CD8$^+$ T-cells and their interaction with other cells in damage to islet β-cells

F. Susan Wong*†, L. Khai Siew* and Li Wen†

*Department of Cellular and Molecular Medicine, University of Bristol, Bristol BS8 1TD, U.K. and †Section of Endocrinology, Yale School of Medicine, New Haven, CT 06520, U.S.A.

Abstract
The autoimmune attack on pancreatic β-cells is orchestrated by a variety of cells that produce cytokines and other toxic mediators. CD8$^+$ T-cells work together with other lymphocytes and antigen-presenting cells to mediate this damage and have been shown in animal models to be important both in the early stages of diabetes development and in the final effector stages. Recently, there has also been much interest in studying CD8$^+$ T-cells that may play a role in human Type 1 diabetes and identifying their antigenic targets. The present paper will focus on the activation of CD8$^+$ T-cells and their interaction with other cells of the immune system and discuss the target antigens and mechanisms of damage that the CD8$^+$ T-cells use in the attack on the islet β-cell.

Introduction
Damage to pancreatic β-cells in Type 1 diabetes occurs through a variety of mechanisms that include CD8$^+$ T-cellular cytotoxic damage and cytokines. The cellular infiltrate within the islets consists of a number of cell types including CD8$^+$ T-cells, CD4$^+$ T-cells, B-cells, macrophages and dendritic cells. In studying the immunology of Type 1 diabetes, there has been considerable interest in which cells cause damage and what they recognize, the cytotoxic mediators produced, how the immune cells damage the islet β-cells and how these cells may be controlled. Ultimately, the hope in understanding these factors is to identify pathways that may be amenable to intervention to prevent the damage to the islets.

Why are CD8$^+$ T-cells important?
Many studies carried out in the NOD (non-obese diabetic) mouse model of Type 1 diabetes have indicated that CD8$^+$ T-cells inflict islet β-cell damage both at an early stage in diabetes development and at the final effector phase [1–3]. Although there is less direct evidence in humans about the early phases of disease, at the time of diagnosis of diabetes, in biopsies of the pancreas, a significant proportion of the infiltrating cells are CD8$^+$ T-cells [4]. Furthermore, in post-mortem samples of Type 1 diabetes patients, CD8$^+$ T-cells are also present within the islets [5]. There is clearly development of CD8$^+$ T-cell memory against islet autoantigens, as shown by the recurrence of diabetes in identical twins, one of whom had received half a pancreas from their non-diabetic twin, only for the graft to be destroyed within 6 weeks with a predominance of CD8$^+$ T-cells in the islet infiltrate [6].

What do these CD8$^+$ T-cells recognize?
In the last few years, there have been a number of studies examining the target autoantigens of CD8$^+$ T-cells in human Type 1 diabetes, using candidate autoantigens identified in NOD mouse studies. In the NOD mouse, proinsulin [7], IGRP (islet-specific glucose-6-phosphatase catalytic subunit-related protein) [8], GAD (glutamic acid decarboxylase) [9] and a ubiquitous antigen DMK (dystrophia myotonica kinase) [10] have been identified as important for CD8$^+$ T-cells. In human studies, so far limited to testing peripheral blood lymphocytes, a variety of reactivities to putative autoantigens have been found, the first of which was to GAD65 [11–13], but, more recently, these specificities have included proinsulin [14–16], ppIAPP (pre-pro-islet amyloid polypeptide protein) [17–19] and GFAP (glial fibrillary acidic protein) [19]. By the time diabetes occurs, it appears that there may be a broad spread of reactivity, which is different for each patient, and it will be a major challenge to identify those antigenic epitopes that are important in the early phases of pathogenesis [20]. Suffice it to say, the NOD mouse has given us important insights into these antigenic targets, and there is considerable similarity between the antigens recognized in humans and in this animal model. It is notable that, in the past, epitopes have been put forward related to their binding affinity to the MHC, but there is increasing evidence that low-binding affinity epitopes may be of considerable importance. Thus the means of identification of relevant epitopes may not be quite so straightforward. The study of antigenic epitopes that have relevance to human disease may also be aided by newer models which incorporate human HLA (histocompatibility locus antigen) molecules into mouse models. Using NOD mice that express...
HLA-A2, epitopes of proinsulin and IGRP have been identified that demonstrate cytotoxic responses towards islet targets [21,22]. These models will give some insight into T-cells, restricted by HLA-A2, that may be important in the diabetes disease process. However, humans are outbred, and subjects expressing HLA-A2 only represent half of all individuals that develop diabetes. Thus novel means of detecting responses will be required to ascertain targets of attack for any particular individual.

