**Identifying the ‘Achilles Heel’ of type 1 diabetes**

Manuela Battaglia,

Jane H. Buckner, Translational Research Program, Benaroya Research Institute at Virginia Mason, Seattle, WA, USA

Megan Levings, BC Children's Hospital Research Institute, Vancouver, BC V5Z 4H4, Canada.

Sarah J. Richardson, Institute of Biomedical and Clinical Science, University of Exeter Medical School, RILD Building, Barrack Road, Exeter, EX2 5DW, UK

F. Susan Wong, Division of Infection and Immunity, Cardiff University School of Medicine, Cardiff, CF14 4XN, UK

Timothy I. Tree, Department of Immunobiology, School of Immunology & Microbial Sciences (SIMS), King's College London, London, United Kingdom and NIHR Biomedical Research Centre, Guy's and St Thomas' NHS Foundation Trust and King's College London, London, United Kingdom.

**Summary**

When Thetis dipped her son Achilles into the River Styx to make him immortal, she held him by the heel, which was not submerged, and thus created a weak spot that proved deadly for Achilles. Millennia later, Achilles heel is part of today's lexicon meaning an area of weakness or a vulnerable spot that causes failure. Also implied is that an Achilles heel is often missed, forgotten or underappreciated, until it is under attack, and then failure is fatal. Paris killed Achilles with an arrow 'guided by the Gods'.

At the International Congress of the Immunology of Diabetes Society, 2018, five leading experts were asked to present the case for a particular cell/element that could represent the Achilles heel of T1D. Their arguments are summarized here, to make this case.

## Introduction

Since the 1970s, we have had evidence suggesting that type 1 diabetes has a multifactorial pathogenesis, involving genetics, environmental influences that include viruses and immune responses. Sophisticated technology has facilitated imaging of the immune target, the pancreatic insulin-producing beta cell, as well as identification of numerous immune components that may be involved. Yet we are still working to understand how type 1 diabetes is initiated, and how the disease process evolves, in order to design therapies that may halt progression,

For nearly a century, insulin replacement therapy has been the only treatment option for individuals diagnosed with type 1 diabetes (T1D). A detailed understanding of disease pathogenesis would aid rational selection of therapies aimed at halting disease progress that destroys the insulin-producing pancreatic beta cells and even, in the future, prevent diabetes onset.

In this debate, five experts have discussed the case for elements of the disease pathogenesis to be the “Achilles Heel” of type 1 diabetes. Each of these elements – cells of the innate immune system, B cells, $CD8^+$ T cells, regulatory T cells (Tregs) and enteroviruses, have each been proposed to play an important role in the pathogenesis of type 1 diabetes. Has a convincing case been made for any of these to be the “Achilles Heel”?

**Cells of the innate immune system (Manuela Battaglia)**

For this debate, we have been tasked with convincing you that there is a specific cell type that is the Achilles heel of type 1 diabetes. I would posit that T1D is not a single disease as multiple mechanisms are likely to lead to the same clinical manifestation; therefore, I start by asserting that I don't think that *the* Achilles heel exists in T1D. I will here defend the hypothesis that some individuals have an abnormal innate interferon-related immune response that, in some circumstances, can lead to the development of T1D.

Type I interferons (IFNs) are polypeptides secreted by infected cells and have three major functions: (i) induce cell-intrinsic antimicrobial states in infected and cells in close proximity that limit the spread of infectious agents, particularly viral pathogens; (ii) modulate innate immune responses in a balanced manner that promotes antigen presentation and natural killer cell functions, while restraining pro-inflammatory pathways and cytokine production; and (iii) activate the adaptive immune system, thus promoting the development of high-affinity antigen-specific T and B cell responses and immunological memory. Type I IFNs are protective in acute viral infections but can also have deleterious effects in autoimmune diseases (1).

The clearest scientific evidence that type I IFNs contribute to T1D development comes from the evidence that individuals with hepatitis C, undergoing type I IFN therapy, have an increased risk of developing T1D by 10-18 fold, as compared to that of the corresponding general population. This complication typically appears abruptly, is manifested by severe hyperglycemia accompanied by a high titer of anti-islet antibodies, and it is often associated with autoimmune thyroid disease (2). Of note, patients with other diseases, including multiple sclerosis and hairy cell leukemia, receiving type I IFN therapy, have higher risk of developing T1D (3).

Type I IFNs are a catastrophic feature of the islet microenvironment as they are consistently found in the islet auto-inflammatory milieu and represent a viable signal that may precipitate diabetogenicity in T1D. Type I IFN cytokines can impair insulin secretory function, possibly through induction of endoplasmic reticulum stress, as well as by impairing mitochondrial bioenergetics. These cytokines also enhance the autoimmune surveillance of pancreatic β cells through induction of the immunoproteasome, de novo synthesis of MHC class I and genes responsible for the peptide loading complex, as well as enhanced surface expression of MHC class I. This increased capacity for antigen presentation results in a functional ability of cytotoxic CD8+ T lymphocyte-mediated β cell destruction (nicely reviewed in (3)) .

Our recent transcriptomic data generated on purified neutrophils from children at-risk of developing T1D, as well as those with overt disease, demonstrate that neutrophil-RNA expression is unique and distinct from that of age- and gender-matched non-diabetic individuals. Of note, this unique signature is already present in T1D family-related donors but who are autoAb negative and it is superimposable on that of individuals with overt T1D. Such a signature is characterized by the high expression of IFN-sensitive genes, suggesting the presence of an IFN-rich environment in genetically predisposed individuals (4). These data corroborate similar previous findings reporting an IFN-rich signature in whole blood of relatives that had already been identified in autoAb negative individuals (5,6). Overall, it is tempting to speculate that a specific genetic background predisposes individuals with a heightened innate immune reactive system who, when challenged maybe repetitively or excessively, may respond erroneously and this leads to uncontrolled innate immune reactivity.

Genetic predisposition is therefore the primary risk factor for the initiation of T1D autoimmunity and can be attributed to the complex interplay of more than 50 genetic loci that

may impact immune function, pancreatic activity and regenerative capacity and many other key features. For example, IFIH1 encodes the protein MDA5, a cytoplasmic sensor of viral double-stranded RNA and the non-synonymous SNP found in IFIH1 results in a gene variant that may diminish ATPase activity of MDA5 activity, leading to deranged constitutive provocation of type I IFNs as well as blunted viral sensing (reviewed in [3]). Compelling evidence in primary human islets has revealed that presence of the homozygous risk allele decreases the innate response to Coxsackievirus B3 (7). One could envisage that the IFIH risk variant might predispose β-cells to persistent enteroviral infection while concurrently promoting deleterious type I IFN production in and around the islet microenvironment.

As only a small number of at risk individuals (who might carry the predisposing gene variant/s) will eventually develop T1D, it is likely that counter-regulatory mechanisms are in place. For instance, Hayday and colleagues have demonstrated that the presence of neutralizing self-reactive antibodies specific for type I IFNs is associated with protection against T1D in people with AIRE mutations and immunopositivity to GAD (8). This further supports an important pathogenic role for IFNs in human T1D and an active mechanism of regulation in some individuals.

In summary, one of the Achilles heels in T1D is likely to be the combination of a genetic background that presents individuals with a heightened innate immune reactive response that in some individuals is kept in check by physiological regulatory mechanisms, while in others it remains uncontrolled. In the latter, an IFN-rich environment is present and this creates a highly toxic milieu for the beta-cells, with consequent development of T1D (Figure). On the basis of the current evidence, additional studies are required to clarify further the role of type I IFNs in

human T1D pathogenesis and determine whether they might represent an interesting therapeutic opportunity.

