

## Review

# 100 years post-insulin: immunotherapy as the next frontier in type 1 diabetes

James A. Pearson<sup>1,\*†,⊕</sup>, Eoin F. McKinney<sup>2,3,4</sup> and Lucy S. K. Walker<sup>5,†</sup>

<sup>1</sup>Diabetes Research Group, Division of Infection and Immunity, School of Medicine, Cardiff University, Cardiff, Wales, UK, <sup>2</sup>Cambridge Institute of Therapeutic Immunology and Infectious Disease, Jeffrey Cheah Biomedical Centre, Cambridge, England, UK, <sup>3</sup>Department of Medicine, University of Cambridge School of Clinical Medicine, Cambridge, England, UK, <sup>4</sup>Cambridge Centre for Artificial Intelligence in Medicine, University of Cambridge, Cambridge, England, UK and <sup>5</sup>Division of Infection and Immunity, Institute of Infection and Transplantation, University College London, Royal Free Campus, London, UK

<sup>†</sup>These authors contributed equally to this work.

\*Correspondence: James A. Pearson, Division of Infection and Immunity, School of Medicine, Cardiff University, Heath Park, Cardiff CF14 4XN, UK. Email: [pearsonj1@cardiff.ac.uk](mailto:pearsonj1@cardiff.ac.uk)

Received 6 October 2021; Revised 15 November 2021; Accepted 20 November 2021

## Summary

Type 1 diabetes (T1D) is an autoimmune disease characterised by T cell-mediated destruction of the insulin-producing  $\beta$  cells in the pancreas. Similar to other autoimmune diseases, the incidence of T1D is increasing globally. The discovery of insulin 100 years ago dramatically changed the outlook for people with T1D, preventing this from being a fatal condition. As we celebrate the centenary of this milestone, therapeutic options for T1D are once more at a turning point. Years of effort directed at developing immunotherapies are finally starting to pay off, with signs of progress in new onset and even preventative settings. Here, we review a selection of immunotherapies that have shown promise in preserving  $\beta$  cell function and highlight future considerations for immunotherapy in the T1D setting.

**Keywords:** type 1 diabetes, human, NOD mouse, immunotherapy

## Introduction

T1D is a complex T cell-mediated autoimmune disease, resulting in destruction of the insulin-producing  $\beta$  cells and a deficiency in insulin secretion. Prior to the

discovery of insulin in 1921, individuals with T1D would have died within a year or two of diagnosis [1]; however, since the discovery and mass production of insulin, T1D is no longer a death sentence and the condition can

Abbreviations: ATG: Anti-thymocyte globulin; CD: Cluster of differentiation; CTLA-4: Cytotoxic T lymphocyte-associated antigen 4; Fc: Fragment crystallisable; FMT: Fecal microbiota transplant; GCSF: Granulocyte colony-stimulating factor; HbA1c: Haemoglobin A1c; IFN $\gamma$ : Interferon gamma; Ig: Immunoglobulin; IL: Interleukin; MMTT: Mixed-meal tolerance test; NK: Natural killer; NOD: Non-obese diabetic; T1D: Type 1 diabetes; TCR: T-cell receptor; Tfh: Follicular helper T cells; TGF $\beta$ : Transforming growth factor beta; TIGIT: T-cell immunoreceptor with Ig and ITIM domains; TNF $\alpha$ : Tumour necrosis factor-alpha; TNFR: Tumour necrosis factor receptor; Treg: Regulatory T cell; ZnT8: Zinc transporter 8.

© The Author(s) 2021. Published by Oxford University Press on behalf of the British Society for Immunology.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

be managed by exogenous insulin, either delivered by multiple daily injections or a pump. Nevertheless, over time many patients develop complications including cardiovascular disease, retinopathy, neuropathy, and nephropathy.

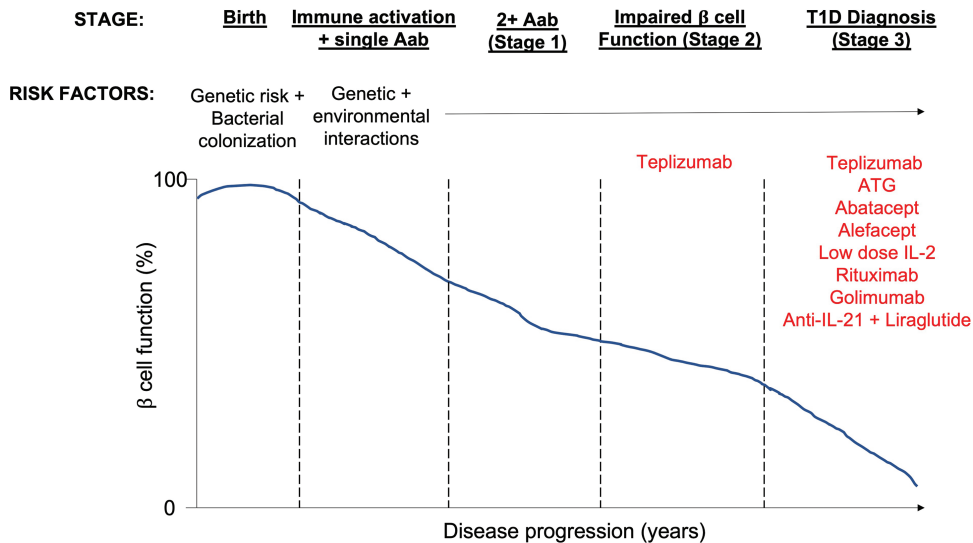
Clinical diagnosis of T1D occurs relatively late in the disease process when a large number of  $\beta$  cells have been destroyed by islet autoantigen-specific T cells; however, there are several preclinical stages in which the immune response has already been triggered and is actively responding to pancreatic islet antigens (Fig. 1; [2]). This presents a window of opportunity to potentially intervene and reset the immune system prior to extensive tissue damage. Autoantibodies, secreted by B cells, can be detected against a number of islet antigens, with multiple autoantibody specificities associated with an increased risk of progression to T1D diagnosis [3–7]. These autoantibodies, alongside genetic susceptibility, provide a crucial biomarker pre-diagnosis to identify those at most risk and who may be the best candidates for future immunotherapy aimed at delaying or preventing T1D development [8].

Based on successful experiments in the Bio-breeding rat model of type 1 diabetes [9], early attempts at immunomodulation included the use of the calcineurin inhibitor cyclosporin [10]. Of 30 patients treated within

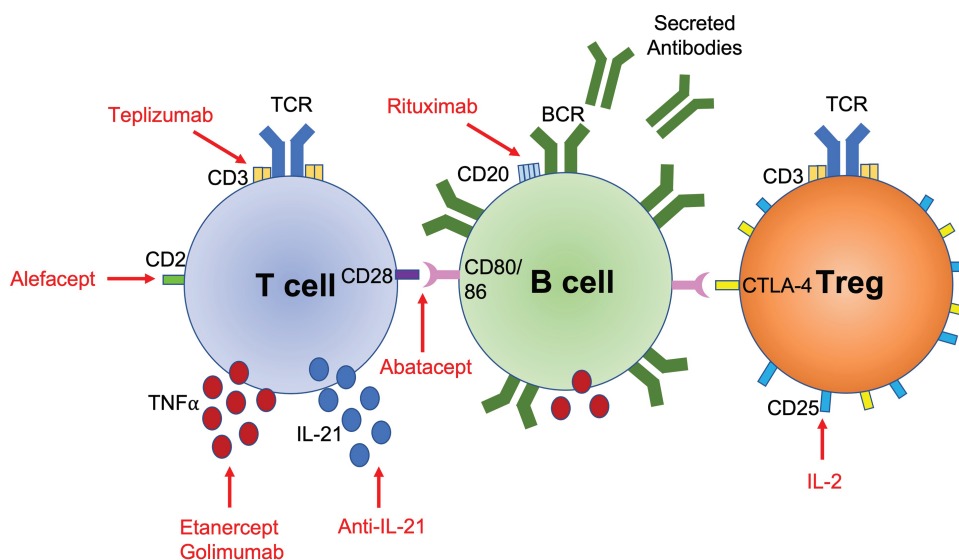
6 weeks of diagnosis, 16 reverted to having normal C-peptide levels and became insulin-independent, an unprecedented result. The use of corticosteroids plus daily azathioprine also showed beneficial outcomes in new onset T1D, with 50% of the treatment group showing C-peptide levels  $>0.5$  nmol/l (three being insulin-independent) compared to 15% of the control group (none being insulin-independent) [11]. Although these approaches were not pursued due to problematic side effects, the trials were nevertheless important in demonstrating the potential of immunomodulation in T1D. There have since been multiple immunotherapy studies aimed at curtailing the loss of  $\beta$  cells by targeting the key immune cells involved in the disease process, as well as cytokines that they produce (Fig. 2). In addition, therapies which may boost immune regulation have also been studied. Here, we review the key successful non-antigen-specific immunotherapies that show most promise in preserving  $\beta$  cell function or even delaying T1D development. Antigen-specific approaches are not covered in this article and have been recently reviewed elsewhere [12].

## T cells in T1D

$CD4^+$  and  $CD8^+$  T cells orchestrate the inflammatory process that culminates in the destruction of the islet  $\beta$



**Figure 1** Stages of T1D development and immune interventions. From birth individuals inherit a genetic predisposition to developing T1D, as well as a collection of colonising bacteria. In those individuals with a risk of developing T1D, the immune interactions with environmental modifiers can lead to inappropriate activation of the immune system driving autoreactive T and B cells and the secretion of detectable autoantibodies (stage 1). The immune response impairs the function and survival of the insulin-producing islet  $\beta$  cells, resulting in a dysglycemic state (stage 2) and finally the clinical diagnosis of T1D when a sufficient number of beta cells have been destroyed (stage 3). Immunotherapy studies have largely been conducted in those with recent-onset T1D, with the exception of Teplizumab, which has also been conducted in ‘at risk’ individuals. Key trials discussed in this article are highlighted in the figure.