**Mechanisms of islet β-cell damage**

CD8+ T-cells, known as CTLs (cytotoxic T-lymphocytes), damage targets in a variety of ways. Although there have been many studies trying to identify whether one specific pathway is used in preference to another, it seems likely that all of the mechanisms by which CD8+ T-cells damage cells are used by these islet-reactive CTLs. These include production of cytokotoxic cytokines such as IFNγ (interferon γ) and TNFα (tumour necrosis factor α), that have a direct cytotoxic action on the islets. In addition, these cytokines have an important role in up-regulation of MHC class I molecules, which could increase immune recognition of the cells. These CD8+ T-cell-generated inflammatory cytokines can work together with cytokines produced by macrophages, such as IL-1 (interleukin)-1, to have a synergistic damaging effect on islet β-cells. The CTLs also exocytose granules from secretory lysosomes which contain cytotoxic proteins that include the pore-forming protein, perforin, and serine esterases, called granymes. When T-cells are activated through recognition of specific MHC-peptide complexes on the surface of the target cells, there is rapid polarization within the CTLs so that the secretory lysosomes containing lytic granules move along microtubules towards the MTOC (microtubule-organizing centre) and dock on to the plasma membrane [23]. There, the contents of the granules are released into a cleft formed between the CTL and the target cell, shown in schematic form in Figure 1. Perforin, homologous with the C9 component of complement, inserts into the target cell membrane and polymerizes, creating a pore. This facilitates the entry of granzymes into the cytosol of the target cells where cell death is initiated by cleavage of cytosolic cellular substrates.

There are a number of different granzymes, 11 identified in mice and five in humans, with non-overlapping targets, which makes granzyme-mediated killing particularly effective [23]. Perforin causes damage to the cell membrane, allowing pore formation, and this facilitates the entry of the granzymes into the target cell, inducing apoptosis via both caspase-dependent and -independent pathways [24]. The actions of granzyme B, a prominent granzyme involved in CTL function are summarized in Figure 2. In addition to damage caused by perforin and granzymes, CTLs also express Fas ligand which is found in lytic granules [25] and thus is also released together with the other cytotoxic proteins targeted to the secretory cleft. Islets are known to express Fas in an inflammatory setting [26] and this is a further means of inducing apoptosis by means of Fas–Fas ligand interactions.

There has been much debate about which of these mechanisms is dominant in diabetes. Various studies in transgenic and knockout animals have indicated that a combination of these mechanisms is likely, as no single pathway has been shown to be dominant. Perforin-knockout mice crossed on to the NOD genetic background have a considerably reduced incidence of diabetes [27]. Similarly, NOD.lpr mice that have functional Fas deficiency are also protected; however, these mice are subject to other immunodeficiency problems and thus the protection from diabetes may be difficult to assess. Other experiments, however, using NOD mice and targeting the Fas–Fas ligand pathway by using a dominant-negative point mutation in a death domain of Fas, Fast(cg) [28], as well as dominant-negative expression of FADD, involved in the Fas–Fas ligand death pathway [29], showed delayed and reduced incidence of diabetes, indicating that this pathway plays a role in islet β-cell damage. The ability to stain for LAMP-1 (lysosome-associated membrane protein 1) (CD107a) on the surface of CD8+ T-cells indicates cytotoxic granule exocytosis and is a useful means of measuring cytotoxic degranulation [30]. When a high concentration of antigen is presented to insulin-reactive CD8+ T-cells, such as in studies using peptides, there is considerable granule release (F.S. Wong and L.K. Siew, unpublished work). In addition, increasing amounts of IFNγ...
Figure 2 | Granzyme B activates caspase-dependent nuclear cell death pathways and caspase-independent mitochondrial death pathways