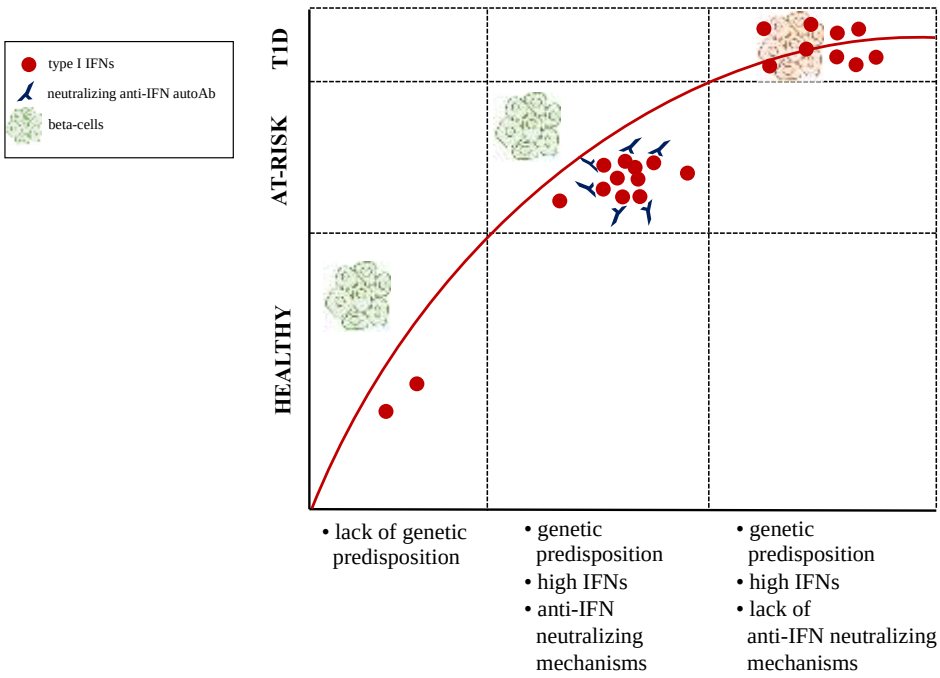


**Figure 1. Intersection of genetic predisposition and immune responses involving type 1 interferons**

**B cells (Jane Buckner)**

For this debate, we have been tasked with convincing you that our assigned immune cell type, in my case the B cell, is the Achilles heel of type 1 diabetes but first we need to decide what makes an immune cell type an Achilles heel for T1D. I would posit that to be the Achilles heel, an immune cell needs to contribute to the following: 1. Disease initiation; 2. Disease progression; and 3. Beta cell damage. It also needs to be difficult to control and underappreciated. Here, I will present evidence that B cells meet all of these criteria and indeed are the immune cell that most warrant recognition as the Achilles heel of T1D.

B cells have multiple immune functions all of which may contribute to T1D pathogenesis. They produce antibodies, present antigens, produce inflammatory cytokines and have a regulatory function (9,10). In T1D, B cells clearly have a key role in disease initiation based on both mouse and human studies. In the NOD mouse model, B cell ablation prevents development of diabetes (11–13), and this appears to be primarily due to their function as antigen presenting cells (14,15). Furthermore, studies of human cohorts at risk for developing T1D also implicate B cells in disease initiation, with the presence of two or more autoantibodies targeting islets as a hallmark of early stage T1D prior to clinical diagnosis (16). There is also emerging evidence that B cell homeostasis and function is impaired in “at-risk” cohorts (10,17,18). Reported alterations include increased frequency of transitional B cells, reduced frequency of anergic B cells and dynamic alterations in both BCR and IL-21 responses. Some of these alterations appear to be present only in pre-symptomatic T1D whereas others persist after clinical diagnosis, suggesting a role in disease progression. Further evidence that B cells contribute to disease progression is provided by the rituximab clinical trial, which showed that B cell depletion with this anti-CD20 monoclonal antibody resulted in preservation of C-peptide (19). Although more research is needed, there are also

data suggesting B cells contribute to beta cell damage. Rapid loss of β cell function in individuals with new-onset T1D is associated with a B cell signature, a finding most pronounced in the young (20). B cells have been detected near or within pancreatic islets from individuals with recent onset T1D (21). Interestingly, the abundance of B cells in the islets stratified with age at diagnosis with CD20hi individuals diagnosed at a younger age than those individuals that were CD20lo, suggesting that B cells may be more important in childhood-onset T1D. In addition, a recent study found a significant reduction in the number of primary B cell follicles in the pancreatic lymph nodes of individuals with recent-onset T1D compared to a non-diabetic control group (22). Mechanistic studies in mice also suggest that B cells contribute beta cell damage by promoting CD8 T cell survival (23) as well as through production of inflammatory cytokines and their role as antigen presenting cells.

B cells also pose unique therapeutic challenges. Despite anti-B cell therapy, islet autoantibodies persist after B cell depletion (19), and B cells arising after depletion are still autoreactive (24). In addition, responses to therapies that target B cells may only be seen in a subgroup of individuals with T1D. Those who were young and had a B cell immunotype were more likely to respond to B cell depletion (20), while individuals showing a poor response to abatacept, a T cell targeted therapy, had a transient increase in B cell activation after therapy (25). Additionally, preservation of regulatory B cells may also enhance responses to B cell targeted therapies (26). This suggests that therapeutic approaches targeting B cells will need to be both selective about which patients receive treatment, and the B cell type that is targeted. Lastly, a review of the literature highlights how underappreciated B cells are in the field of T1D research. On April 15, 2020, a PubMed search for “B cell and type 1 diabetes”

resulted in only 1,081 publications; in contrast a search for “T cell and type 1 diabetes” identified 5,909 publications.

In summary, B cells are involved in disease initiation, disease progression, and beta cell damage, and are difficult to target therapeutically. Importantly, our understanding of B cells is still incomplete, with an underappreciated role in T1D pathogenesis until recently. Achilles knew he had a weak point- but left it unshielded – B cells have been telling us they are important- have we been ignoring them?

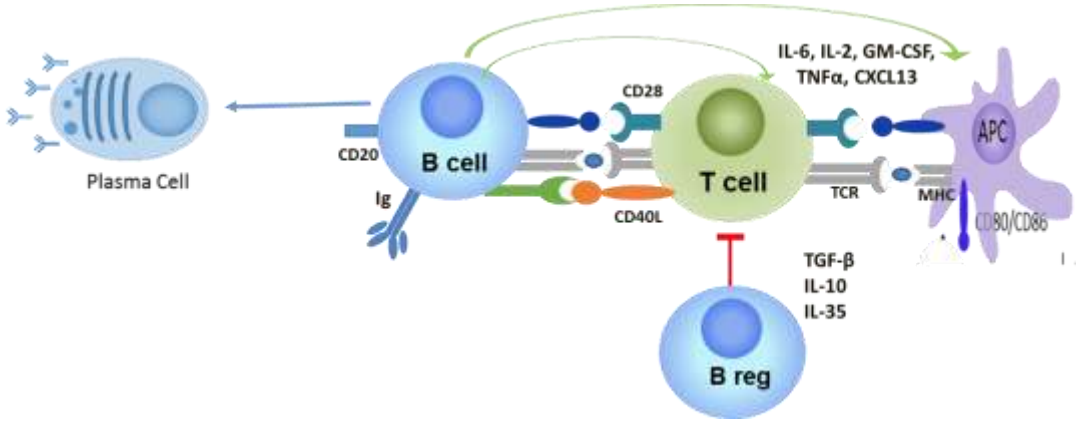


Figure 2. B cells influence islet autoimmunity at multiple levels.

**CD8$^+$ T cells (F. Susan Wong)**

In this debate, I propose that cytotoxic CD8$^+$ T cells are the Achilles heel in type 1 diabetes. In type 1 diabetes, there is infiltration of the pancreatic islets with immune cells and a loss of the production of insulin, implying beta cell damage and destruction (27). If a modern definition of Achilles heel is a vulnerable spot that is missed, and underappreciated, the CD8$^+$ T cells cause failure of beta cells by attacking in a situation where their effector role causes immunopathology and beta cell damage. This may occur at disease initiation and certainly progression, in a setting where highly effective cytotoxic CD8$^+$ T cells are either inappropriately activated, or correctly functioning but inadequately regulated.

Cytotoxic CD$^+$8 T cells are vital effectors in the adaptive immune system and are required for protection against viruses and tumours, and can respond to very low amounts of antigen. They kill target cells by a variety of means that include release of cytotoxic granules containing perforin and granzymes, production of cytotoxic cytokines, as well as induce apoptosis by CD95-CD95 ligand interactions. They can also become memory cells, which will rapidly kill target cells on reactivation. In recent years, it has become clear that CD8 T cells may be highly promiscuous, and are able to respond to a spectrum of antigens, possibly becoming activated by high avidity targets and then able to recognise autoantigenic targets with low-affinity (28).

