identified as a putative signal transducer³⁸². However, a subsequent study showed that LAG-3 remained functional despite having a mutated EP domain³⁸⁰. It is possible that LAP recruitment was not completely abrogated in that case, or that other proteins compensated for a lack of LAP binding. As the LAG-3 pathway is increasingly targeted in the clinic, understanding how LAG-3 transmits an inhibitory signal will be of great importance.

Human and mouse CD4⁺ T cell lines stimulated with cognate peptide in the presence of LAG-3-blocking antibodies showed sustained proliferation, and increased production of IL-2, IL-4, TNF- α and IFN- γ *in vitro*^{375, 383}. This prompted the development and study of LAG-3 gene (*Lag3*) knockout mice. Unlike CTLA-4 or PD-1, LAG-3 deficiency on the B6, B6.SJL, B10.D2, or BALB/c backgrounds does not lead to overt autoimmunity^{384–386}. However, LAG-3-deficient B6 mice show CD4⁺ and CD8⁺ T cell accumulation in the spleen and Peyer's patches as mice age³⁷⁵, while LAG-3-deficient B6.SJL mice are more susceptible to mercury-induced autoimmunity compared to wild-type animals³⁸⁷. These subtle phenotypes suggest that the effects of LAG-3 deficiency might be more prominent in autoimmune-prone mice. Indeed, PD-1/LAG-3 double gene knockout B6 and BALB/c mice rapidly succumb to autoimmunity, demonstrating that these two inhibitory pathways synergize to control self-tolerance^{385, 386}.

In NOD mice, LAG-3 deficiency or blockade accelerates T1D, with all mice becoming diabetic by 15 weeks of age^{297, 386}. Disease acceleration is due to increased CD4⁺ and CD8⁺ T_{eff} cell infiltration and proliferation in the pancreas, despite a relative increase in T_{reg} cells.²⁹⁷ While anti-LAG-3 agonist antibody treatment has not been attempted in NOD mice, one study tested the effects of LAG-3 shedding on T1D progression. Treating NOD mice with a catalytic oxidant to prevent LAG-3 cleavage significantly delayed spontaneous and adoptively transferred T1D³⁸⁸. It is possible that modulating redox balance via catalytic oxidant treatment also altered other aspects of T_{eff} or T_{reg} cell biology; therefore, future studies could include treating NOD mice with sLAG-3 or engineering mice with a cleavage-resistant LAG-3 variant to monitor T1D development.

Since T_{reg} cells express LAG-3 constitutively, several studies have attempted to address how LAG-3 deficiency or blockade impacts T_{reg} cell function and maintenance in autoimmunity. While one study reported impaired suppression by LAG-3-deficient T_{reg} cells,³⁸⁹ others reported no difference between wild-type and LAG-3-deficient T_{reg} cells, and described LAG-3-deficient T_{eff} cells to be more resistant to T_{reg} cell-mediated suppression³⁹⁰. To resolve the role of LAG-3 in T_{eff} versus T_{reg} cells in NOD T1D, Zhang *et al.* generated

NOD.*Lag3*^{fl/fl-YFP} mice and crossed them to NOD.*Cd4*^{Cre} or NOD.*Foxp3*^{Cre} mice³⁹¹. The resulting progeny had either LAG-3–deficient CD4⁺ and CD8⁺ T cells or LAG-3–deficient T_{reg} cells, respectively. All NOD.*Cd4*^{Cre}*Lag3*^{fl/fl-YFP} mice developed diabetes by 15 weeks of age, recapitulating the findings with global NOD.LAG-3–deficient mice^{297, 386}. However, NOD.*Foxp3*^{Cre}*Lag3*^{fl/fl-YFP} mice were significantly protected from T1D. Transcriptome analysis of LAG-3–sufficient and –deficient intra-islet T_{reg} cells showed that the IL-2/STAT5 signature was increased in the absence of LAG-3. Furthermore, LAG-3–deficient intra-islet T_{reg} cells had increased proliferation and survival compared to wild-type cells, but were not more suppressive on a per cell basis³⁹¹. This elegant study showed that LAG-3 limits both T_{eff} and T_{reg} cell proliferative capacity, and that LAG-3 expression by T_{reg} cells in T1D might account for their inability to suppress T_{eff} cell responses in islets.

