

Identifying the 'Achilles Heel' of type 1 diabetes

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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

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.

Since the 1970s, we have had evidence suggesting that type 1 diabetes has a multifactorial pathogenesis, involving genetics and 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,

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 (1) 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 (2).

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 (3). Of note, patients with other diseases, including multiple

sclerosis and hairy cell leukemia, receiving type I IFN therapy, have higher risk of developing T1D (4).

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 (4)) .

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 (5). 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 (6,7). 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 (8). One could envisage that the *IFIH1* 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 (9). This further supports an important pathogenic role for IFNs in human T1D and an active mechanism of regulation in some individuals.

In summary, the Achilles Heel 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 1). 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.

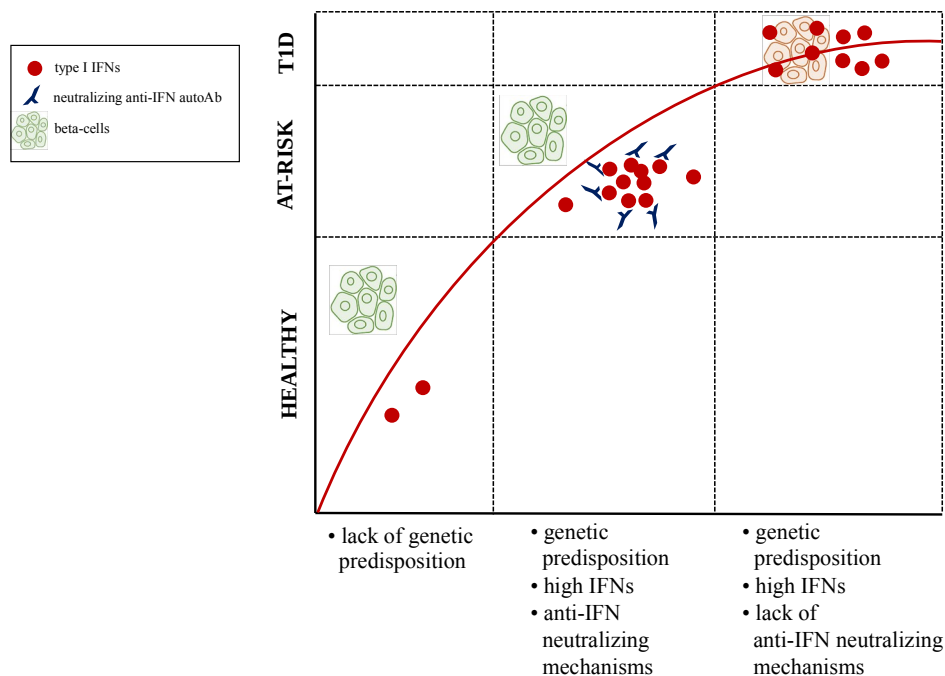


Figure 1. Intersection of genetic predisposition and immune responses involving type 1 interferons

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 (10,11). 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 (12–14), and this appears to be primarily due to their function as antigen presenting cells (15,16). 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 (17). There is also emerging evidence that B cell homeostasis and function is impaired in “at-risk” cohorts (11,18,19). 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 (20). 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 (21). B cells have been detected near or within pancreatic islets from individuals with recent onset T1D (22). 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 (23). Mechanistic studies in mice also suggest that B cells contribute beta cell damage by promoting CD8 T cell survival (24) 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 (20), and B cells arising after depletion are still autoreactive (25). 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 (21), while individuals showing a poor response to abatacept, a T cell targeted therapy, had a transient increase in B cell activation after therapy (26). Additionally, preservation of regulatory B cells may also enhance responses to B cell targeted therapies (27). 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?