**Figure 2** Immune intervention targets. Schematic illustrating key immunotherapies that have been tested in the T1D setting and their immune cell targets. Many immunotherapies target markers expressed by T cells, others target B cells or cytokines. Immunotherapies can also target regulatory T cells (Tregs) e.g. IL-2, which helps to expand and boost Treg suppression, preventing the destruction of the islet  $\beta$  cells. Immunotherapies are in red with arrows indicating their targets. While a B cell is shown interacting with T cell CD28, this costimulation signal can come from CD80/86 on other immune cells such as macrophages and dendritic cells.

cells, leading to the development of T1D. Many of the genes associated with susceptibility to type 1 diabetes are active in T cells and the strongest genetic contribution to disease maps to the human leukocyte antigen genes, which function to present antigens to T cells. T cells infiltrate the pancreatic islets in people with diabetes [13, 14] and in mouse models, diabetes can be transferred from one animal to another by the adoptive transfer of T cells [15]. Thus, extensive effort has been directed at the development of immunotherapies to target T cells.

### Anti-CD3 immunotherapy

In 1979, a mouse hybridoma cell line was developed that produced an IgG2a monoclonal antibody, named Orthoclone (OKT3), against a T cell surface antigen [16], later identified as the  $\epsilon$  chain of the CD3 receptor [17]. In 1981, the first patients were administered OKT3, which was shown to successfully reverse allograft rejection [18, 19]. In 1985, OKT3 became commercially available for use in transplantation, making it the first-in-human approved monoclonal antibody; however, by the late 1980s, OKT3 use in the clinic became limited following the severe cytokine release from activated T cells [20–22]. This cytokine release was induced by OKT3 crosslinking with the T-cell receptor/CD3 complex; however, binding of the Fc portion of OKT3 by Fc-receptor-expressing cells further enhanced the crosslinking and thus the severity of

the cytokine release. Activation of the T cell also varied with the antibody isotype of the OKT3 antibody, with IgG2a having the strongest immunostimulatory effect [23, 24]. As OKT3 was a mouse anti-human antibody, human anti-mouse antibodies were also raised against OKT3, which resulted in clearance of OKT3 and a reduction in efficacy [20]. Thus, to improve clinical efficacy and tolerance, OKT3 antibodies were humanised and developed with modified Fc portions to prevent Fc binding by Fc receptors and thus severe cytokine release, while preserving their suppressive effects. Teplizumab is a modified OKT3 antibody, with the same binding region as OKT3 but the amino acids at positions 234 and 235 of the human IgG1 were substituted with alanine (hOKT3  $\gamma$ 1(Ala-Ala)) [25].

Pivotal pre-clinical studies by Lucienne Chatenoud and colleagues showed short-term anti-CD3 treatment (5-day course) was able to induce disease remission in up to 80% of recently diagnosed diabetic non-obese diabetic (NOD) mice, and this was associated with a transient and partial T cell depletion, with numbers returning to normal within 15–20 days [26, 27]. This protection was not due to deletion of autoreactive T cells, as insulinitis was only transiently reduced, and spleen cells from these mice could transfer diabetes to irradiated mice [27]. The protective effect of anti-CD3 treatment in mice may relate to the induction of regulatory T cells (Treg) and immunosuppressive cytokines (TGF $\beta$ ) [28–30] and, partial TCR

signalling leading to the clonal anergy or age-dependent deletion of specific T cells [31, 32]. Transgenic NOD mice were also developed to express human CD3 [33], providing a useful preclinical model for testing humanised anti-CD3 antibodies. Following anti-CD3 treatment, diabetes in these mice was reversed and again, in line with previous data [29], protection was TGF $\beta$ -dependent and associated with enhanced Treg function [33].

Given the success of the pre-clinical studies of anti-CD3 treatment in NOD mice, Herold and colleagues recruited 24 newly diagnosed individuals with T1D (within 6 weeks of diagnosis), half of whom received an escalating dose of Teplizumab each day for 2 weeks, while the placebo group received no antibody [34]. Importantly, 12 months after treatment, two thirds of the Teplizumab-treated group had C-peptide responses that were equivalent or higher than their response at study entry, whereas 10 out of the 12 control participants exhibited a decline in C-peptide response. Similarly, a phase II study of another humanised anti-CD3 antibody (Otelixizumab) in 80 individuals with new onset T1D also showed a slower deterioration of  $\beta$  cell function in those receiving anti-CD3 treatment [35]. Preservation of even a small amount of residual insulin secretion, measured by C-peptide, can provide long-standing health benefits [36]. Later anti-CD3 studies confirmed this preservation of insulin secretion by the  $\beta$  cells could be maintained for many years [37, 38], with the latest data indicating up to 7 years post-diagnosis [39]. A phase III trial of Teplizumab in 516 individuals however failed to meet its primary endpoint, a composite outcome comprising insulin dose and haemoglobin A1c (HbA1c) which had not been previously validated [40]. However, exploratory analyses showed that C-peptide declined less in the treated group than in the placebo group and that 5% of patients were not taking insulin at 1 year compared with no patients in the placebo group.

As mentioned above, the fact that islet autoantibodies are produced many years prior to diabetes development provides a window of opportunity for therapeutic intervention. Herold and colleagues set out to exploit this window by administering Teplizumab to high-risk relatives of patients with T1D who had dysglycemia and the presence of 2 or more islet autoantibodies but had not yet been diagnosed with T1D [41]. This study successfully delayed the development of T1D in these individuals, with 57% of the teplizumab group being diabetes free compared to 28% of the placebo group. An extended follow-up study (median of 923 days) found that 50% of the Teplizumab-treated group were still diabetes free compared to 22% of the placebo group [42]. These data have changed the landscape for immunotherapy in T1D,

providing the first evidence that T cell-directed therapies administered in at risk individuals can alter the future disease course.

This prevention of  $\beta$  cell destruction has been associated with a combination of induced regulatory T cell responses [43], partially exhausted CD8<sup>+</sup> T cells, characterised by TIGIT and killer cell lectin-like receptor G1 expression, which were associated with improved clinical efficacy [42, 44], and reduced proinflammatory cytokines [42]. The success of this intervention in delaying T1D development is groundbreaking and Teplizumab is likely to be the first immunotherapy licensed for delaying, and possibly preventing, the development of T1D. There is still work to be done: trials in T1D present significant challenges in the areas of recruitment and endpoints so accumulating sufficient data is problematic and the US Food and Drug Administration rejected a request for Teplizumab approval in July 2021; however, momentum is clearly building for immunotherapies to be approved in the T1D setting and Teplizumab looks to be at the forefront. Further longitudinal studies are needed to identify the duration for which T1D can be delayed following a single course of additional anti-CD3 treatment and whether additional doses, or other combination treatments, could be administered later to maximise clinical efficacy.

### Anti-thymocyte globulin (ATG)

ATG is a polyclonal IgG targeting multiple T cell antigens and mediating cellular depletion: in NOD mice, similar to anti-CD3 administration, ATG treatment was able to reverse diabetes in mice with recent-onset disease [45]. Initial small studies in humans with recent-onset T1D suggested ATG administration may help preserve  $\beta$  cell function [46, 47]; however, in a phase II randomised multi-center, placebo-controlled trial involving 58 individuals within 100 days of T1D diagnosis, ATG (6.5 mg/kg administered over a 4-day course) did not preserve  $\beta$  cell function [48, 49]. This failure was linked to a decrease in the Treg to T-effector memory ratio in ATG treated individuals between baseline and 6 months, since effector memory CD4<sup>+</sup> T cells were poorly depleted relative to the other T cell subsets examined [48]. A subsequent trial in 25 individuals with established T1D (between 4 months and 2 years post-diagnosis) used a lower dose of ATG (2.5 mg/kg administered as 0.5 mg/kg on day 1 and 2 mg/kg on day 2) and combined the treatment with Granulocyte colony-stimulating factor (GCSF) in line with previous preclinical data [45]. This approach appeared to result in protection of the  $\beta$  cells; on average, subjects who received placebo experienced a 39% reduction in C-peptide over 1 year, while those who received

ATG/GCSF experienced a 4.3% increase over the same time period [50]. The relative resistance of CD4<sup>+</sup> effector memory T cells to depletion was also evident in this trial, but Tregs appeared to be preserved [51, 52]. A further trial in 89 individuals within 100 days of T1D diagnosis confirmed that low-dose ATG (2.5 mg/kg) resulted in clinical benefit, with C-peptide levels 57% higher in recipients of low-dose ATG, compared with recipients of placebo, at the 1-year timepoint [53]. Side-by-side comparison with the ATG/GCSF combination revealed that the low-dose ATG monotherapy was favourable [53], and a separate study confirmed that GCSF alone did not preserve  $\beta$  cell function [54]. Thus, low-dose ATG remains an interesting candidate for further development. Notably, attempts to compare  $\beta$  cell preserving interventions across multiple clinical trials identified low-dose ATG and anti-CD3 immunotherapy as the treatments showing the greatest impact on C-peptide preservation [55].

### Abatacept

A further example of a T cell-directed immunotherapy is the cytotoxic T lymphocyte-associated antigen 4 (CTLA-4)-Ig fusion protein, Abatacept. CTLA-4 is naturally expressed at high levels in regulatory T cells; it interacts with the same ligands as the T cell costimulatory receptor CD28 but binds to them with higher affinity. CTLA-4-Ig fusion proteins such as Abatacept therefore bind to the costimulatory ligands CD80 and CD86 on antigen-presenting cells and inhibit their interaction with T cell CD28 ([56]; Fig. 2). Since CD28 costimulation provides an important 'second signal' to promote full T cell activation, inhibiting this with CTLA4-Ig would be expected to be immunosuppressive. When CTLA-4-Ig was administered to NOD mice early, around the time insulinitis first develops, only 11% of the mice went on to become diabetic compared with 87% in the control-treated group, while later administration had little effect [57]. Surprisingly, however, mice expressing CTLA-4-Ig transgenically from birth showed exacerbated diabetes development, with 100% of mice diabetic by 24 weeks compared with only 8.3% of the non-transgenic NOD mice [58]. This is now thought to reflect the role of CD28 in the development of Tregs which have immunosuppressive function [59]. In the context of Treg deficiency, and an absence of CD28 signalling since birth, it was proposed that alternative costimulatory pathways had compensated for the lack of CD28 in these mice [58]. Therapeutic targeting of the CD28 pathway therefore requires careful consideration regarding impacts on the Treg population, and timing of administration is likely to be important.