Are CD8$^+$ T cells involved in disease initiation? It is difficult to know for certain about human type 1 diabetes. Certainly, for one of the most studied mouse models, the Non obese diabetic (NOD) mouse, they are clearly involved in the early stages of disease. In the absence of MHC class I, required for antigen presentation to CD8$^+$ T cells, few CD8$^+$ T cells develop and neither insulitis nor diabetes occurs (29,30). In humans, CD8$^+$ T cells are found infiltrating the pancreatic islets at the time of diagnosis of type 1 diabetes (31). They are also found in post mortem samples of people who have died having had recently diagnosed type 1 diabetes, and are the most abundant infiltrating immune cell (32,33). The CD8$^+$ T cells within the pancreas can be shown to be specifically responsive to insulin and other islet

autoantigens (34). Along with this can be seen upregulation of MHC class I on the islets of Langerhans (35), and this is not observed in the islets that are not infiltrated, and also not found in non-diabetic controls (36). The very presence of these $CD8^+$ T cells does not necessarily imply that they are initiators, but certainly they are the most abundant cell to be found within the islets, in the studies focused on pancreatic cell infiltration.

How would the $CD8^+$ T cells become activated to initiate damage the beta cells? For many years, there has been debate about whether molecular mimicry is a mechanism for activation of autoreactive T cells in diabetes, with a number of viral epitopes proposed to be potential initiators. Recently, gut bacterial peptides have been shown to be able to activate $CD8^+$ T cells in NOD mice. This was exemplified by a peptide from fusobacteria found in the gut, able to stimulate islet-specific glucose-6-phosphatase related protein (IGRP)-reactive CD8 T cells (37). Similarly, a human proinsulin-specific clone can recognise a peptide of C. aspargiforme (38). Thus, there is the means to activate these pathogenic $CD8^+$ T cells, without invoking an infection. Furthermore, there is also the possibility of a viral infection in the pancreas, with some enteroviruses specifically able to target islet beta cells (39) (see section below). As $CD8^+$ T cells are important effectors that are central to the immune response to viral infection, their collateral effects in dealing with a viral infection within the pancreas could be particularly important, in both initiation and progression of islet cell damage.

So, are $CD8^+$ T cells involved in damage to the islets? Undoubtedly, islet-reactive T cells can kill islet beta cells. The initial discovery that proinsulin was an important antigen for $CD8^+$ T cells in the NOD mouse model showed very clearly that insulin-reactive $CD8^+$ T cells can not only kill islet beta cells in vitro but also in vivo, leading directly to the onset of diabetes and clear histopathological evidence that the islets had been destroyed (41,42). These findings were followed by the cloning of a proinsulin-specific $CD8^+$ T cell clone from an individual who had type 1 diabetes, which was shown to have the capacity to kill islet cells in vitro (43). Transplantation studies by Sutherland and colleagues have given very strong evidence that $CD8^+$ T cells are able to damage and destroy islets in vivo in humans (40).

Pancreatic transplantation was carried out in identical twins discordant for type 1 diabetes, and when the non-diabetic twin donated a portion of pancreas to the diabetic twin, unfortunately, type 1 diabetes recurred rapidly, and histology showed a predominance of $CD8^+$ T cells. $^+$It is interesting that more recently, it has been noted that alterations in frequency of a CD57+ subset of memory CD8 T cells correlates with changes in c-peptide levels after diagnosis of type 1 diabetes (44).

Finally, if CD8 T cells are the Achilles heel, could they be targeted to prevent or treat type 1 diabetes? Whilst there have not been any immunotherapies that have had a lasting effect on reducing beta cell loss, as measured by decrease of c-peptide, recently, a number of strategies have slowed the initial rate of loss of beta cell function. In a study where the anti-CD2 monoclonal antibody Alefacept was given in two 12-week courses over 36 weeks – the treated individuals exhibited a delay in C-peptide loss (45,46). Interestingly, there was a reduction in the $CD8^+$ central memory cells, correlated with this delay in c-peptide decline. An alternative therapeutic manoeuvre involved administration of plasmid-encoded proinsulin, and a short duration of c-peptide preservation was associated with reduction in proinsulin-specific CD8 T cells (47). Although these are correlative observations, if $CD8^{++}$ T cells could be directly targeted, this might in the future, be an important avenue to pursue.

In conclusion, we could consider that the major vulnerability in type 1 diabetes is the beta cell itself, as the target organ that becomes damaged, and requires protection. $CD8^+$ cytotoxic T cells will attack cells, which they specifically recognise and that display signals, such as increased MHC class I. Other processes that can lead to an increase in this vulnerability include beta cell stress, and viral infection and these increase the visibility to the $CD8^+$ T cells, which if unchecked will damage the beta cells. Thus, whilst a major strength in terms of the vitally important protection given by $CD8^+$ T cells fighting off infectious agents, and tumours, this strength could be considered the Achilles heel in type 1 diabetes, where normal function of $CD8^+$ T cells is deleterious, and should be targeted for control.

**Regulatory T cells (Megan Levings)**

According to Wikipedia, an Achilles heel is "a weakness in spite of overall strength, which can lead to downfall." T1D is an immunologically complex disease mediated by a coordinated network of innate and adaptive immune cells. I argue that at the top of this network of immune cells is the regulatory T cell (Treg): a cell type which possesses strong immunosuppressive function yet has several points of weakness which can lead to their functional demise. A key concept is that there are likely many origins of Treg weakness in T1D. Their loss of function could be the consequence of genetics, environment, intrinsic dysfunction, and/or changes in the susceptibility of effector cells to suppression (48). Although multiple roads can lead to the downfall of Tregs, the common outcome is the failure to keep the autoreactive immune response at bay, thus unleashing the destructive power of islet-cell reactive effector T cells, which we all have in circulation (49).

When one gives a talk or writes a review about Tregs, it is common to use analogies such as "conductors" or "police of the immune system", "peacekeepers", or "firefighters". These comparisons are meant to convey the message that this single cell type is at the top of a cellular hierarchy, with the power to orchestrate and control many aspects of immune function. It important to consider that although the best-known effect of Tregs is control over other T cells, through their broad, typically cell-type agnostic, immunosuppressive mechanisms they control many different types of immune responses. For example, beyond conventional T cells, they control B cells (50,51), NK cells (52), $\gamma\delta$ T cells (53), antigen presenting cells (54) and even neutrophils (55)! Essentially, all the other cell types that my learned co-authors have argued for. As long as Tregs are functioning properly, these other immune cells don't have a chance. Moreover, a recent development is that Tregs not only exert control over immune

cells, but also of islets themselves, with an emerging literature - albeit almost exclusively in mice so far - describing the important role of Tregs in promoting beta cell survival and regeneration (56).

Perhaps the strongest evidence that Tregs are *the* Achilles heel in T1D comes from the study of IPEX: the X-linked monogenic immunodeficiency arising from mutations in FOXP3, the key Treg lineage-defining transcription factor (57). Children affected by IPEX have a variety of conditions, but a unifying feature is T1D, which manifests in the majority of patients, often at birth. Imaging and autopsy studies in IPEX reveal destruction of the pancreas and intense lymphocytic infiltrates in many tissues, highlighting the strength of the autoreactive response in the absence of Tregs (58,59). Moreover, other rare monogenic mutations which affect Treg function, for example in *CD25*, *CTLA4,* can also cause T1D, lending more support to the argument that without these cells, islet-directed immunity is unleashed (60).

But beyond the rare monogeic causes of T1D, it is also important to consider the prevalence of genetic effects on Treg function in the common polygenic form of the disease. Classical GWAS studies repeatedly uncover genetic risk factors associated with a variety of Treg relevant genes (61,62). For example, genes with high odds ratios include *CD25*, *PTPN2*, *PTPN22*, *CTLA4*, and *IL2*, all genes which can affect Treg function (63,64).

It has been challenging to pinpoint exactly how Tregs are dysfunctional in T1D. In addition to the fact that multiple roads can lead to dysfunction, seeking the answer to this question in humans has been difficult due to the limitations of studying peripheral blood, which may not capture relevant antigen specific cells, the lack of a standardized, reproducible and antigen-specific suppression assay, and the fact that the commonly used in vitro suppression may not even measure function which is relevant in vivo. Because of the difficulty in accurately

quantifying Tregs by flow cytometry, over the years there have been a myriad of reports of Tregs being higher or lower, and functional or dysfunctional in T1D (48). There are also reports of Treg "instability", manifested as increased pro-inflammatory cytokine production (65,66). A consistent finding is their relative unresponsiveness to IL-2 (67,68) and we have shown diminished production of chemokines that are important for attracting their targets of suppression (69). We also studied Tregs using a gene signature-based approach, initially defining a core Treg transcriptome, then applying this signature to unfractionated cells in blood. These data showed that Tregs from both children and adults with T1D have changes in their core transcriptional signature, and that monitoring this signature can be used to track changes in Tregs over time in the context of clinical trials (70,71).