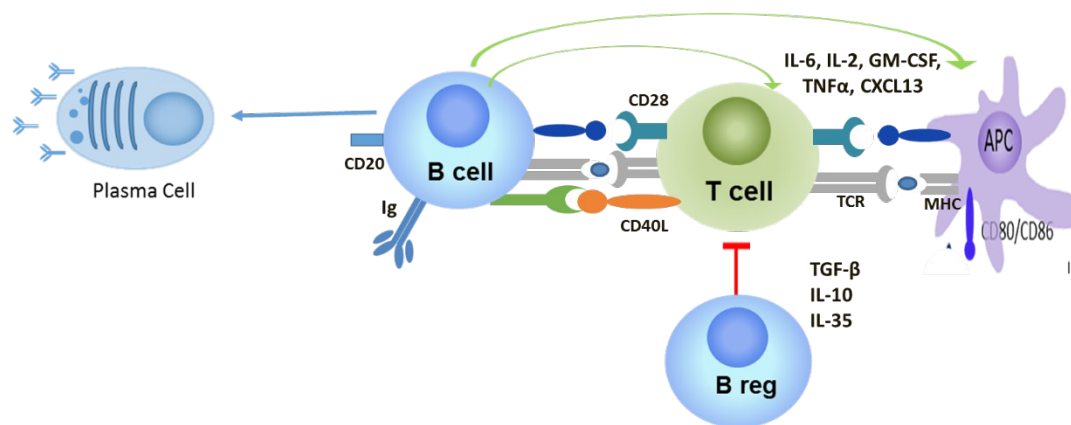


Figure 2. B cells influence islet autoimmunity at multiple levels.

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 (28). 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 (29).

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 insulinitis nor diabetes occurs (30,31). In humans, CD8⁺ T cells are found infiltrating the pancreatic islets at the time of diagnosis of type 1 diabetes (32). 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 (33,34). The CD8⁺ T cells within the pancreas can be shown to be specifically responsive to insulin and other islet

autoantigens (35). Along with this can be seen upregulation of MHC class I on the islets of Langerhans (36), and this is not observed in the islets that are not infiltrated, and also not found in non-diabetic controls (37). 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 (38). Similarly, a human proinsulin-specific clone can recognise a peptide of *C. aspariforme* (39). 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 (40) (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 (44).

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 (45).

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 (46,47). 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 (48). 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 (49). 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 (50).

When one gives a talk or writes a review about Tregs, it is common to use analogies such as "conductors", "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 is 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 (51,52), NK cells (53), $\gamma\delta$ T cells (54), antigen presenting cells (55) and even neutrophils (56)! 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 (57).

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 (58). 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 (59,60). Moreover, other rare monogenic mutations which affect Treg function, for example in *CD25* or *CTLA4*, can also cause T1D, lending more support to the argument that without these cells, islet-directed immunity is unleashed (61).

But beyond the rare monogenic 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 (62,63). For example, genes with high odds ratios include *CD25*, *PTPN2*, *PTPN22*, *CTLA4*, and *IL2*, all genes which can affect Treg function (64,65).

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 (49). There are also reports of Treg "instability", manifested as increased pro-inflammatory cytokine production (66,67). A consistent finding is their relative unresponsiveness to IL-2 (68,69) and we have shown diminished production of chemokines that are important for attracting their targets of suppression (70). 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 (71,72).

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 favourable for Treg function. It can be anticipated that the polyclonal Treg cell therapy studies which provided important safety data (73), will soon advance to testing of antigen-specific Tregs (74). Close to 20 years since the power of this approach was first demonstrated in NOD mice (75,76), 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? (77). 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 (78–81). 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 (40)). The examination of pancreata from children who died following an acute CVB infection shows selective targeting of the islets (82–85). Furthermore, isolated human islets are highly susceptible to infection *in vitro* (41,78,86), 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 (86–88), 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 (87). 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 (86,89,90) and Ingenuity Pathway Analysis (IPA) of these demonstrates that the “top hit” pathways involve cellular sensing of infections and responses to interferons (IFNs) (91). Beta cells are known to be highly responsive to interferons (92), 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 (8,93). 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 (80). 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 (94). The circulating enterovirus isolated from affected individuals was shown to infect and impact upon beta cell function *in vitro* (94,95). 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 (79,81)). 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 (80). 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 (40,79,96)).

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 (37,97,98); these findings correlate with evidence of low levels of infection as assessed by the presence of viral protein and RNA (83,97–100). One key hallmark feature in the pancreas of T1D donors is the dramatic hyperexpression of HLA-class I (37,101), 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, which is far easier said than done. Encouragingly though, efforts

are now underway (40,78,102) to generate an anti-enteroviral vaccine, which targets multiple CVB serotypes with first-in-man trials scheduled in 2020/2021. 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.

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