The CTLA-4-Ig molecule Abatacept was trialled in 112 patients (6–45 years of age) diagnosed with T1D within the last 100 days. Infusions were given intravenously on days 1, 14, and 28 and then monthly for 2 years. Abatacept was able to slow the decline in  $\beta$  cell destruction and function for an estimated 9.6 months [60], with higher C-peptide levels still observed in the treated group, compared to placebo, 1 year after therapy cessation [61]. This protective effect was associated with a reduced CD4<sup>+</sup> central memory T cell population and B cell population, as well as increased Tregs and naive CD4<sup>+</sup> T cells [62]. Recent data suggest that analysis of circulating follicular helper T cells (Tfh) prior to Abatacept treatment may prove useful in predicting the subsequent clinical response [63]. Following on from the study in new onset T1D, Abatacept is currently being trialled in people at risk of T1D development (NCT01773707).

### Alefacept

Alefacept is another Ig fusion protein comprising two LFA-3 molecules bound to the Fc portion of human IgG1. It binds to CD2 and mediates depletion of antigen experienced effector/memory T cells. Memory T cells are an attractive target in autoimmune disease and are believed to be less sensitive to costimulation blockade drugs such as Abatacept. Alefacept inhibits the proliferation of T cells in mixed lymphocyte reactions in a manner that depends on Fc-receptor binding [64]. In the T1DAL study, Alefacept was delivered by intramuscular injection in two 12-week courses to 33 individuals within 100 days of T1D diagnosis, while 16 individuals received placebo treatment [65]. The primary endpoint was not met, since the difference between C-peptide measurements in a 2h mixed-meal tolerance test (MMTT) at 12 months was not significant ( $P = 0.065$ ); however, the secondary endpoint, involving C-peptide measurement in a 4-h MMTT at the same timepoint was met ( $P = 0.019$ ). It was suggested that the curtailed recruitment following voluntary withdrawal of Alefacept by the manufacturer may have reduced the power to detect the impact of treatment. Follow-up analysis suggested beneficial effects were maintained 15 months after therapy cessation, with Alefacept-treated individuals exhibiting higher C-peptide levels than placebo-treated individuals, a significantly lower insulin requirement, and substantially lower rates of hypoglycaemia [66].

### IL-2 therapy

An additional immunotherapeutic approach directed at T cells centres on the selective expansion of Tregs using the cytokine IL-2. Although IL-2 was originally described as

a growth factor for conventional T cells, it subsequently became clear that a major biological function for IL-2 is to regulate immune responses by supporting the homeostasis of Tregs. A role for IL-2 in immune regulation indicated why defects in IL-2, or in genes that contribute to IL-2 signalling, are associated with autoimmune diseases, including T1D [67, 68]. Since Tregs express high levels of CD25, a component of the high-affinity IL-2 receptor, this sparked the idea that they might be selectively targeted by low doses of IL-2. The use of IL-2 to suppress immune responses is an extraordinary example of the same agent being used at different doses for opposing purposes, since high-dose IL-2 is used to promote anti-tumour responses in cancer patients.

In preclinical models, low-dose IL-2 was able to expand Tregs and reverse established type 1 diabetes [69]. However, a human phase I study in which IL-2 was combined with rapamycin gave disappointing results: nine individuals within 4 years of T1D diagnosis were included and although Treg frequencies increased, C-peptide transiently decreased and this coincided with an increase in NK cells and eosinophils [70]. In the light of this, the field has moved in two directions: one involving careful dosing to identify regimens that activate Tregs without activating effector cells [71], and the other involving the generation of mutant IL-2 therapeutics aimed at avoiding the detrimental activation of NK cells, eosinophils, and effector T cells. Encouragingly, doses of IL-2 that can be safely administered have now been identified and can be further explored in larger patient groups [72]. At the same time, numerous IL-2 mutant approaches are in development [73–75]. Boosting Treg numbers by cell therapy in combination with IL-2 administration is also being explored, however, the data again reinforce the need for IL-2 mutant approaches since NK cells, mucosal-associated invariant T cells and CD8<sup>+</sup> T cells were also affected by IL-2 administration [76]. While we do not cover cell therapy in this review, it should be noted that Treg cell therapy is an area of emerging interest as has been discussed elsewhere [77, 78].

## B cells in T1D

B cells are also implicated in the development of T1D. In animal models, B cell deficiency [79] or B cell depletion [80] inhibits the onset of diabetes and to our knowledge, only one individual lacking B cells, caused by X-linked agammaglobulinemia, has developed T1D [81]. Autoantibodies, secreted by B cells, can be detected against a number of islet antigens including insulin, glutamic acid decarboxylase, tyrosine phosphatase-related islet antigen 2 (IA-2) and zinc transporter 8 (ZnT8) [3–6]; however, autoantibodies themselves are not believed

to be pathogenic. Importantly, there is a substantially increased risk in those individuals who have 2 or more islet antigen-specific autoantibodies, with an 84% risk of developing T1D by 18 years of age [7]. Thus, the presence of autoantibodies provides an important biomarker pre-diagnosis, when individuals are still normoglycemic, that correlates with disease progression risk. B cells are also potent antigen-presenting cells, capable of activating autoantigen-specific T cells to cause diabetes [79]. B cells have been shown to infiltrate the islets, with increased islet CD20<sup>+</sup> B cell presence associated with enhanced  $\beta$  cell destruction, and diagnosis of T1D at an earlier age, compared to those with fewer CD20<sup>+</sup> islet B cells [13, 50, 82]. Thus, B cells have also been targeted in immunotherapy trials in T1D.

## Anti-CD20 immunotherapy

Rituximab binds to CD20 expressed on the surface of B cells, leading to their destruction mediated via antibody-dependent cell-mediated cytotoxicity, apoptosis, and complement-dependent cytotoxicity [83, 84]. It is important to note that Rituximab does not deplete all B cells, as plasma cells, which secrete antibodies, do not express CD20 and other B cell subsets such as B1 cells, germinal centre B cells and tissue-resident B cells may be less sensitive to depletion. Rituximab has shown efficacy in a number of different autoimmune diseases including systemic lupus erythematosus and rheumatoid arthritis. To explore the benefit of B cell depletion in T1D, pre-clinical studies were conducted by studying transgenic hCD20 NOD mice, expressing human CD20 on B cells [80]. In these hCD20 NOD mice, a single cycle of anti-CD20 antibody administration (9-day treatment cycle: 0.5 mg/ml at day 0, followed by three injections of 0.25 mg/ml at 3-day intervals), was able to both delay and reduce the development of T1D. Importantly, similar to anti-CD3 immunotherapy [26], anti-CD20 administration was able to reverse diabetes, with over one third of mice in remission. Studies evaluating the repopulation of cells in NOD mice found that the protection of anti-CD20 mice was associated with increased regulatory immune cells [80, 85–87] and reduced proinflammatory cytokines secretion by, and activation of, islet T cells [88].

Following the positive outcomes in pre-clinical models, Rituximab was trialled in individuals with recent-onset T1D. The therapy was administered by intravenous infusion in 4 doses over a period of 22 days, with 49 individuals receiving Rituximab and 29 receiving placebo [89]. The primary outcome measure was stimulated C-peptide levels in a MMTT 1 year after the initial infusion. Results from this clinical trial suggested clear benefit of B cell depletion: mean C-peptide levels

were 20% higher in the Rituximab-treated group (0.56 pmol/ml in the Rituximab group vs. 0.47 pmol/ml in the placebo group) and treatment also significantly reduced glycated haemoglobin levels (6.76% vs. 7.00%) and decreased the required insulin dose ( $0.39\text{U} \pm 0.22/\text{kg}$  of body weight vs.  $0.48\text{U} \pm 0.23/\text{kg}$ ). In common with other clinical trials, the initial improvements were short-lived and C-peptide continued to decline thereafter. Of note, CD19<sup>+</sup> B cell counts had substantially recovered by 6 months post-treatment initiation suggesting the possibility that benefits might be increased if the depletion could be sustained. In addition, the participants recruited to this study were aged 8–40 years of age [89]; however, given the prevalence of B cells in the pancreas of individuals diagnosed with T1D before 7 years of age [50], it is possible that Rituximab may show enhanced clinical efficacy in younger individuals and possibly in the ‘at risk’ population.

### Targeting inflammatory cytokines in T1D

IL-1 $\beta$  secretion increases with progression to diabetes and islet  $\beta$  cell destruction [90, 91]; however, two randomised, double-blind, placebo-controlled trials administering either Canakinumab (a human anti-IL-1 monoclonal antibody) or Anakinra (a human IL-1 receptor antagonist) were conducted, which failed to show any protective effects. This is in line with data from NOD mouse studies of IL-1 receptor- or IL-1 $\beta$ -deficient NOD mice, where no protection from diabetes development was observed [92, 93]. Likewise, blocking IL-6Ra in a recent trial in individuals with newly diagnosed T1D does not appear to provide benefit [94].

TNF $\alpha$  has been a target of interest in T1D for some time. Studies in NOD mice have yielded complex results, suggesting that TNF $\alpha$  plays site-specific, cell type-specific and age-dependent roles [95]. Administering anti-TNF $\alpha$  antibody to neonatal mice robustly inhibited the development of diabetes and this was associated with decreased T cell responses to islet antigens [96]; however, protection was weaker if treatment was initiated in adult mice, and administering TNF $\alpha$  itself exacerbated disease in neonates but paradoxically delayed it in adults. TNFR1-deficient NOD mice are however protected from the development of T1D [97].