It is also noteworthy that both academia and industry are clearly convinced that Tregs represent an Achilles heel, on the basis of the large number of clinical studies specifically aimed at targeting these cells. Examples of Treg targeted therapies include those that aim to directly manipulate the IL-2/IL2R pathway, or to indirectly boost their numbers or activity using approaches such as tolerogenic DCs or cytokine modulation to create an environment favorable for Treg function. It can be anticipated that the polyclonal Treg cell therapy studies which provided important safety data (72), will soon advance to testing of antigen-specific Tregs (73). Close to 20 years since the power of this approach was first demonstrated in NOD mice (74,75), perhaps there will finally be a way to fix the Achilles heel of T1D.

**The virus infected beta cell (Sarah Richardson).**

In the 1920s, Gunderson originally proposed that diabetes may be ‘of infectious origin? (76). Since then many studies have provided additional support for the role of viruses, particularly enteroviruses (EV), the focus of this review, in the development of islet autoimmunity and progression to Type 1 diabetes (77–80). As alluded to earlier, for a cell or a pathological agent to be the Achilles Heel of T1D, they need to contribute to the following: 1. *Disease initiation*; 2. *Disease progression* and 3. *Beta cell damage.* However, in the case for EVs, I propose that we think of the ’beta cell’ itself as the Achilles Heel, and then, the enterovirus as the arrow that targets it. I will also re-order the contributions to: 1. *Beta cell damage*, followed by 2. *Disease initiation* and finally, 3. *Disease progression*, for reasons that will soon become clear.

1. *Beta cell targeting and damage by enteroviruses*

Evidence of beta cell damage by select EV serotypes (Coxsackie B (CVB) and echoviruses) has been demonstrated both *in vivo* and *in* vitro (reviewed in (39)). The examination of pancreata from children who died following an acute CVB infection shows selective targeting of the islets (81–84). Furthermore, isolated human islets are highly susceptible to infection *in vitro* (41,77,85), particularly with EVs associated with the development of islet autoimmunity, and T1D in epidemiological studies. These viruses have tropism for beta cells above other pancreatic endocrine cells (85–87), and the recent finding that human beta cells express a specific isoform of the Coxsackie and Adenovirus Receptor (CAR), could explain why they are selectively targeted (86). This isoform was unexpectedly localised to the insulin granule membrane and therefore, could, under situations where the

host has viremia (virus in the blood), facilitate infection of the beta cells during the process of insulin secretion.

Importantly, a large proportion of genes associated with genetic susceptibility to T1D are expressed in beta cells (85,88,89) and Ingenuity Pathway Analysis (IPA) of these demonstrates that the “top hit” pathways involve cellular sensing of infections and responses to interferons (IFNs) (90). Beta cells are known to be highly responsive to interferons (91), which are rapidly induced and distributed systemically in response to a viral infection. The ability of the host to rapidly produce an immune response against the virus, and the capacity of the beta cells themselves to control the infection will determine the extent and level of damage in a given individual. Crucially, several of the T1D associated single nucleotide polymorphisms (SNPs) are associated with differential responses to both viral infection and IFN stimulation (7,92). It is not inconceivable, therefore, that Achilles ‘the beta cell’ carrying these risk SNPs would have altered susceptibility to infection, and/ or an aberrant immunological response following infection, which could impact on both the degree of initial damage and his ability to resolve it.

*2. Disease initiation*

As with any viral infection, recruitment of innate immune cells to the affected site facilitates the presentation of the antigens released from damaged cells (in this case derived from the beta cells) to the adaptive immune system. In the context of T1D, individuals at risk of developing disease, have an increased propensity to recognise self-derived antigens and have deficiencies in regulating responses against these self-antigens (covered elsewhere in this review). The toxic combination of a viral infection, which causes beta cell damage and specific presentation of beta cell antigens to an immune system primed to recognise self-antigen,

without appropriate regulation, could promote *disease initiation* in these genetically susceptible individuals.

In support of this hypothesis, epidemiological studies have demonstrated an association between enteroviral infections and the appearance of islet autoantibodies (evidence of an adaptive host response against the beta cell antigens). A meta-analysis in 2011, found that individuals with islet autoimmunity were over 3.7-times more likely to have evidence of an enterovirus infection (79). It is also worth considering the evidence from studies in Cuba, where an enterovirus epidemic led to the development of islet autoantibodies in a significant number of individuals (93). The circulating enterovirus isolated from affected individuals was shown to infect and impact upon beta cell function *in vitro* (93,94). However, the majority of these individuals did not develop T1D. What this study therefore demonstrates is that enteroviruses can infect beta cells, they can cause sufficient damage to induce an adaptive response directed against beta cells, but this alone is not sufficient to initiate the development of T1D. Progression to T1D needs a breakdown in immune tolerance (in the case of Achilles – 'friendly fire') and potentially also a 'smouldering fire' (chronic infection) to facilitate the continued recruitment of immune cells to the islets.

*3. Disease Progression*

The evidence for the role of enteroviruses in beta cell damage and disease initiation is compelling, but could they also contribute to progression? There is circumstantial evidence that they can. Enteroviral infections have been associated with accelerated progression from islet autoantibody positivity to clinical onset of diabetes in many studies around the world (reviewed in (78,80)). A meta-analysis of these demonstrated that individuals at clinical onset of T1D are nearly 10-times more likely to exhibit evidence of an enteroviral infection compared

to controls (79). However, the emerging evidence at onset does not support the presence of an acute infection, rather studies of the blood and pancreas from individuals with T1D, suggest the presence of a low level, chronic infection (reviewed in (39,78,95)).

Why might this be important? Studies in the pancreas of individuals with T1D demonstrate that islets, which still contain residual beta cells (even several years after diagnosis), have clear evidence of aberrant IFN and anti-viral responses (36,96,97); these findings correlate with evidence of low levels of infection as assessed by the presence of viral protein and RNA (82,96–99). One key hallmark feature in the pancreas of T1D donors is the dramatic hyperexpression of HLA-class I (36,100), which can facilitate the recognition and targeting of beta cells by infiltrating, potentially self-reactive, CD8+ T cells. A low level, chronic – smouldering infection within the pancreatic beta cells could be sufficient to maintain an environment that facilitates the recruitment of self-reactive immune cells over a protracted period, ultimately creating a progressively destructive process in the islets – eventually leading to clinical diagnosis.

So, was Achilles' Heel (the beta cell), first damaged by an infection that did not resolve; which when combined with the presence of islet-reactive immune cells and a breakdown in immune tolerance, resulted in a festering wound that brought about his demise? The answer: maybe. How do we know? Most of the studies described here report an association of infection with the clinical biomarkers accompanying the development of T1D, importantly they do not demonstrate causality. The only way to definitively prove that enteroviruses contribute to the initiation and progression of the disease is to prevent the infection in the first place and assess the impact of this on disease development. Effectively, we want to give Achilles 'a pair of boots' to protect his heels (Figure 3), which is far easier said than done. Encouragingly though,

efforts are now underway (39,77,101) to generate an anti-enteroviral vaccine, which targets multiple CVB serotypes with first-in-man trials scheduled for Spring 2020. The intention is to immunise young individuals who are genetically at-risk of developing T1D and follow them to assess the impact on the development of islet autoantibodies and onset of clinical disease. Another approach, currently being trialled at the University of Oslo, involves the use of the anti-viral drugs, ribavirin and pleconaril, which are given to individuals at the onset of disease to promote clearance of chronic infection with the hope that this could help preserve residual beta cell mass. The field eagerly awaits the outcome of these efforts and Achilles 'the beta cell' looks forward to his new boots!

## Conclusion

We will leave the reader to judge whether, indeed, there is a single "Achilles Heel" in type 1 diabetes. At the conference, In the spirit of debate, voting was carried out and was won by Sarah Richardson, who deftly adjusted the proposition that the beta cells, infected by enteroviruses, are the Achilles heel in type 1 diabetes. In providing the arguments in support of the individual component/cell/process that is the Achilles heel in type 1 diabetes, all the debate participants agreed that this is not a single entity, with the beta cell representing a weakness that is targeted for death, and a number of other potential contributors to this process. The challenge remains as to how we use the evidence to progress the therapeutic targeting, with the aim of providing robust immunotherapeutic treatment for halting disease progression and future prevention.