While some antibody blockade studies concluded that LAG-3 only affects CD4⁺ T cells³⁸³, others showed that CD8⁺ T cells are also affected in the absence of LAG-3 signaling^{392, 393}. To regulate the CD8⁺ T cell compartment, LAG-3 must engage other binding partners besides MHCII. Indeed, LAG-3 has been shown to bind to galectin-3³⁹⁴, liver sinusoidal endothelial cell lectin (LSECtin)³⁹⁵, and, most recently, to fibrinogen-like protein 1 (FGL1)³⁹⁶. Importantly, blocking FGL1–LAG-3 interaction reinvigorated CD8⁺ T cell function in a tumor model, but did not impact T_{reg} cells³⁹⁶. While it remains to be determined whether FGL1–LAG-3 signaling plays a role in restraining CD8⁺ T cells in T1D, this exciting discovery will undoubtedly prompt the development of selective LAG-3–ligand targeting antibodies that allow for precise control of LAG-3 signaling in cancer and in autoimmunity.

Conclusions

Decades of research have shown that CD4⁺ and CD8⁺ T cells as well as B cells are indispensable for T1D pathogenesis. TCR and BCR transgenic cells and advances in tetramer technology have allowed many groups to investigate the phenotype and regulation of islet-reactive lymphocytes and to pinpoint tolerance checkpoint failures. Besides T_{reg} cell expansion and infusion⁶⁴, promising therapeutic approaches include antigen-coupled splenocytes¹³⁷ or nanoparticles³⁹⁷, and monoclonal antibodies blocking islet-peptide–MHCII complexes^{138, 398}. Considerable effort has also been dedicated towards harnessing the T cell inhibitory receptors CTLA-4 and PD-1, and future studies will undoubtedly explore modulating LAG-3 to treat autoimmunity. With an increase in spontaneous and checkpoint blockade–induced T1D incidence, there is a rising need for developing and validating these, and other, immunomodulating strategies that either stop T1D progression prior to extensive beta cell destruction or allow beta cell recovery.

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Figure 1.

Beta cell destruction involves CD4⁺ and CD8⁺ T cells, inflammatory M1 macrophages, and B cells. CD8⁺ T cells have been shown to directly kill beta cells via TCR-mediated peptide–MHCII complex recognition. Islet-resident inflammatory M1 macrophages secrete proinflammatory cytokines that lead to beta cell death, while islet-infiltrating CD4⁺ T cells both secrete cytokines and potentially directly contact beta cells. B cells secrete autoantibodies and present islet antigens to CD8⁺ and CD4⁺ T cells to further potentiate their activation. In addition to directly contributing to beta cell death through cytokine secretion or contact, CD4⁺ T cells help activate B cells, stimulate macrophage polarization into the M1 phenotype, and prime dendritic cells to effectively stimulate CD8⁺ T cell responses.

Figure 2.

CD4⁺ T and B cells interact in the germinal center to promote (auto)antibody production. T follicular helper (T_{FH}) cells interact with germinal center (GC) B cells via peptide–MHCII, provide CD40-ligand, and secrete IL-4 and IL-21 to promote affinity maturation, proliferation, and, ultimately, immunoglobulin class switching. T follicular regulatory cells (T_{FR}) suppress this interaction via IL-10, CTLA-4, and PD-1.

Figure 3.

In the draining lymph nodes, autoreactive $CD4^+$ T cells are held in check through deletion, differentiation into anergic cells, differentiation into peripheral T_{reg} cells, and inhibitory receptor expression. $CD4^+$ T cell interaction with antigen-presenting cells in the lymph node can result in strong TCR stimulation and activation-induced cell death (deletion), differentiation into an anergic phenotype (T_{an} ; $FR4^+CD73^+$), skewing toward an effector phenotype (T_{eff}), or differentiation into the T_{reg} cell lineage. Anergic cells are thought to arise due to insufficient costimulation during activation, while peripheral T_{reg} cells differentiate in the presence of $TGF-\beta$. T_{eff} cells express CTLA-4, PD-1, and LAG-3 sequentially after antigen encounter. These receptors regulate T_{eff} cell function and are involved in controlling T_{reg} cell-mediated suppression. As a result of chronic antigen stimulation, effector cells may become exhausted (T_{ex}). Recent evidence suggests that in some cases anergic cells serve as pre-cursors to T_{reg} cells and could contribute to immune regulation in that way as well.