Serum TNF is increased in individuals with recent-onset T1D [98] and TNF $\alpha$  is known to be toxic to the islet  $\beta$  cells [99]. Thus, TNF $\alpha$ -targeting therapies were administered to newly diagnosed T1D patients to test whether they could preserve  $\beta$  cell function. Etanercept, a recombinant soluble TNF-receptor fusion protein that binds to TNF $\alpha$  was trialled in 18 subjects with newly

diagnosed T1D. In this randomised, double-blind, placebo-controlled feasibility study over 24 weeks, Etanercept was shown to increase mean C-peptide levels by 39% from baseline whereas a mean decrease of 20% was observed in the placebo group [100]. More recently, Golimumab, an anti-TNF $\alpha$  monoclonal antibody previously approved for the treatment of rheumatoid arthritis and ulcerative colitis, has also been tested in newly diagnosed individuals with T1D [101]. This phase II randomised, double-blind, placebo-controlled study involved 56 participants receiving Golimumab and 28 participants receiving placebo, and resulted in significantly higher C-peptide and lower insulin use in the treatment group after 52 weeks. Since reagents that block the TNF $\alpha$  pathway are widely used in rheumatology settings and approved for use in patients as young as 2 years of age, this requires further investigation in T1D.

Interleukin 21 (IL-21) has also gained some traction as a target for T1D immunotherapy. IL-21 is the characteristic cytokine made by follicular helper T cells (T<sub>fh</sub> cells), that provide help for B cell antibody production, and therefore plays an important role in humoral immunity [102]. NOD mice lacking IL-21 or IL-21 receptor were protected from diabetes development, while transgenic expression of IL-21 in pancreatic islets was sufficient to induce diabetes in non-autoimmune prone (C57BL/6) mice [103, 104]. In a TCR transgenic mouse model of diabetes, T cells responding to islet antigen showed a T<sub>fh</sub> phenotype with high IL-21 expression, and the pancreas-infiltrating T cells were shown to express IL-21, IFN $\gamma$ , and TNF $\alpha$  [105]. In humans, a genetic region encompassing the IL-2 and IL-21 genes is associated with T1D [106] and an increased proportion of effector memory CD4<sup>+</sup> T cells secreting IL-21 and elevated T<sub>fh</sub> cells have been reported in people with T1D compared to healthy controls [105, 107]. Interestingly, the gene expression of cells responding to pro-insulin in genetically at risk children showed elements of a T<sub>fh</sub> signature (including *IL-21*), with a transition to a Th1-like signature (with decreased *IL-21* and increased *IFNG* and *TNF*) after the appearance of autoantibodies [108]. A phase II randomised double-blind, double-dummy, placebo-controlled study was conducted in recent-onset individuals with T1D, where they received either anti-IL21, anti-IL-21 with liraglutide, liraglutide alone or placebo (77 individuals per treatment arm) [109]. Liraglutide is a glucagon-like peptide 1 receptor agonist, which works by increasing insulin secretion from the pancreas and decreasing glucagon release. Thus, liraglutide improves  $\beta$  cell function. Von Herrath and colleagues found that in all treated groups, HbA1C was lowered compared to placebos; however, in the combination of anti-IL-21 with

liraglutide, a smaller reduction in C-peptide following a MMITT was observed, suggesting enhanced  $\beta$  cell function in the combination group, compared to single treatment groups. It will be important to enlarge the study and determine how long the effects may last following cessation of treatment.

### Microbial-derived therapeutics

Environmental factors such as the intestinal bacterial composition are important for shaping the immune response and modulating susceptibility to T1D. Altered bacterial composition has been reported in both individuals with T1D, and those 'at risk' of T1D development [110–118]. These changes in bacterial composition have also been linked to the development of early  $\beta$  cell auto-antibody responses [114]. Studies in NOD mice have also suggested that antibiotic administration, through depleting components of the bacterial composition, can alter immune responses and susceptibility to T1D development [119–126]. In humans, while antibiotic use alters the gut bacterial composition, particularly in the first few months of life [118], it does not seem to strongly associate with the development of islet autoimmunity or T1D [117, 127], although it can reduce beneficial *Bifidobacteria* (a probiotic) members [118]. Probiotics, bacteria with potential health benefits, have also been studied for their role in modulating susceptibility to T1D. *L. casei*, VSL#3 (a mixture of *B. longum*, *B. infantis*, *B. breve*, *L. acidophilus*, *L. casei*, *L. delbrueckii subsp. L. bulgaricus*, *L. plantarum*, and *Streptococcus salivarius subsp. Thermophilus*) and IRT5 (a mixture of *L. acidophilus*, *L. casei*, *L. reuteri*, *Bifidobacterium bifidum*, and *Streptococcus thermophilus*) have all been shown to protect NOD mice from developing T1D by promoting tolerogenic immune responses and reducing inflammatory Th1 cells [128–131]. *Lactobacillus johnsonii* N6.2, another probiotic, has also been shown to protect bio-breeding diabetes-prone rats from developing T1D [132, 133]. This probiotic has also been used in human studies whereby in a double-blind randomised trial in healthy adults, *L. johnsonii* N6.2 was safe and induced tolerogenic immune responses [134]. Studies are currently ongoing in children, adolescents and adults with T1D to identify safety and tolerance to *L. johnsonii* N6.2, as well as the immunological responses (NCT03961854 and NCT03961347). *Bifidobacterium longum subsp. infantis* is also being evaluated as a probiotic (NCT04769037), due to its ability to metabolise human milk oligosaccharides, which have significant impacts on inducing tolerogenic immune properties [135]. To date, only one human study has shown success in limiting  $\beta$  cell destruction. A faecal microbiota transplant (FMT) study

conducted in individuals with recent-onset (<6 weeks) T1D, showed participants receiving autologous FMTs, compared to allogenic (healthy control) FMTs, had improved preservation of  $\beta$  cell function for the 12 months individuals were followed post-FMT [136]. Thus, the area of microbial-derived therapeutics for T1D is still in an early stage of development and more work is required to determine whether it can be harnessed to modulate the immune response and deliver long-term clinical benefits.

### Future directions

In the 100 years since the discovery of insulin, there is still no cure for T1D; however, the promise of immunotherapy is gradually starting to be realised, with early signs of progress in both prevention and new onset settings. Key challenges moving forward lie in discerning which interventions are best suited to which disease stage; intervening after the emergence of symptomatic disease (stage 3; Fig. 1) will likely require memory cell targeting, while preventative interventions (stage 1/2) in children will need to have excellent safety profiles. A better appreciation of disease endotypes, for example, related to age of diagnosis [137], will ultimately inform the stratification of individuals to different treatment options. Using biomarkers, such as Tfh [63], or Treg and soluble IL-2R [72] to unpick the heterogeneity in clinical response will also be key.

Capitalising on the window to prevent T1D development will require extensive screening initiatives to identify at-risk individuals. Studies suggest that 95% of children who progress to clinical diabetes in puberty have autoantibodies by the age of 5; however, the time from seroconversion to clinical disease can vary enormously, taking over a decade in some cases [138]. The timing and risk/benefit profile of candidate interventions therefore need to be carefully considered. Intervening at stage 2, where dysglycemia is evident, permits focus on those at highest risk and decreases the duration of clinical trials; however, it is possible that some treatments may be less effective at this later stage of disease.

There are many other immunotherapy approaches in T1D that are not discussed here due to space considerations. Examples include the non-depleting anti-CD40 antibody Iscalimab (NCT04129528) and the JAK1/JAK2 inhibitor Baricitinib (NCT04774224). There is also interest in repurposing therapies with proven utility in other autoimmune conditions, such as Hydroxychloroquine (NCT03428945) which is used in systemic lupus erythematosus and rheumatoid arthritis, and the IL-12/IL-23 targeting drug Ustekinumab (NCT03941132) which is used in psoriasis.



Antigen-specific immunotherapies are likely to be important contributors to the future therapeutic landscape, and their specificity may prove beneficial from a safety perspective. Early data suggest administering antigen-specific therapies following IL-2-mediated Treg expansion may be a useful strategy [139]. Indeed, there is increasing interest in combination approaches, perhaps leveraging two immunotherapies such as the Rituximab/Abatacept combination currently being tested (NCT03929601), or perhaps combining an immunotherapy with strategies to augment  $\beta$  cell function. The latter goal will be boosted by recent advances in the generation of stem cell-derived  $\beta$  cells [140] and human islet-like organoids [141].

With effective immunotherapies gradually starting to emerge, it will be important to establish mechanisms that allow more individuals to be offered the option of participating in clinical trials at T1D diagnosis so that candidate interventions can be compared. Combined with large-scale initiatives to identify at-risk individuals, such as the trailblazing public health screening approach taken by Ziegler and colleagues [142], it seems that the new landscape for T1D treatment and prevention is beginning to take shape.

## Acknowledgements

This review was submitted on behalf of the British Society for Immunology Autoimmunity Affinity Group which includes the authors and Dr Kathryn Steel, Kings College London, and Dr Mohini Gray, University of Edinburgh. The Editor-in-Chief, Tim Elliott, and handling editor, Marianne Boes, would like to thank the following reviewers, Emma Hamilton-Williams and Remi Creusot, for their contribution to the publication of this article.

## Funding

This work was funded by a Medical Research Council Career Development Award (MRT010525/1) and a JDRF UK grant (1-SGA-2021-002) to J.A.P. E.F.M. is supported by a JDRF award (2-SRA-2018-644-M-B). L.S.K.W. is funded by a Medical Research Council Programme Grant (MR/N001435/1), a Wellcome Trust Investigator Award (220772/Z/20/Z), and a Diabetes UK project grant (20/0006172).

## Author contributions

J.A.P. and L.S.K.W. wrote and edited the manuscript. E.M. contributed to the editing of the manuscript.

## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Data availability

No data are available as this is a review article.