1. Ivashkiv LB, Donlin LT. Regulation of type I interferon responses. Nat Rev Immunol [Internet]. 2014 Jan 23;14(1):36–49. Available from: http://www.nature.com/articles/nri3581

2. Zornitzki T. Interferon therapy in hepatitis C leading to chronic type 1 diabetes. World J Gastroenterol [Internet]. 2015;21(1):233. Available from: http://www.wjgnet.com/1007-9327/full/v21/i1/233.htm

3. Newby BN, Mathews CE. Type I Interferon Is a Catastrophic Feature of the Diabetic Islet Microenvironment. Front Endocrinol (Lausanne) [Internet]. 2017 Sep 14;8. Available from: http://journal.frontiersin.org/article/10.3389/fendo.2017.00232/full

4. Vecchio F, Lo Buono N, Stabilini A, Nigi L, Dufort MJ, Geyer S, et al. Abnormal neutrophil signature in the blood and pancreas of presymptomatic and symptomatic type 1 diabetes. JCI Insight [Internet]. 2018 Sep 20;3(18). Available from: https://insight.jci.org/articles/view/122146

5. Ferreira RC, Guo H, Coulson RMR, Smyth DJ, Pekalski ML, Burren OS, et al. A Type I Interferon Transcriptional Signature Precedes Autoimmunity in Children Genetically at Risk for Type 1 Diabetes. Diabetes [Internet]. 2014 Jul 1;63(7):2538–50. Available from: http://diabetes.diabetesjournals.org/cgi/doi/10.2337/db13-1777

6. Kallionpaa H, Elo LL, Laajala E, Mykkanen J, Ricano-Ponce I, Vaarma M, et al. Innate Immune Activity Is Detected Prior to Seroconversion in Children With HLA-Conferred Type 1 Diabetes Susceptibility. Diabetes [Internet]. 2014 Jul 1;63(7):2402–14. Available from: http://diabetes.diabetesjournals.org/cgi/doi/10.2337/db13-1775

7. Domsgen E, Lind K, Kong L, Hühn MH, Rasool O, van Kuppeveld F, et al. An IFIH1 gene polymorphism associated with risk for autoimmunity regulates canonical antiviral defence pathways in Coxsackievirus infected human pancreatic islets. Sci Rep [Internet]. 2016 Dec 21;6(1):39378. Available from: http://www.nature.com/articles/srep39378

8. Meyer S, Woodward M, Hertel C, Vlaicu P, Haque Y, Kärner J, et al. AIRE-Deficient Patients Harbor Unique High-Affinity Disease-Ameliorating Autoantibodies. Cell [Internet]. 2016 Jul;166(3):582–95. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0092867416307929

9. Fillatreau S. B cells and their cytokine activities implications in human diseases. Clin Immunol [Internet]. 2018 Jan;186:26–31. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1521661617305326

10. Smith MJ, Simmons KM, Cambier JC. B cells in type 1 diabetes mellitus and diabetic kidney disease. Nat Rev Nephrol [Internet]. 2017 Nov 17;13(11):712–20. Available from: http://www.nature.com/articles/nrneph.2017.138

11. Akashi T. Direct evidence for the contribution of B cells to the progression of insulitis and the development of diabetes in non-obese diabetic mice. Int Immunol [Internet]. 1997 Aug 1;9(8):1159–64. Available from: https://academic.oup.com/intimm/article-lookup/doi/10.1093/intimm/9.8.1159

12. Xiu Y, Wong CP, Bouaziz J-D, Hamaguchi Y, Wang Y, Pop SM, et al. B Lymphocyte Depletion by CD20 Monoclonal Antibody Prevents Diabetes in Nonobese Diabetic Mice despite Isotype-Specific Differences in FcγR Effector Functions. J Immunol [Internet]. 2008 Mar 1;180(5):2863–75. Available from: http://www.jimmunol.org/lookup/doi/10.4049/jimmunol.180.5.2863

13. Hu C, Rodriguez-Pinto D, Du W, Ahuja A, Henegariu O, Wong FS, et al. Treatment with CD20-specific antibody prevents and reverses autoimmune diabetes in mice. J Clin Invest [Internet]. 2007 Dec 3;117(12):3857–67. Available from: http://content.the-jci.org/articles/view/32405

14. Noorchashm H, Lieu YK, Noorchashm N, Rostami SY, Greeley SA, Schlachterman A, et al. I-Ag7-mediated antigen presentation by B lymphocytes is critical in overcoming a checkpoint in T cell tolerance to islet beta cells of nonobese diabetic mice. J Immunol [Internet]. 1999 Jul 15;163(2):743–50. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10395666

15. Marino E, Tan B, Binge L, Mackay CR, Grey ST. B-Cell Cross-Presentation of Autologous Antigen Precipitates Diabetes. Diabetes [Internet]. 2012 Nov 1;61(11):2893–905. Available from: http://diabetes.diabetesjournals.org/cgi/doi/10.2337/db12-0006

16. Ziegler AG, Rewers M, Simell O, Simell T, Lempainen J, Steck A, et al. Seroconversion to Multiple Islet Autoantibodies and Risk of Progression to Diabetes in Children. JAMA [Internet]. 2013 Jun 19;309(23):2473. Available from: http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.2013.6285

17. Habib T, Long SA, Samuels PL, Brahmandam A, Tatum M, Funk A, et al. Dynamic Immune Phenotypes of B and T Helper Cells Mark Distinct Stages of T1D Progression. Diabetes [Internet]. 2019 Jun;68(6):1240–50. Available from: http://diabetes.diabetesjournals.org/lookup/doi/10.2337/db18-1081

18. Smith MJ, Rihanek M, Wasserfall C, Mathews CE, Atkinson MA, Gottlieb PA, et al. Loss of B-Cell Anergy in Type 1 Diabetes Is Associated With High-Risk HLA and Non-HLA Disease Susceptibility Alleles. Diabetes [Internet]. 2018 Apr;67(4):697–703. Available from: http://diabetes.diabetesjournals.org/lookup/doi/10.2337/db17-0937

19. Pescovitz MD, Greenbaum CJ, Krause-Steinrauf H, Becker DJ, Gitelman SE, Goland R, et al. Rituximab, B-Lymphocyte Depletion, and Preservation of Beta-Cell Function. N Engl J Med [Internet]. 2009 Nov 26;361(22):2143–52. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMoa0904452

20. Dufort MJ, Greenbaum CJ, Speake C, Linsley PS. Cell type–specific immune phenotypes predict loss of insulin secretion in new-onset type 1 diabetes. JCI Insight [Internet]. 2019 Feb 21;4(4). Available from: https://insight.jci.org/articles/view/125556

21. Leete P, Willcox A, Krogvold L, Dahl-Jørgensen K, Foulis AK, Richardson SJ, et al. Differential Insulitic Profiles Determine the Extent of β-Cell Destruction and the Age at Onset of Type 1 Diabetes. Diabetes [Internet]. 2016 May;65(5):1362–9. Available from: http://diabetes.diabetesjournals.org/lookup/doi/10.2337/db15-1615

22. Willcox A, Richardson SJ, Walker LSK, Kent SC, Morgan NG, Gillespie KM. Germinal centre frequency is decreased in pancreatic lymph nodes from individuals with recent-onset type 1 diabetes. Diabetologia [Internet]. 2017 Jul 17;60(7):1294–303. Available from: http://link.springer.com/10.1007/s00125-017-4221-7

23. Brodie GM, Wallberg M, Santamaria P, Wong FS, Green EA. B-Cells Promote Intra-Islet CD8+ Cytotoxic T-Cell Survival to Enhance Type 1 Diabetes. Diabetes [Internet]. 2008 Apr 1;57(4):909–17. Available from: http://diabetes.diabetesjournals.org/cgi/doi/10.2337/db07-1256

24. Chamberlain N, Massad C, Oe T, Cantaert T, Herold KC, Meffre E. Rituximab does not

reset defective early B cell tolerance checkpoints. J Clin Invest [Internet]. 2015 Dec 7;126(1):282–7. Available from: https://www.jci.org/articles/view/83840

25. Linsley PS, Greenbaum CJ, Speake C, Long SA, Dufort MJ. B lymphocyte alterations accompany abatacept resistance in new-onset type 1 diabetes. JCI Insight [Internet]. 2019 Feb 21;4(4). Available from: https://insight.jci.org/articles/view/126136

26. Hamad ARA, Ahmed R, Donner T, Fousteri G. B cell-targeted immunotherapy for type 1 diabetes: What can make it work? Discov Med [Internet]. 2016;21(115):213–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27115172

27. Pearson JA, Wong FS, Wen L. The importance of the Non Obese Diabetic (NOD) mouse model in autoimmune diabetes. Journal of Autoimmunity. 2016.