Perforin facilitates entry of granzyme B into the target cells. Granzyme B cleaves pro-caspase 3 (pathway A), leading to activation of caspase 3, and downstream cleaving ICAD (inhibitor of caspase-activated DNase), liberating CAD (caspase-activated DNase). This can then cause DNA fragmentation in the nucleus. Granzyme B can also cleave ICAD directly. Granzyme B interacts with BID (pathway B) to disrupt the outer mitochondrial membrane, releasing a number of pro-apoptotic factors including cytochrome c (which activates pro-caspase 9), HtrA2 (which blocks apoptosis inhibitors) and endonuclease G (which activates DNA damage). Cytochrome c binds to APAF1 (apoptotic protease-activating factor 1) recruiting pro-caspase 9 which is activated by autocatalysis, and this in turn can activate pro-caspase 3. It is thought that granzyme B may also disrupt the mitochondrial membrane through a separate unknown mechanism (pathway C). BID, BHS-interacting domain death agonist, tBID, truncated BID. Based on data from [24].

Interactions with other cells of the immune system in damaging islets

Although CTLs are clearly important in damage to islets, it is also clear that a network of interactions lead to damage to the islets and that these cells are not the sole effectors causing diabetes. Many strategies that have been successful in altering the course of disease in animal models and also strategies that are currently under test in humans have not targeted CD8+ T-cells directly. Understanding the role of the other cellular players in the disease process, both destructive cell subsets as well as regulatory T-cells, is important in considering how the immune system may deviate from damage to the islet β-cells. CD4+ T-cells have been studied for many years as important players in the destructive cellular processes and, while individual T-cell clones may be effective in damaging islets, it is clear that they are not solely responsible. It is likely that they play a number of roles that may facilitate CD8+ T-cell damage. These include activating dendritic cells and other antigen-presenting cells through CD40–CD154 interactions which ‘license’ dendritic cells to activate naïve CD8+ T-cells [37], and for generation of CD8+ T-cell memory [38,39]. They are able to potently produce cytokines, such as IL-2 which can increase the effect of CD8+ T-cells, as well as chemokines which facilitate trafficking to the islets. These interactions are likely to increase the pathogenicity of CD8+ T-cells.
Recently, there has been much interest in the role of regulatory T-cells and their interaction with pathogenic T-cells and how these cells may be harnessed for protection against islet damage. There are a number of subsets of regulatory T-cells, which include CD4⁺CD25⁺ thymically derived regulatory cells as well as induced subsets of Tr1 (T-regulatory 1) cells, which are induced to produce IL-10, and Th3 cells which are induced to produce TGF-β (transforming growth factor β). How regulatory T-cells interact with pathogenic T-cells is a matter of considerable ongoing research as to whether this is by direct effects on CD8⁺ T-cells, or indirectly through antigen-presenting cells, reducing their activation of T-cells. One means by which CD4⁺CD25⁺ T-cells can affect CD8⁺ T-cells is to impair the granule exocytosis after CD8⁺ T-cells have formed conjugates with the target cell [40]. This appears to be dependent on the inhibitory cytokine TGF-β and can be reversed on removal of regulatory T-cells. This is not the only means by which CD8⁺ T-cells may be ‘regulated’, and more insights are likely to emerge in the near future.

There are many other possible interactions leading to diabetes and also means of regulation, which could help tolerate and prevent disease, but it is not possible in this short review to cover all these aspects. However, an understanding of the cellular components of islet β-cell damage in Type 1 diabetes is of critical importance if we are to design means of diverting the immune response and preventing β-cell damage caused by a large number of cytotoxic effects of CD8⁺ T-cells.

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References


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