## References

1. Brostoff JM, Keen H, Brostoff J. A diabetic life before and after the insulin era. *Diabetologia* 2007;50(6):1351–3. <https://doi.org/10.1007/s00125-007-0641-0>
2. Insel RA, Dunne JL, Atkinson MA, et al. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. *Diabetes Care* 2015;38(10):1964–74. <https://doi.org/10.2337/dc15-1419>
3. Palmer JP, Asplin CM, Clemons P, et al. Insulin antibodies in insulin-dependent diabetes before insulin treatment. *Science* 1983;222(4630):1337–9.
4. Baekkeskov S, Aanstoot HJ, Christgau S, et al. Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature* Sep 1990;347(6289):151–6. <https://doi.org/10.1038/347151a0>
5. Bonifacio E, Lampasona V, Genovese S, Ferrari M, Bosi E. Identification of protein tyrosine phosphatase-like IA2 (islet cell antigen 512) as the insulin-dependent diabetes-related 37/40K autoantigen and a target of islet-cell antibodies. *J Immunol* 1995;155(11):5419–26.
6. Wenzlau JM, Juhl K, Yu L, et al. The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. *Proc Natl Acad Sci USA* 2007;104(43):17040–5. <https://doi.org/10.1073/pnas.0705894104>
7. Ziegler AG, Rewers M, Simell O, et al. Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. *JAMA* 2013;309(23):2473–9. <https://doi.org/10.1001/jama.2013.6285>
8. Dayan CM, Besser REJ, Oram RA, et al. Preventing type 1 diabetes in childhood. *Science* 2021;373(6554):506–10. <https://doi.org/10.1126/science.aba4742>
9. Laupacis A, Stiller CR, Gardell C, et al. Cyclosporin prevents diabetes in BB Wistar rats. *Lancet* 1983;1(8314-5):10–2. [https://doi.org/10.1016/s0140-6736\(83\)91558-1](https://doi.org/10.1016/s0140-6736(83)91558-1)
10. Stiller CR, Dupré J, Gent M, et al. Effects of cyclosporine immunosuppression in insulin-dependent diabetes mellitus of recent onset. *Science* 1984;223(4643):1362–7. <https://doi.org/10.1126/science.6367043>
11. Silverstein J, Maclaren N, Riley W, Spillar R, Radjenovic D, Johnson S. Immunosuppression with azathioprine and prednisone in recent-onset insulin-dependent diabetes mellitus. *N Engl J Med* 1988;319(10):599–604. <https://doi.org/10.1056/NEJM198809083191002>
12. Thomas R, Carballido JM, Wesley JD, Ahmed ST. Overcoming obstacles in the development of antigen-specific immunotherapies for type 1 diabetes. *Front Immunol* 2021;12:730414. <https://doi.org/10.3389/fimmu.2021.730414>
13. Willcox A, Richardson SJ, Bone AJ, Foulis AK, Morgan NG. Analysis of islet inflammation in human type 1 diabetes. *Clin Exp Immunol* 2009;155(2):173–81. [https://doi.org/CEI3860\[pii\]10.1111/j.1365-2249.2008.03860.x](https://doi.org/CEI3860[pii]10.1111/j.1365-2249.2008.03860.x)
14. Coppieters KT, Dotta F, Amirian N, et al. Demonstration of islet-autoreactive CD8 T cells in insulinitic lesions from recent onset and long-term type 1 diabetes patients. *J Exp Med* 2012;209(1):51–60. <https://doi.org/10.1084/jem.20111187>

15. Christianson SW, Shultz LD, Leiter EH. Adoptive transfer of diabetes into immunodeficient NOD-scid/scid mice. Relative contributions of CD4+ and CD8+ T-cells from diabetic versus prediabetic NOD.NON-Thy-1a donors. *Diabetes* 1993;42(1):44–55.
16. Kung P, Goldstein G, Reinherz EL, Schlossman SF. Monoclonal antibodies defining distinctive human T cell surface antigens. *Science* 1979;206(4416):347–9. <http://doi.org/10.1126/science.314668>
17. Kjer-Nielsen L, Dunstone MA, Kostenko L, et al. Crystal structure of the human T cell receptor CD3 epsilon gamma heterodimer complexed to the therapeutic mAb OKT3. *Proc Natl Acad Sci USA* 2004;101(20):7675–80. <http://doi.org/10.1073/pnas.0402295101>
18. Cosimi AB, Burton RC, Colvin RB, et al. Treatment of acute renal allograft rejection with OKT3 monoclonal antibody. *Transplantation* 1981;32(6):535–9. <http://doi.org/10.1097/00007890-198112000-00018>
19. Cosimi AB, Colvin RB, Burton RC, et al. Use of monoclonal antibodies to T-cell subsets for immunologic monitoring and treatment in recipients of renal allografts. *N Engl J Med* 1981;305(6):308–14. <http://doi.org/10.1056/NEJM198108063050603>
20. Thistlethwaite JR, Stuart JK, Mayes JT, et al. Complications and monitoring of OKT3 therapy. *Am J Kidney Dis.* 1988;11(2):112–9. [http://doi.org/10.1016/s0272-6386\(88\)80192-6](http://doi.org/10.1016/s0272-6386(88)80192-6)
21. Chatenoud L, Ferran C, Reuter A, et al. Systemic reaction to the anti-T-cell monoclonal antibody OKT3 in relation to serum levels of tumor necrosis factor and interferon-gamma [corrected]. *N Engl J Med.* 1989;320(21):1420–1. <http://doi.org/10.1056/NEJM198905253202117>
22. Abramowicz D, Schandene L, Goldman M, et al. Release of tumor necrosis factor, interleukin-2, and gamma-interferon in serum after injection of OKT3 monoclonal antibody in kidney transplant recipients. *Transplantation.* 1989;47(4):606–8. <http://doi.org/10.1097/00007890-198904000-00008>
23. Hirsch R, Bluestone JA, DeNenno L, Gress RE. Anti-CD3 F(ab')<sub>2</sub> fragments are immunosuppressive in vivo without evoking either the strong humoral response or morbidity associated with whole mAb. *Transplantation.* 1990;49(6):1117–23. <http://doi.org/10.1097/00007890-199006000-00018>
24. Parlevliet KJ, ten Berge IJ, Yong SL, Surachno J, Wilmink JM, Schellekens PT. In vivo effects of IgA and IgG2a anti-CD3 isotype switch variants. *J Clin Invest.* 1994;93(6):2519–25. <http://doi.org/10.1172/JCI117262>
25. Alegre ML, Peterson LJ, Xu D, et al. A non-activating “humanized” anti-CD3 monoclonal antibody retains immunosuppressive properties in vivo. *Transplantation.* 1994;57(11):1537–43.
26. Chatenoud L, Thervet E, Primo J, Bach JF. Anti-CD3 antibody induces long-term remission of overt autoimmunity in nonobese diabetic mice. *Proc Natl Acad Sci USA* 1994;91(1):123–7. <http://doi.org/10.1073/pnas.91.1.123>
27. Chatenoud L, Primo J, Bach JF. CD3 antibody-induced dominant self tolerance in overtly diabetic NOD mice. *J Immunol.* 1997;158(6):2947–54.
28. You S, Belghith M, Cobbold S, et al. Autoimmune diabetes onset results from qualitative rather than quantitative age-dependent changes in pathogenic T-cells. *Diabetes.* 2005;54(5):1415–22. <http://doi.org/10.2337/diabetes.54.5.1415>
29. Belghith M, Bluestone JA, Barriot S, Mégret J, Bach JF, Chatenoud L. TGF-beta-dependent mechanisms mediate restoration of self-tolerance induced by antibodies to CD3 in overt autoimmune diabetes. *Nat Med* 2003;9(9):1202–8. <http://doi.org/10.1038/nm924>
30. You S, Leforban B, Garcia C, Bach JF, Bluestone JA, Chatenoud L. Adaptive TGF-beta-dependent regulatory T cells control autoimmune diabetes and are a privileged target of anti-CD3 antibody treatment. *Proc Natl Acad Sci USA* 2007;104(15):6335–40. <http://doi.org/10.1073/pnas.0701171104>
31. Smith JA, Tso JY, Clark MR, Cole MS, Bluestone JA. Nonmitogenic anti-CD3 monoclonal antibodies deliver a partial T cell receptor signal and induce clonal anergy. *J Exp Med* 1997;185(8):1413–22. <http://doi.org/10.1084/jem.185.8.1413>
32. Yang W, Hussain S, Mi QS, Santamaria P, Delovitch TL. Perturbed homeostasis of peripheral T cells elicits decreased susceptibility to anti-CD3-induced apoptosis in prediabetic nonobese diabetic mice. *J Immunol* 2004;173(7):4407–16. <http://doi.org/10.4049/jimmunol.173.7.4407>
33. Kuhn C, You S, Valette F, et al. Human CD3 transgenic mice: preclinical testing of antibodies promoting immune tolerance. *Sci Transl Med* 2011;3(68):68ra10. <http://doi.org/10.1126/scitranslmed.3001830>
34. Herold KC, Hagopian W, Auger JA, et al. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *N Engl J Med.* 2002;346(22):1692–8. <http://doi.org/10.1056/NEJMoa012864>
35. Keymeulen B, Vandemeulebroucke E, Ziegler AG, et al. Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. *N Engl J Med* 2005;352(25):2598–608. <http://doi.org/10.1056/NEJMoa043980>
36. Gubitosi-Klug RA, Braffett BH, Hitt S, et al. Residual  $\beta$  cell function in long-term type 1 diabetes associates with reduced incidence of hypoglycemia. *J Clin Invest* 2021;131(3):e143011. <http://doi.org/10.1172/JCI143011>
37. Herold KC, Gitelman SE, Masharani U, et al. A single course of anti-CD3 monoclonal antibody hOKT3gamma1(Ala-Ala) results in improvement in C-peptide responses and clinical parameters for at least 2 years after onset of type 1 diabetes. *Diabetes* 2005;54(6):1763–9. <http://doi.org/10.2337/diabetes.54.6.1763>
38. Herold KC, Gitelman S, Greenbaum C, et al. Treatment of patients with new onset Type 1 diabetes with a single course of anti-CD3 mAb Teplizumab preserves insulin production for up to 5 years. *Clin Immunol* 2009;132(2):166–73. <http://doi.org/10.1016/j.clim.2009.04.007>