28. Wooldridge L, Ekeruche-Makinde J, van den Berg HA, Skowera A, Miles JJ, Tan MP, et al. A Single Autoimmune T Cell Receptor Recognizes More Than a Million Different Peptides. J Biol Chem [Internet]. 2012 Jan 6;287(2):1168–77. Available from: http://www.jbc.org/lookup/doi/10.1074/jbc.M111.289488

29. Wicker LS, Leiter EH, Todd JA, Renjilian RJ, Peterson E, Fischer PA, et al. Beta 2-microglobulin-deficient NOD mice do not develop insulitis or diabetes. Diabetes [Internet]. 1994 Mar 1;43(3):500–4. Available from: http://diabetes.diabetesjournals.org/cgi/doi/10.2337/diabetes.43.3.500

30. Serreze D V., Leiter EH, Christianson GJ, Greiner D, Roopenian DC. Major histocompatibility complex class I-deficient NOD-B2mnull mice are diabetes and insulitis resistant. Diabetes [Internet]. 1994 Mar 1;43(3):505–9. Available from: http://diabetes.diabetesjournals.org/cgi/doi/10.2337/diabetes.43.3.505

31. Itoh N, Hanafusa T, Miyazaki A, Miyagawa J, Yamagata K, Yamamoto K, et al. Mononuclear cell infiltration and its relation to the expression of major histocompatibility complex antigens and adhesion molecules in pancreas biopsy specimens from newly diagnosed insulin-dependent diabetes mellitus patients. J Clin Invest [Internet]. 1993 Nov 1;92(5):2313–22. Available from: http://www.jci.org/articles/view/116835

32. Willcox A, Richardson SJ, Bone AJ, Foulis AK, Morgan NG. Analysis of islet inflammation in human type 1 diabetes. Clin Exp Immunol [Internet]. 2009 Feb;155(2):173–81. Available from: http://doi.wiley.com/10.1111/j.1365-2249.2008.03860.x

33. Foulis AK, Liddle CN, Farquharson MA, Richmond JA, Weir RS. The histopathology of the pancreas in Type I (insulin-dependent) diabetes mellitus: a 25-year review of deaths in patients under 20 years of age in the United Kingdom. Diabetologia [Internet]. 1986 May;29(5):267–74. Available from: http://link.springer.com/10.1007/BF00452061

34. Coppieters KT, Dotta F, Amirian N, Campbell PD, Kay TWH, Atkinson MA, et al. Demonstration of islet-autoreactive CD8 T cells in insulitic lesions from recent onset and long-term type 1 diabetes patients. J Exp Med [Internet]. 2012 Jan 16;209(1):51–60. Available from: https://rupress.org/jem/article/209/1/51/40983/Demonstration-of-isletautoreactive-CD8-T-cells-in

35. Foulis A, Farquharson M, Meager A. IMMUNOREACTIVE α-INTERFERON IN INSULIN-SECRETING β CELLS IN TYPE 1 DIABETES MELLITUS. Lancet [Internet]. 1987 Dec;330(8573):1423–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0140673687911287

36. Richardson SJ, Rodriguez-Calvo T, Gerling IC, Mathews CE, Kaddis JS, Russell MA, et al. Islet cell hyperexpression of HLA class I antigens: a defining feature in type 1 diabetes. Diabetologia [Internet]. 2016 Nov 9;59(11):2448–58. Available from: http://link.springer.com/10.1007/s00125-016-4067-4

37. Tai N, Peng J, Liu F, Gulden E, Hu Y, Zhang X, et al. Microbial antigen mimics activate diabetogenic CD8 T cells in NOD mice. J Exp Med [Internet]. 2016 Sep 19;213(10):2129–46. Available from: https://rupress.org/jem/article/213/10/2129/42010/Microbial-antigen-mimics-activate-diabetogenic-CD8

38. Cole DK, Bulek AM, Dolton G, Schauenberg AJ, Szomolay B, Rittase W, et al. Hotspot autoimmune T cell receptor binding underlies pathogen and insulin peptide cross-reactivity. J Clin Invest [Internet]. 2016 May 16;126(6):2191–204. Available from: https://www.jci.org/articles/view/85679

39. Richardson SJ, Morgan NG. Enteroviral infections in the pathogenesis of type 1 diabetes: new insights for therapeutic intervention. Curr Opin Pharmacol [Internet]. 2018 Dec;43:11–9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1471489217302205

40. Sutherland DE, Sibley R, Xu XZ, Michael A, Srikanta AM, Taub F, et al. Twin-to-twin pancreas transplantation: reversal and reenactment of the pathogenesis of type I diabetes. Trans Assoc Am Physicians. 1984;97:80–7.

41. Wong FS, Visintin I, Wen L, Flavell RA, Janeway CA. CD8 T cell clones from young nonobese diabetic (NOD) islets can transfer rapid onset of diabetes in NOD mice in the absence of CD4 cells. J Exp Med [Internet]. 1996 Jan 1;183(1):67–76. Available from: https://rupress.org/jem/article/183/1/67/58343/CD8-T-cell-clones-from-young-nonobese-diabetic-NOD

42. Wong FS, Karttunen J, Dumont C, Wen L, Visintin I, Pilip IM, et al. Identification of an MHC class I-restricted autoantigen in type 1 diabetes by screening an organ-specific cDNA library. Nat Med [Internet]. 1999 Sep;5(9):1026–31. Available from: http://www.nature.com/articles/nm0999_1026

43. Skowera A, Ellis RJ, Varela-Calviño R, Arif S, Huang GC, Van-Krinks C, et al. CTLs are targeted to kill β cells in patients with type 1 diabetes through recognition of a glucose-regulated preproinsulin epitope. J Clin Invest. 2008 Oct 1;118(10):3390–402.

44. Yeo L, Woodwyk A, Sood S, Lorenc A, Eichmann M, Pujol-Autonell I, et al. Autoreactive T effector memory differentiation mirrors β cell function in type 1 diabetes. J Clin Invest [Internet]. 2018 Aug 1;128(8):3460–74. Available from: https://www.jci.org/articles/view/120555

45. Rigby MR, DiMeglio LA, Rendell MS, Felner EI, Dostou JM, Gitelman SE, 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 [Internet]. 2013 Dec;1(4):284–94. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2213858713701116

46. Rigby MR, Harris KM, Pinckney A, DiMeglio LA, Rendell MS, Felner EI, et al. Alefacept provides sustained clinical and immunological effects in new-onset type 1 diabetes patients. J Clin Invest [Internet]. 2015 Aug 3;125(8):3285–96. Available from: http://www.jci.org/articles/view/81722

47. Roep BO, Solvason N, Gottlieb PA, Abreu JRF, Harrison LC, Eisenbarth GS, et al. Plasmid-Encoded Proinsulin Preserves C-Peptide While Specifically Reducing Proinsulin-Specific CD8+ T Cells in Type 1 Diabetes. Sci Transl Med [Internet]. 2013 Jun 26;5(191):191ra82-191ra82. Available from: https://stm.sciencemag.org/lookup/doi/10.1126/scitranslmed.3006103

48. Hull CM, Peakman M, Tree TIM. Regulatory T cell dysfunction in type 1 diabetes: what's broken and how can we fix it? Vol. 60, Diabetologia. Springer Verlag; 2017. p. 1839–50.