39. Perdigoto AL, Preston-Hurlburt P, Clark P, *et al.* Treatment of type 1 diabetes with teplizumab: clinical and immunological follow-up after 7 years from diagnosis. *Diabetologia* 2019;62(4):655–64. <http://doi.org/10.1007/s00125-018-4786-9>
40. Sherry N, Hagopian W, Ludvigsson J, *et al.* Teplizumab for treatment of type 1 diabetes (Protégé study): 1-year results from a randomised, placebo-controlled trial. *Lancet* 2011;378(9790):487–97. [http://doi.org/10.1016/S0140-6736\(11\)60931-8](http://doi.org/10.1016/S0140-6736(11)60931-8)
41. Herold KC, Bundy BN, Long SA, *et al.* An Anti-CD3 Antibody, Teplizumab, in Relatives at Risk for Type 1 Diabetes. *N Engl J Med* 2019;381:603–13. <http://doi.org/10.1056/NEJMoa1902226>
42. Sims EK, Bundy BN, Stier K, *et al.* Teplizumab improves and stabilizes beta cell function in antibody-positive high-risk individuals. *Sci Transl Med* 2021;13(583):eabc8980. <http://doi.org/10.1126/scitranslmed.abc8980>
43. Waldron-Lynch F, Henegariu O, Deng S, *et al.* Teplizumab induces human gut-tropic regulatory cells in humanized mice and patients. *Sci Transl Med* 2012;4(118):118ra12. <http://doi.org/10.1126/scitranslmed.3003401>
44. Long SA, Thorpe J, DeBerg HA, *et al.* Partial exhaustion of CD8 T cells and clinical response to teplizumab in new-onset type 1 diabetes. *Sci Immunol* 2016;1(5): aai7793 <http://doi.org/10.1126/sciimmunol.aai7793>
45. Parker MJ, Xue S, Alexander JJ, *et al.* Immune depletion with cellular mobilization imparts immunoregulation and reverses autoimmune diabetes in nonobese diabetic mice. *Diabetes* 2009;58(10):2277–84. <http://doi.org/10.2337/db09-0557>
46. Eisenbarth GS, Srikanta S, Jackson R, *et al.* Anti-thymocyte globulin and prednisone immunotherapy of recent onset type 1 diabetes mellitus. *Diabetes Res* 1985;2(6):271–6.
47. Saudek F, Havrdova T, Boucek P, Karasova L, Novota P, Skibova J. Polyclonal anti-T-cell therapy for type 1 diabetes mellitus of recent onset. *Rev Diabet Stud* 2004;1(2):80–8. <http://doi.org/10.1900/RDS.2004.1.80>
48. Gitelman SE, Gottlieb PA, Rigby MR, *et al.* Antithymocyte globulin treatment for patients with recent-onset type 1 diabetes: 12-month results of a randomised, placebo-controlled, phase 2 trial. *Lancet Diabetes Endocrinol.* 2013;1(4):306–16. [http://doi.org/10.1016/S2213-8587\(13\)70065-2](http://doi.org/10.1016/S2213-8587(13)70065-2)
49. Gitelman SE, Gottlieb PA, Felner EI, *et al.* Antithymocyte globulin therapy for patients with recent-onset type 1 diabetes: 2 year results of a randomised trial. *Diabetologia* 2016;59(6):1153–61. <http://doi.org/10.1007/s00125-016-3917-4>
50. Haller MJ, Gitelman SE, Gottlieb PA, *et al.* Anti-thymocyte globulin/G-CSF treatment preserves  $\beta$  cell function in patients with established type 1 diabetes. *J Clin Invest* 2015;125(1):448–55. <http://doi.org/10.1172/JCI78492>
51. Haller MJ, Gitelman SE, Gottlieb PA, *et al.* Antithymocyte globulin plus G-CSF combination therapy leads to sustained immunomodulatory and metabolic effects in a subset of responders with established type 1 diabetes. *Diabetes* 2016;65(12):3765–75. <http://doi.org/10.2337/db16-0823>
52. Haller MJ, Schatz DA, Skyler JS, *et al.* Low-dose anti-thymocyte globulin (ATG) preserves  $\beta$ -cell function and improves HbA. *Diabetes Care* 2018;41(9):1917–25. <http://doi.org/10.2337/dc18-0494>
53. Haller MJ, Atkinson MA, Wasserfall CH, *et al.* Mobilization without immune depletion fails to restore immunological tolerance or preserve beta cell function in recent onset type 1 diabetes. *Clin Exp Immunol* 2016;183(3):350–7. <http://doi.org/10.1111/cei.12731>
54. Jacobsen LM, Bundy BN, Greco MN, *et al.* Comparing beta cell preservation across clinical trials in recent-onset type 1 diabetes. *Diabetes Technol Ther* 2020;22(12):948–53. <http://doi.org/10.1089/dia.2020.0305>
55. Edner NM, Carlesso G, Rush JS, Walker LSK. Targeting co-stimulatory molecules in autoimmune disease. *Nat Rev Drug Discov* 2020;19(12):860–83. <http://doi.org/10.1038/s41573-020-0081-9>
56. Lenschow DJ, Ho SC, Sattar H, *et al.* Differential effects of anti-B7-1 and anti-B7-2 monoclonal antibody treatment on the development of diabetes in the nonobese diabetic mouse. *J Exp Med* 1995;181(3):1145–55. <http://doi.org/10.1084/jem.181.3.1145>
57. Lenschow DJ, Herold KC, Rhee L, *et al.* CD28/B7 regulation of Th1 and Th2 subsets in the development of autoimmune diabetes. *Immunity* 1996;5(3):285–93. [http://doi.org/10.1016/s1074-7613\(00\)80323-4](http://doi.org/10.1016/s1074-7613(00)80323-4)
58. Salomon B, Lenschow DJ, Rhee L, *et al.* B7/CD28 costimulation is essential for the homeostasis of the CD4+CD25+ immunoregulatory T cells that control autoimmune diabetes. *Immunity* 2000;12(4):431–40. [http://doi.org/10.1016/s1074-7613\(00\)80195-8](http://doi.org/10.1016/s1074-7613(00)80195-8)
59. Orban T, Bundy B, Becker DJ, *et al.* Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled trial. *Lancet* 2011;378(9789):412–9. [http://doi.org/10.1016/S0140-6736\(11\)60886-6](http://doi.org/10.1016/S0140-6736(11)60886-6)
60. Orban T, Bundy B, Becker DJ, *et al.* Costimulation modulation with abatacept in patients with recent-onset type 1 diabetes: follow-up 1 year after cessation of treatment. *Diabetes Care* 2014;37(4):1069–75. <http://doi.org/10.2337/dc13-0604>
61. Orban T, Beam CA, Xu P, *et al.* Reduction in CD4 central memory T-cell subset in costimulation modulator abatacept-treated patients with recent-onset type 1 diabetes is associated with slower C-peptide decline. *Diabetes* 2014;63(10):3449–57. <http://doi.org/10.2337/db14-0047>
62. Edner NM, Heuts F, Thomas N, *et al.* Follicular helper T cell profiles predict response to costimulation blockade in type 1 diabetes. *Nat Immunol* 2020;21(10):1244–55. <http://doi.org/10.1038/s41590-020-0744-z>
63. da Silva AJ, Brickelmaier M, Majeau GR, *et al.* Alefacept, an immunomodulatory recombinant LFA-3/IgG1 fusion protein, induces CD16 signaling and CD2/CD16-dependent apoptosis of CD2(+) cells. *J Immunol* 2002;168(9):4462–71. <http://doi.org/10.4049/jimmunol.168.9.4462>
64. Rigby MR, DiMeglio LA, Rendell MS, *et al.* Targeting of memory T cells with alefacept in new-onset type 1