49. Culina S, Lalanne AI, Afonso G, Cerosaletti K, Pinto S, Sebastiani G, et al. Islet-reactive CD8 + T cell frequencies in the pancreas, but not in blood, distinguish type 1 diabetic patients from healthy donors. Sci Immunol [Internet]. 2018 Feb 2;3(20):eaao4013. Available from: https://immunology.sciencemag.org/lookup/doi/10.1126/sciimmunol.aao4013

50. Fonseca VR, Ribeiro F, Graca L. T follicular regulatory (Tfr) cells: Dissecting the complexity of Tfr-cell compartments. Immunol Rev [Internet]. 2019 Mar 15;288(1):112–27. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/imr.12739

51. Weingartner E, Golding A. Direct control of B cells by Tregs: An opportunity for long-term modulation of the humoral response. Cell Immunol [Internet]. 2017 Aug;318:8–16. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0008874917300813

52. Pedroza-Pacheco I, Madrigal A, Saudemont A. Interaction between natural killer cells and regulatory T cells: perspectives for immunotherapy. Cell Mol Immunol [Internet]. 2013 May 25;10(3):222–9. Available from: http://www.nature.com/articles/cmi20132

53. Okeke EB, Uzonna JE. The Pivotal Role of Regulatory T Cells in the Regulation of Innate Immune Cells. Front Immunol [Internet]. 2019 Apr 9;10. Available from: https://www.frontiersin.org/article/10.3389/fimmu.2019.00680/full

54. Josefowicz SZ, Lu L-F, Rudensky AY. Regulatory T Cells: Mechanisms of Differentiation and Function. Annu Rev Immunol [Internet]. 2012 Apr 23;30(1):531–64. Available from: http://www.annualreviews.org/doi/10.1146/annurev.immunol.25.022106.141623

55. Himmel ME, Crome SQ, Ivison S, Piccirillo C, Steiner TS, Levings MK. Human CD4+FOXP3+ regulatory T cells produce CXCL8 and recruit neutrophils. Eur J Immunol [Internet]. 2011 Feb;41(2):306–12. Available from: http://doi.wiley.com/10.1002/eji.201040459

56. Dirice E, Kahraman S, De Jesus DF, El Ouaamari A, Basile G, Baker RL, et al. Increased β-cell proliferation before immune cell invasion prevents progression of type 1 diabetes. Nat Metab [Internet]. 2019 May 6;1(5):509–18. Available from: http://www.nature.com/articles/s42255-019-0061-8

57. Bacchetta R, Barzaghi F, Roncarolo M-G. From IPEX syndrome to FOXP3 mutation: a lesson on immune dysregulation. Ann N Y Acad Sci [Internet]. 2018 Apr;1417(1):5–22. Available from: http://doi.wiley.com/10.1111/nyas.13011

58. Costa-Carvalho BT, de Moraes-Pinto MI, de Almeida LC, de Seixas Alves MT, Maia RP, de Souza RL, et al. A Remarkable Depletion of Both Naïve CD4+ and CD8+ with High Proportion of Memory T Cells in an IPEX Infant with a FOXP3 Mutation in the Forkhead Domain. Scand J Immunol [Internet]. 2008 Jul;68(1):85–91. Available from: http://doi.wiley.com/10.1111/j.1365-3083.2008.02055.x

59. Rubio-Cabezas O, Minton JAL, Caswell R, Shield JP, Deiss D, Sumnik Z, et al. Clinical Heterogeneity in Patients With FOXP3 Mutations Presenting With Permanent Neonatal Diabetes. Diabetes Care [Internet]. 2009 Jan 1;32(1):111–6. Available from: http://care.diabetesjournals.org/cgi/doi/10.2337/dc08-1188

60. Cepika A-M, Sato Y, Liu JM-H, Uyeda MJ, Bacchetta R, Roncarolo MG. Tregopathies: Monogenic diseases resulting in regulatory T-cell deficiency. J Allergy Clin Immunol [Internet]. 2018 Dec;142(6):1679–95. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0091674918315173

61. Nyaga DM, Vickers MH, Jefferies C, Perry JK, O'Sullivan JM. The genetic architecture of type 1 diabetes mellitus. Mol Cell Endocrinol [Internet]. 2018 Dec;477:70–80. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0303720718301886

62. Pociot F, Akolkar B, Concannon P, Erlich HA, Julier C, Morahan G, et al. Genetics of Type 1 Diabetes: What's Next? Diabetes [Internet]. 2010 Jul 1;59(7):1561–71. Available from: http://diabetes.diabetesjournals.org/cgi/doi/10.2337/db10-0076

63. Long A, Buckner JH. Intersection between genetic polymorphisms and immune deviation in type 1 diabetes. Curr Opin Endocrinol Diabetes Obes [Internet]. 2013 Aug;20(4):285–91. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=01266029-201308000-00006

64. Valta M, Gazali AM, Viisanen T, Ihantola EL, Ekman I, Toppari J, et al. Type 1 diabetes linked PTPN22 gene polymorphism is associated with the frequency of circulating regulatory T cells. Eur J Immunol. 2019;

65. Marwaha AK, Crome SQ, Panagiotopoulos C, Berg KB, Qin H, Ouyang Q, et al. Cutting edge: Increased IL-17-secreting T cells in children with new-onset type 1 diabetes. J Immunol [Internet]. 2010/09/03. 2010;185(7):3814–8. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=20810982

66. McClymont SA, Putnam AL, Lee MR, Esensten JH, Liu W, Hulme MA, et al. Plasticity of human regulatory T cells in healthy subjects and patients with type 1 diabetes. J Immunol [Internet]. 2011/03/04. 2011;186(7):3918–26. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21368230

67. Long SA, Cerosaletti K, Bollyky PL, Tatum M, Shilling H, Zhang S, et al. Defects in IL-2R signaling contribute to diminished maintenance of FOXP3 expression in CD4(+)CD25(+) regulatory T-cells of type 1 diabetic subjects. Diabetes [Internet]. 2009/10/31. 2010;59(2):407–15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19875613

68. Cerosaletti K, Schneider A, Schwedhelm K, Frank I, Tatum M, Wei S, et al. Multiple Autoimmune-Associated Variants Confer Decreased IL-2R Signaling in CD4+CD25hi T Cells of Type 1 Diabetic and Multiple Sclerosis Patients. Jacobson S, editor. PLoS One [Internet]. 2013 Dec 23;8(12):e83811. Available from: https://dx.plos.org/10.1371/journal.pone.0083811

69. Patterson SJ, Pesenacker AM, Wang AY, Gillies J, Mojibian M, Morishita K, et al. T regulatory cell chemokine production mediates pathogenic T cell attraction and suppression. J Clin Invest [Internet]. 2016 Feb 8;126(3):1039–51. Available from: https://www.jci.org/articles/view/83987

70. Pesenacker AM, Chen V, Gillies J, Speake C, Marwaha AK, Sun AC, et al. Treg gene signatures predict and measure type 1 diabetes trajectory. JCI Insight [Internet]. 2019 Feb 7; Available from: http://insight.jci.org/articles/view/123879

71. Pesenacker AM, Wang AY, Singh A, Gillies J, Kim Y, Piccirillo CA, et al. A Regulatory T-Cell Gene Signature Is a Specific and Sensitive Biomarker to Identify Children With New-Onset Type 1 Diabetes. Diabetes [Internet]. 2016 Apr;65(4):1031–9. Available from: http://diabetes.diabetesjournals.org/lookup/doi/10.2337/db15-0572

72. Bluestone JA, Buckner JH, Fitch M, Gitelman SE, Gupta S, Hellerstein MK, et al. Type 1 diabetes immunotherapy using polyclonal regulatory T cells. Sci Transl Med [Internet]. 2015 Nov 25;7(315):315ra189-315ra189. Available from: https://stm.sciencemag.org/lookup/doi/10.1126/scitranslmed.aad4134

73. Ferreira LMR, Muller YD, Bluestone JA, Tang Q. Next-generation regulatory T cell therapy. Nat Rev Drug Discov [Internet]. 2019 Oct 20;18(10):749–69. Available from: http://www.nature.com/articles/s41573-019-0041-4

74. Tang Q, Henriksen KJ, Bi M, Finger EB, Szot G, Ye J, et al. In vitro-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. J Exp Med [Internet]. 2004;199(11):1455–65. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15184499

75. Tarbell K V, Yamazaki S, Olson K, Toy P, Steinman RM. CD25+ CD4+ T cells, expanded with dendritic cells presenting a single autoantigenic peptide, suppress autoimmune diabetes. J Exp Med [Internet]. 2004/06/09. 2004;199(11):1467–77. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15184500

76. Gundersen E. Is Diabetes of Infectious Origin? J Infect Dis [Internet]. 1927 Sep 1;41(3):197–202. Available from: https://academic.oup.com/jid/article-lookup/doi/10.1093/infdis/41.3.197

77. Dunne JL, Richardson SJ, Atkinson MA, Craig ME, Dahl-Jørgensen K, Flodström-Tullberg M, et al. Rationale for enteroviral vaccination and antiviral therapies in human type 1 diabetes. Diabetologia [Internet]. 2019 May 23;62(5):744–53. Available from: http://link.springer.com/10.1007/s00125-019-4811-7

78. Hyöty H. Viruses in type 1 diabetes. Pediatr Diabetes [Internet]. 2016 Jul;17:56–64. Available from: http://doi.wiley.com/10.1111/pedi.12370

79. Yeung W-CG, Rawlinson WD, Craig ME. Enterovirus infection and type 1 diabetes mellitus: systematic review and meta-analysis of observational molecular studies. BMJ [Internet]. 2011 Feb 3;342(feb03 1):d35–d35. Available from: http://www.bmj.com/cgi/doi/10.1136/bmj.d35

80. Craig ME, Nair S, Stein H, Rawlinson WD. Viruses and type 1 diabetes: A new look at an old story. Pediatric Diabetes. 2013.

81. Foulis AK, McGill M, Farquharson MA, Hilton DA. A search for evidence of viral infection in pancreases of newly diagnosed patients with IDDM. Diabetologia. 1997;

82. Richardson SJ, Willcox A, Bone AJ, Foulis AK, Morgan NG. The prevalence of enteroviral capsid protein vp1 immunostaining in pancreatic islets in human type 1 diabetes.

Diabetologia [Internet]. 2009 Jun 6;52(6):1143–51. Available from: http://link.springer.com/10.1007/s00125-009-1276-0

83. Ylipaasto P, Klingel K, Lindberg AM, Otonkoski T, Kandolf R, Hovi T, et al. Enterovirus infection in human pancreatic islet cells, islet tropism in vivo and receptor involvement in cultured islet beta cells. Diabetologia. 2004;

84. BENNETTJENSON A. PANCREATIC ISLET-CELL DAMAGE IN CHILDREN WITH FATAL VIRAL INFECTIONS. Lancet [Internet]. 1980 Aug;316(8190):354–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0140673680903499

85. M H, A L, O K, G F. Tropism Analysis of Two Coxsackie B5 Strains Reveals Virus Growth in Human Primary Pancreatic Islets but not in Exocrine Cell Clusters In Vitro. Open Virol J [Internet]. 2013 Apr 5;7(1):49–56. Available from: https://openvirologyjournal.com/VOLUME/7/PAGE/49/

86. Ifie E, Russell MA, Dhayal S, Leete P, Sebastiani G, Nigi L, et al. Unexpected subcellular distribution of a specific isoform of the Coxsackie and adenovirus receptor, CAR-SIV, in human pancreatic beta cells. Diabetologia [Internet]. 2018 Nov 3;61(11):2344–55. Available from: http://link.springer.com/10.1007/s00125-018-4704-1

87. Frisk G, Diderholm H. Tissue culture of isolated human pancreatic islets infected with different strains of Coxsackievirus B4: Assessment of virus replication and effects on islet morphology and insulin release. Exp Diabesity Res. 2000;

88. Bergholdt R, Brorsson C, Palleja A, Berchtold LA, Floyel T, Bang-Berthelsen CH, et al. Identification of Novel Type 1 Diabetes Candidate Genes by Integrating Genome-Wide Association Data, Protein-Protein Interactions, and Human Pancreatic Islet Gene Expression. Diabetes [Internet]. 2012 Apr 1;61(4):954–62. Available from: http://diabetes.diabetesjournals.org/cgi/doi/10.2337/db11-1263

89. Eizirik DL, Sammeth M, Bouckenooghe T, Bottu G, Sisino G, Igoillo-Esteve M, et al. The Human Pancreatic Islet Transcriptome: Expression of Candidate Genes for Type 1 Diabetes and the Impact of Pro-Inflammatory Cytokines. Gibson G, editor. PLoS Genet [Internet]. 2012 Mar 8;8(3):e1002552. Available from: https://dx.plos.org/10.1371/journal.pgen.1002552

90. Marroqui L, Dos Santos RS, Fløyel T, Grieco FA, Santin I, Op de beeck A, et al. TYK2 , a Candidate Gene for Type 1 Diabetes, Modulates Apoptosis and the Innate Immune Response in Human Pancreatic β-Cells. Diabetes [Internet]. 2015 Nov;64(11):3808–17. Available from: http://diabetes.diabetesjournals.org/lookup/doi/10.2337/db15-0362

91. Hultcrantz M, Hühn MH, Wolf M, Olsson A, Jacobson S, Williams BR, et al. Interferons induce an antiviral state in human pancreatic islet cells. Virology [Internet]. 2007 Oct;367(1):92–101. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0042682207003509

92. Cinek O, Tapia G, Witsø E, Kramna L, Holkova K, Rasmussen T, et al. Enterovirus RNA in Peripheral Blood May Be Associated with the Variants of rs1990760, a Common Type 1 Diabetes Associated Polymorphism in IFIH1. von Herrath MG, editor. PLoS One [Internet]. 2012 Nov 7;7(11):e48409. Available from: https://dx.plos.org/10.1371/journal.pone.0048409

93. Sarmiento L, Frisk G, Anagandula M, Cabrera-Rode E, Roivainen M, Cilio CM. Expression of Innate Immunity Genes and Damage of Primary Human Pancreatic Islets

by Epidemic Strains of Echovirus: Implication for Post-Virus Islet Autoimmunity. von Herrath MG, editor. PLoS One [Internet]. 2013 Nov 1;8(11):e77850. Available from: https://dx.plos.org/10.1371/journal.pone.0077850

94. Sarmiento L, Cubas-Dueñas I, Cabrera-Rode E. Evidence of association between type 1 diabetes and exposure to enterovirus in Cuban children and adolescents. MEDICC Rev [Internet]. 2013 Jan;15(1):29–32. Available from: http://www.scielosp.org/scielo.php?script=sci_arttext&pid=S1555-79602013000100007&lng=en&nrm=iso&tlng=en

95. Morgan NG, Richardson SJ. Enteroviruses as causative agents in type 1 diabetes: loose ends or lost cause? Trends Endocrinol Metab [Internet]. 2014 Dec;25(12):611–9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1043276014001520

96. Krogvold L, Edwin B, Buanes T, Frisk G, Skog O, Anagandula M, et al. Detection of a Low-Grade Enteroviral Infection in the Islets of Langerhans of Living Patients Newly Diagnosed With Type 1 Diabetes. Diabetes [Internet]. 2015 May;64(5):1682–7. Available from: http://diabetes.diabetesjournals.org/lookup/doi/10.2337/db14-1370

97. Richardson SJ, Leete P, Bone AJ, Foulis AK, Morgan NG. Expression of the enteroviral capsid protein VP1 in the islet cells of patients with type 1 diabetes is associated with induction of protein kinase R and downregulation of Mcl-1. Diabetologia [Internet]. 2013 Jan 14;56(1):185–93. Available from: http://link.springer.com/10.1007/s00125-012-2745-4

98. Dotta F, Censini S, van Halteren AGS, Marselli L, Masini M, Dionisi S, et al. Coxsackie B4 virus infection of beta cells and natural killer cell insulitis in recent-onset type 1 diabetic patients. Proc Natl Acad Sci [Internet]. 2007 Mar 20;104(12):5115–20. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.0700442104

99. Busse N, Paroni F, Richardson SJ, Laiho JE, Oikarinen M, Frisk G, et al. Detection and localization of viral infection in the pancreas of patients with type 1 diabetes using short fluorescently-labelled oligonucleotide probes. Oncotarget [Internet]. 2017 Feb 21;8(8). Available from: http://www.oncotarget.com/fulltext/14896

100. Foulis AK, Farquharson MA, Hardman R. Aberrant expression of Class II major histocompatibility complex molecules by B cells and hyperexpression of Class I major histocompatibility complex molecules by insulin containing islets in Type 1 (insulin-dependent) diabetes mellitus. Diabetologia [Internet]. 1987 May;30(5):333–43. Available from: http://link.springer.com/10.1007/BF00299027

101. Stone VM, Hankaniemi MM, Svedin E, Sioofy-Khojine A, Oikarinen S, Hyöty H, et al. A Coxsackievirus B vaccine protects against virus-induced diabetes in an experimental mouse model of type 1 diabetes. Diabetologia [Internet]. 2018 Feb 18;61(2):476–81. Available from: http://link.springer.com/10.1007/s00125-017-4492-z