- diabetes (T1DAL study): 12 month results of a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet Diabetes Endocrinol* 2013;1(4):284–94. [http://doi.org/10.1016/S2213-8587\(13\)70111-6](http://doi.org/10.1016/S2213-8587(13)70111-6)
65. Rigby MR, Harris KM, Pinckney A, *et al.* Alefacept provides sustained clinical and immunological effects in new-onset type 1 diabetes patients. *J Clin Invest* 2015;125(8):3285–96. <http://doi.org/10.1172/JCI81722>
  66. Dendrou CA, Wicker LS. The IL-2/CD25 pathway determines susceptibility to T1D in humans and NOD mice. *J Clin Immunol* 2008;28(6):685–96. <http://doi.org/10.1007/s10875-008-9237-9>
  67. Garg G, Tyler JR, Yang JH, *et al.* Type 1 diabetes-associated IL2RA variation lowers IL-2 signaling and contributes to diminished CD4+CD25+ regulatory T cell function. *J Immunol* 2012;188(9):4644–53. <http://doi.org/10.4049/jimmunol.1100272>
  68. Grinberg-Bleyer Y, Baeyens A, You S, *et al.* IL-2 reverses established type 1 diabetes in NOD mice by a local effect on pancreatic regulatory T cells. *J Exp Med* 2010;207(9):1871–8. <http://doi.org/10.1084/jem.20100209>
  69. Long SA, Rieck M, Sanda S, *et al.* Rapamycin/IL-2 combination therapy in patients with type 1 diabetes augments Tregs yet transiently impairs  $\beta$ -cell function. *Diabetes* 2012;61(9):2340–8. <http://doi.org/10.2337/db12-0049>
  70. Seelig E, Howlett J, Porter L, *et al.* The DILfrequency study is an adaptive trial to identify optimal IL-2 dosing in patients with type 1 diabetes. *JCI Insight* 2018;3(19): e99306. <http://doi.org/10.1172/jci.insight.99306>
  71. Rosenzweig M, Salet R, Lorenzon R, *et al.* Low-dose IL-2 in children with recently diagnosed type 1 diabetes: a Phase I/II randomised, double-blind, placebo-controlled, dose-finding study. *Diabetologia* 2020;63(9):1808–21. <http://doi.org/10.1007/s00125-020-05200-w>
  72. Peterson LB, Bell CJM, Howlett SK, *et al.* A long-lived IL-2 mutein that selectively activates and expands regulatory T cells as a therapy for autoimmune disease. *J Autoimmun* 2018;95:1–14. <http://doi.org/10.1016/j.jaut.2018.10.017>
  73. Trotta E, Bessette PH, Silveria SL, *et al.* A human anti-IL-2 antibody that potentiates regulatory T cells by a structure-based mechanism. *Nat Med* 2018;24(7):1005–14. <http://doi.org/10.1038/s41591-018-0070-2>
  74. Khoryati L, Pham MN, Sherve M, *et al.* An IL-2 mutein engineered to promote expansion of regulatory T cells arrests ongoing autoimmunity in mice. *Sci Immunol* 2020;5(50): aba5264 <http://doi.org/10.1126/sciimmunol.aba5264>
  75. Dong S, Hiam-Galvez KJ, Mowery CT, *et al.* The effect of low-dose IL-2 and Treg adoptive cell therapy in patients with type 1 diabetes. *JCI Insight* 2021;6(18): e147474. <http://doi.org/10.1172/jci.insight.147474>
  76. Ferreira LMR, Muller YD, Bluestone JA, Tang Q. Next-generation regulatory T cell therapy. *Nat Rev Drug Discov* 2019;18(10):749–69. <http://doi.org/10.1038/s41573-019-0041-4>
  77. MacDonald KN, Piret JM, Levings MK. Methods to manufacture regulatory T cells for cell therapy. *Clin Exp Immunol* 2019;197(1):52–63. <http://doi.org/10.1111/cei.13297>
  78. Serreze DV, Chapman HD, Varnum DS, *et al.* B lymphocytes are essential for the initiation of T cell-mediated autoimmune diabetes: analysis of a new “speed congenic” stock of NOD.Ig mu null mice. *J Exp Med* 1996;184(5):2049–53.
  79. Hu CY, Rodriguez-Pinto D, Du W, *et al.* Treatment with CD20-specific antibody prevents and reverses autoimmune diabetes in mice. *J Clin Invest* 2007;117(12):3857–67. <http://doi.org/10.1172/JCI32405>
  80. Martin S, Wolf-Eichbaum D, Duinkerken G, *et al.* Development of type 1 diabetes despite severe hereditary B-cell deficiency. *N Engl J Med* 2001;345(14):1036–40. <http://doi.org/10.1056/NEJMoa010465>
  81. Arif S, Leete P, Nguyen V, *et al.* Blood and islet phenotypes indicate immunological heterogeneity in type 1 diabetes. *Diabetes* 2014;63(11):3835–45. <http://doi.org/10.2337/db14-0365>
  82. Leete P, Willcox A, Krogvold L, *et al.* Differential insulinitic profiles determine the extent of  $\beta$ -cell destruction and the age at onset of type 1 diabetes. *Diabetes* 2016;65(5):1362–9. <http://doi.org/10.2337/db15-1615>
  83. Byrd JC, Kitada S, Flinn IW, *et al.* The mechanism of tumor cell clearance by rituximab in vivo in patients with B-cell chronic lymphocytic leukemia: evidence of caspase activation and apoptosis induction. *Blood* 2002;99(3):1038–43. <http://doi.org/10.1182/blood.v99.3.1038>
  84. Kennedy AD, Solga MD, Schuman TA, *et al.* An anti-C3b(i) mAb enhances complement activation, C3b(i) deposition, and killing of CD20+ cells by rituximab. *Blood* 2003;101(3):1071–9. <http://doi.org/10.1182/blood-2002-03-0876>
  85. Hu C, Du W, Zhang X, Wong FS, Wen L. The role of Gr1+ cells after anti-CD20 treatment in type 1 diabetes in nonobese diabetic mice. *J Immunol* 2012;188(1):294–301. <http://doi.org/10.4049/jimmunol.1101590>
  86. Hu C, Ding H, Zhang X, Wong FS, Wen L. Combination treatment with anti-CD20 and oral anti-CD3 prevents and reverses autoimmune diabetes. *Diabetes* 2013;62(8):2849–58. <http://doi.org/10.2337/db12-1175>
  87. Xiang Y, Peng J, Tai N, *et al.* The dual effects of B cell depletion on antigen-specific T cells in BDC2.5NOD mice. *J Immunol* 2012;188(10):4747–58. <http://doi.org/10.4049/jimmunol.1103055>
  88. Da Rosa LC, Boldison J, De Leenheer E, Davies J, Wen L, Wong FS. B cell depletion reduces T cell activation in pancreatic islets in a murine autoimmune diabetes model. *Diabetologia* 2018;61(6):1397–410. <http://doi.org/10.1007/s00125-018-4597-z>
  89. Pescovitz MD, Greenbaum CJ, Krause-Steinrauf H, *et al.* Rituximab, B-lymphocyte depletion, and preservation of beta-cell function. *N Engl J Med* 2009;361(22):2143–52. <http://doi.org/10.1056/NEJMoa0904452>
  90. Rothe H, Jenkins NA, Copeland NG, Kolb H. Active stage of autoimmune diabetes is associated with the expression of a novel cytokine, IGF, which is located near Idd2. *J Clin Invest* 1997;99(3):469–74. <http://doi.org/10.1172/JCI119181>
  91. Amrani A, Verdager J, Thiessen S, Bou S, Santamaria P. IL-1 $\alpha$ , IL-1 $\beta$ , and IFN- $\gamma$  mark beta cells for Fas-dependent destruction by diabetogenic CD4(+) T

- lymphocytes. *J Clin Invest* 2000;105(4):459–68. <http://doi.org/10.1172/JCI8185>
92. Thomas HE, Irawaty W, Darwiche R, *et al.* IL-1 receptor deficiency slows progression to diabetes in the NOD mouse. *Diabetes* 2004;53(1):113–21. <http://doi.org/10.2337/diabetes.53.1.113>
  93. Wen L, Green EA, Stratmann T, *et al.* In vivo diabetogenic action of CD4+ T lymphocytes requires Fas expression and is independent of IL-1 and IL-18. *Eur J Immunol* 2011;41(5):1344–51. <http://doi.org/10.1002/eji.201041216>
  94. Greenbaum CJ, Serti E, Lambert K, *et al.* IL-6 receptor blockade does not slow  $\beta$  cell loss in new-onset type 1 diabetes. *JCI Insight* 2021;6(21): e150074. <http://doi.org/10.1172/jci.insight.150074>
  95. Green EA, Flavell RA. Tumor necrosis factor -  $\alpha$  and the progression of diabetes in non-obese diabetic mice. *Immunol Rev* 2006: 11–22.
  96. Yang XD, Tisch R, Singer SM, *et al.* Effect of tumor necrosis factor alpha on insulin-dependent diabetes mellitus in NOD mice. I. The early development of autoimmunity and the diabetogenic process. *J Exp Med* 1994;180(3):995–1004.
  97. Kägi D, Ho A, Odermatt B, Zakarian A, Ohashi PS, Mak TW. TNF receptor 1-dependent beta cell toxicity as an effector pathway in autoimmune diabetes. *J Immunol* 1999;162(8):4598–605.
  98. Cavallo MG, Pozzilli P, Bird C, *et al.* Cytokines in sera from insulin-dependent diabetic patients at diagnosis. *Clin Exp Immunol* 1991;86(2):256–9. <http://doi.org/10.1111/j.1365-2249.1991.tb05806.x>
  99. Rabinovitch A, Sumoski W, Rajotte RV, Warnock GL. Cytotoxic effects of cytokines on human pancreatic islet cells in monolayer culture. *J Clin Endocrinol Metab* 1990;71(1):152–6. <http://doi.org/10.1210/jcem-71-1-152>
  100. Mastrandrea L, Yu J, Behrens T, *et al.* Etanercept treatment in children with new-onset type 1 diabetes: pilot randomized, placebo-controlled, double-blind study. *Diabetes Care* 2009;32(7):1244–9. <http://doi.org/10.2337/dc09-0054>
  101. Quattrin T, Haller MJ, Steck AK, *et al.* Golimumab and beta-cell function in youth with new-onset type 1 diabetes. *N Engl J Med* 2020;383(21):2007–17. <http://doi.org/10.1056/NEJMoa2006136>
  102. Tangye SG, Ma CS. Regulation of the germinal center and humoral immunity by interleukin-21. *J Exp Med* 2020;217(1): e20191638. <http://doi.org/10.1084/jem.20191638>
  103. Spolski R, Kashyap M, Robinson C, Yu Z, Leonard WJ. IL-21 signaling is critical for the development of type 1 diabetes in the NOD mouse. *Proc Natl Acad Sci USA* 2008;105(37):14028–33. <http://doi.org/10.1073/pnas.0804358105>
  104. Sutherland AP, Van Belle T, Wurster AL, *et al.* Interleukin-21 is required for the development of type 1 diabetes in NOD mice. *Diabetes* 2009;58(5):1144–55. <http://doi.org/10.2337/db08-0882>
  105. Kenefeck R, Wang CJ, Kapadi T, *et al.* Follicular helper T cell signature in type 1 diabetes. *J Clin Invest* 2015;125(1):292–303. <http://doi.org/10.1172/JCI76238>
  106. Consortium WTCC. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007;447(7145):661–78. <http://doi.org/10.1038/nature05911>
  107. Ferreira RC, Simons HZ, Thompson WS, *et al.* IL-21 production by CD4+ effector T cells and frequency of circulating follicular helper T cells are increased in type 1 diabetes patients. *Diabetologia* 2015;58(4):781–90. <http://doi.org/10.1007/s00125-015-3509-8>
  108. Heninger AK, Eugster A, Kuehn D, *et al.* A divergent population of autoantigen-responsive CD4. *Sci Transl Med* 2017;9(378): aaf8848. <http://doi.org/10.1126/scitranslmed.aaf8848>
  109. von Herrath M, Bain SC, Bode B, *et al.* Anti-interleukin-21 antibody and liraglutide for the preservation of  $\beta$ -cell function in adults with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet Diabetes Endocrinol* 2021;9(4):212–24. [http://doi.org/10.1016/S2213-8587\(21\)00019-X](http://doi.org/10.1016/S2213-8587(21)00019-X)
  110. Giongo A, Gano KA, Crabb DB, *et al.* Toward defining the autoimmune microbiome for type 1 diabetes. *ISME J* 2011;5(1):82–91. <http://doi.org/10.1038/ismej.2010.92>
  111. Murri M, Leiva I, Gomez-Zumaquero JM, *et al.* Gut microbiota in children with type 1 diabetes differs from that in healthy children: a case-control study. *BMC Med* 2013;11:46. <http://doi.org/10.1186/1741-7015-11-46>
  112. de Goffau MC, Luopajarvi K, Knip M, *et al.* Fecal microbiota composition differs between children with  $\beta$ -cell autoimmunity and those without. *Diabetes* 2013;62(4):1238–44. <http://doi.org/10.2337/db12-0526>
  113. de Goffau MC, Fuentes S, van den Bogert B, *et al.* Aberrant gut microbiota composition at the onset of type 1 diabetes in young children. *Diabetologia* 2014;57(8):1569–77. <http://doi.org/10.1007/s00125-014-3274-0>
  114. Kostic AD, Gevers D, Siljander H, *et al.* The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. *Cell Host Microbe* 2015;17(2):260–73. <http://doi.org/10.1016/j.chom.2015.01.001>
  115. Vatanen T, Kostic AD, d’Hennezel E, *et al.* Variation in Microbiome LPS Immunogenicity Contributes to Autoimmunity in Humans. *Cell* 2016;165(4):842–53. <http://doi.org/10.1016/j.cell.2016.04.007>
  116. de Groot PF, Belzer C, Aydin Ö, *et al.* Distinct fecal and oral microbiota composition in human type 1 diabetes, an observational study. *PLoS One* 2017;12(12):e0188475. <http://doi.org/10.1371/journal.pone.0188475>
  117. Stewart CJ, Ajami NJ, O’Brien JL, *et al.* Temporal development of the gut microbiome in early childhood from the TEDDY study. *Nature* 2018;562(7728):583–8. <http://doi.org/10.1038/s41586-018-0617-x>
  118. Vatanen T, Franzosa EA, Schwager R, *et al.* The human gut microbiome in early-onset type 1 diabetes from the TEDDY study. *Nature* 2018;562(7728):589–94. <http://doi.org/10.1038/s41586-018-0620-2>
  119. Hansen CH, Krych L, Nielsen DS, *et al.* Early life treatment with vancomycin propagates Akkermansia muciniphila

- and reduces diabetes incidence in the NOD mouse. *Diabetologia* 2012;55(8):2285–94. <http://doi.org/10.1007/s00125-012-2564-7>
120. Tormo-Badia N, Håkansson A, Vasudevan K, Molin G, Ahrné S, Cilio CM. Antibiotic treatment of pregnant non-obese diabetic (NOD) mice leads to altered gut microbiota and intestinal immunological changes in the offspring. *Scand J Immunol* 2014;80(4):250–60. <http://doi.org/10.1111/sji.12205>
  121. Candon S, Perez-Arroyo A, Marquet C, *et al.* Antibiotics in early life alter the gut microbiome and increase disease incidence in a spontaneous mouse model of autoimmune insulin-dependent diabetes. *PLoS One* 2015;10(5):e0125448. <http://doi.org/10.1371/journal.pone.0125448>
  122. Hu Y, Peng J, Tai N, *et al.* Maternal antibiotic treatment protects offspring from diabetes development in nonobese diabetic mice by generation of tolerogenic APCs. *J Immunol* 2015;195(9):4176–84. <http://doi.org/10.4049/jimmunol.1500884>
  123. Hu Y, Jin P, Peng J, Zhang X, Wong FS, Wen L. Different immunological responses to early-life antibiotic exposure affecting autoimmune diabetes development in NOD mice. *J Autoimmun* 2016;72:47–56. <http://doi.org/10.1016/j.jaut.2016.05.001>
  124. Livanos AE, Greiner TU, Vangay P, *et al.* Antibiotic-mediated gut microbiome perturbation accelerates development of type 1 diabetes in mice. *Nat Microbiol* 2016;1(11):16140. <http://doi.org/10.1038/nmicrobiol.2016.140>
  125. Pearson JA, Agriantonis A, Wong FS, Wen L. Modulation of the immune system by the gut microbiota in the development of type 1 diabetes. *Hum Vaccin Immunother* 2018;14(11):2580–96. <http://doi.org/10.1080/21645515.2018.1514354>
  126. Pearson JA, Kakabadse D, Davies J, *et al.* Altered gut microbiota activate and expand insulin B15-23-reactive CD8+ T-cells. *Diabetes* 2019;68(5):1002–13. <http://doi.org/10.2337/db18-0487>
  127. Kempainen KM, Vehik K, Lynch KF, *et al.* Association between early-life antibiotic use and the risk of islet or celiac disease autoimmunity. *JAMA Pediatr* 2017;171(12):1217–25. <http://doi.org/10.1001/jamapediatrics.2017.2905>
  128. Matsuzaki T, Nagata Y, Kado S, *et al.* Prevention of onset in an insulin-dependent diabetes mellitus model, NOD mice, by oral feeding of *Lactobacillus casei*. *APMIS* 1997;105(8):643–9.
  129. Calcinario F, Dionisi S, Marinaro M, *et al.* Oral probiotic administration induces interleukin-10 production and prevents spontaneous autoimmune diabetes in the non-obese diabetic mouse. *Diabetologia* 2005;48(8):1565–75. <http://doi.org/10.1007/s00125-005-1831-2>
  130. Dolpady J, Sorini C, Di Pietro C, *et al.* Oral probiotic VSL#3 prevents autoimmune diabetes by modulating microbiota and promoting indoleamine 2,3-dioxygenase-enriched tolerogenic intestinal environment. *J Diabetes Res* 2016;2016:7569431. <http://doi.org/10.1155/2016/7569431>
  131. Kim TK, Lee JC, Im SH, Lee MS. Amelioration of autoimmune diabetes of NOD mice by immunomodulating probiotics. *Front Immunol* 2020;11:1832. <http://doi.org/10.3389/fimmu.2020.01832>
  132. Valladares R, Sankar D, Li N, *et al.* *Lactobacillus johnsonii* N6.2 mitigates the development of type 1 diabetes in BB-DP rats. *PLoS One* 2010;5(5):e10507. <http://doi.org/10.1371/journal.pone.0010507>
  133. Lau K, Benitez P, Ardisson A, *et al.* Inhibition of type 1 diabetes correlated to a *Lactobacillus johnsonii* N6.2-mediated Th17 bias. *J Immunol* 2011;186(6):3538–46. <http://doi.org/10.4049/jimmunol.1001864>
  134. Marcial GE, Ford AL, Haller MJ, *et al.* *Lactobacillus johnsonii* N6.2 modulates the host immune responses: a double-blind, randomized trial in healthy adults. *Front Immunol* 2017;8:655. <http://doi.org/10.3389/fimmu.2017.00655>
  135. Henrick BM, Rodriguez L, Lakshmikanth T, *et al.* Bifidobacteria-mediated immune system imprinting early in life. *Cell* 2021;184(15):3884–98.e11. <http://doi.org/10.1016/j.cell.2021.05.030>
  136. de Groot P, Nikolic T, Pellegrini S, *et al.* Faecal microbiota transplantation halts progression of human new-onset type 1 diabetes in a randomised controlled trial. *Gut* 2021;70(1):92–105. <http://doi.org/10.1136/gutjnl-2020-322630>
  137. Leete P, Oram RA, McDonald TJ, *et al.* Studies of insulin and proinsulin in pancreas and serum support the existence of aetiopathological endotypes of type 1 diabetes associated with age at diagnosis. *Diabetologia* 2020;63(6):1258–67. <http://doi.org/10.1007/s00125-020-05115-6>
  138. Parikka V, Näntö-Salonen K, Saarinen M, *et al.* Early seroconversion and rapidly increasing autoantibody concentrations predict prepubertal manifestation of type 1 diabetes in children at genetic risk. *Diabetologia* 2012;55(7):1926–36. <http://doi.org/10.1007/s00125-012-2523-3>
  139. Pham MN, Khoryati L, Jamison BL, *et al.* In vivo expansion of antigen-specific regulatory T cells through staggered Fc $\gamma$ IL-2 mutein dosing and antigen-specific immunotherapy. *Immunohorizons* 2021;5(9):782–91. <http://doi.org/10.4049/imunohorizons.2100051>
  140. Högberg NJ, Maxwell KG, Augsornworawat P, Millman JR. Generation of insulin-producing pancreatic  $\beta$  cells from multiple human stem cell lines. *Nat Protoc* 2021;16(9):4109–43. <http://doi.org/10.1038/s41596-021-00560-y>
  141. Yoshihara E, O'Connor C, Gasser E, *et al.* Immune-evasive human islet-like organoids ameliorate diabetes. *Nature* 2020;586(7830):606–11. <http://doi.org/10.1038/s41586-020-2631-z>
  142. Ziegler AG, Kick K, Bonifacio E, *et al.* Yield of a public health screening of children for islet autoantibodies in Bavaria, Germany. *JAMA* 2020;323(4):339–51. <http://doi.org/10.1001/jama.2019.21565>