Published in final edited form as:

Clin Rev Allergy Immunol. 2011 October ; 41(2): 169–178. doi:10.1007/s12016-010-8220-4.

Viruses and Cytotoxic T Lymphocytes in Type 1 Diabetes

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Abstract

Histopathological studies on pancreas tissues from individuals with recent-onset type 1 diabetes (T1D) consistently find that CD8 T cells substantially contribute to the formation of islet lesions. CD8 T cells reactive against islet-associated antigens can also be found in blood samples from T1D patients. Mechanistic studies on the pathogenic role of this T cell subset have mostly focused on two animal models, i.e., the non-obese diabetic mouse and the virally induced rat insulin promoter–lymphocytic choriomeningitis virus model. Data were obtained in support of a role for viral infection in expanding a population of diabetogenic cytotoxic T lymphocytes. In view of the theorized association of viral infection with initiation of islet autoimmunity and progression to clinically overt disease, CD8 T cells thus represent an attractive target for immunotherapy. We will review here arguments in favor of a pivotal role for CD8 T cells in driving T1D development and speculate on etiologic agents that may provoke their aberrant activation.

Keywords

Type 1 diabetes; Autoimmunity; CD8 T cells; CTL; Beta cells

CD8 T Cells Within Insulitic Lesions and in Peripheral Blood from T1D Patients

Development of islet autoimmunity in type 1 diabetes (T1D) is associated with genetic polymorphisms in the major histocompatibility complex (MHC) region, and autoantibodies can often be detected long before clinical onset [1]. Another hallmark feature that has historically defined T1D as an autoimmune disease is the presence of inflammatory infiltrates or “insulitis” around pancreatic islets [2]. These lesions are typically found in patients around diagnosis, but despite their obvious pathogenic relevance, few studies have been attributed to defining their precise cellular composition. CD4 T cells, B cells, macrophages and, most recently, NK cells were all found to contribute to islet infiltration, with notable variation between individual patients and in particular time of sampling relative to diagnosis [3, 4]. Most reports, however, find that CD8 T cells are present in significant numbers and represent the predominant cellular subset around islets at the time of disease onset [3, 5–7]. A key observation is that even in patients affected by aggressive islet-specific autoimmunity, CD8 T cell numbers are by no means comparable with the degree of

inflammation that is seen in animal models. This discrepancy is even more pronounced when prediabetic, autoantibody-positive individuals are studied as In't Veld and coworkers found signs of insulinitis in less than 10% of islets from triple autoantibody-positive subjects [8]. Collectively, these data suggest that CD8 T cells at least contribute to beta cell destruction before and around disease onset and may be important in driving anti-islet autoimmunity.

Due to the anatomical location of the pancreas and the significant risks associated with biopsy sampling, the effector population in the islets is particularly difficult to access. Therefore, most studies have historically focused on peripheral blood from T1D patients, assuming that this source contains a surrogate T cell subset that is representative of the islet-infiltrating population. Multiple islet antigen-reactive CD8 T cell species were shown to be associated with T1D development and progression, including reactivity against insulin, glutamic acid decarboxylase (GAD65), islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), insulinoma-associated protein 2 (IA-2), and prepro islet amyloid polypeptide (reviewed in [9]). The low frequency of these diabetes-related CD8 T cell specificities in the blood suggests that they only represent the proverbial tip of the iceberg and have long impeded their direct detection. With the advent of tetramer technology, they can now be accurately identified without prior culturing and expansion. A recent study by Velthuis and coworkers introduced and validated the use of combinatorial MHC class I multimers that allow for the simultaneous detection of multiple specificities in small sample volumes [10].

The importance of CD8 T cells in the non-obese diabetic (NOD) mouse has been acknowledged for some time, but results in T1D patients have only recently become available. So what can we learn from determining the repertoire of peripheral islet-specific CD8 T cells in patients? First, fundamental insights can come from determining functional CD8 T cell responses after stimulation with islet autoantigens. In vitro assays show that activation of CD8 T cells from T1D patients with islet autoantigens provokes a potent interferon (IFN) response and that a considerable fraction has acquired a memory phenotype [11–20]. Of interest, and in line with studies in the NOD mouse, binding affinity to HLA class I is generally low, which may explain why these specificities were able to escape thymic selection and peripheral tolerance mechanisms [16, 21]. A particularly exciting study by Skowera and coworkers showed that preproinsulin-reactive cytotoxic T lymphocytes (CTL) exert cytotoxic activity towards human beta cells in vitro [22]. In addition to providing functional relevance for islet-reactive CD8 T cells, the latter study indicates that beta cells may be actively involved in their own demise, as higher compensatory insulin production in later disease stages may lead to increased CD8 recognition and killing.

Second, changes in the frequencies of these cells may enable investigators to better diagnose and track the autoimmune response during natural progression or experimental treatment. Recent reports suggest that frequencies and immunodominance patterns change according to disease progression [23]. Technical advances in MHC multimer design have dramatically improved detection sensitivity and specificity and enable one to successfully discriminate T1D subjects from controls based on CD8 T cell reactivity profiling [10]. Cernea et al. assessed the islet-reactive CD8 T cell population in peripheral blood from T1D patients as

compared to healthy individuals and evaluated the effect of anti-CD3 treatment in a small T1D cohort [24]. As expected, the islet-reactive specificities were found to be overrepresented in T1D patients. Remarkable, however, was the finding that the majority of these islet-antigen-specific cells displayed a naive phenotype. Upon anti-CD3 treatment, there was a notable decrease in frequency of these naive cells accompanied by an increase in memory phenotypes. Selective sequestration of effector phenotypes in the pancreas was hypothesized to account for the predominance of naive cells in peripheral blood under baseline T1D conditions. Anti-CD3 therapy could then induce the expansion of pre-existing antigen-specific cells and alter their phenotype towards regulatory function.

Finally, measuring islet-reactive CD8⁺ T cell frequencies in the peripheral blood of islet transplant recipients may be useful in clinical follow-up [14]. It was shown that proliferation of CD4⁺ T cells specific for GAD and IA-2 is associated with clinical outcome after islet transplantation [25]. Likewise, Pinkse et al. found that detecting elevated numbers of insulin B₁₀₋₁₈-specific CD8 T cells in the blood precedes recurrent autoimmunity against the grafts. In another study, the presence of insulin B₁₀₋₁₈-reactive CD8⁺ T cells after transplantation correlated with a poor clinical outcome [10]. In combination with the growing appreciation of a prime effector role for CD8 T cells in beta cell destruction, monitoring CD8⁺ T cell frequencies may constitute a powerful approach in the prediction of clinical outcome after islet transplantation.

What are the arguments to assume that the islet-specific CD8 T cell peripheral blood mononuclear cell (PBMC) population corresponds with the islet-infiltrating subset? Such data are at present solely derived from experiments in animals [26, 27], and work in the HLA-A2-transgenic “humanized” NOD mouse indeed shows that the PBMC-derived CD8 T cell population parallels the repertoire found in islet lesions [28]. However, direct support for a similar concordance in humans is currently lacking, although islet-reactive CD8 T cells were detected in blood from a T1D pancreas transplant recipient that were also detectable in the inflamed pancreas tissue homogenate, be it in higher frequencies and diversity [29].

In conclusion, islet-specific CD8 T cells act as important effectors in T1D, and monitoring their frequencies, hierarchy, and cytokine profile in peripheral blood may become instrumental in the follow-up and clinical prediction during therapy or after islet transplantation.

Viral Infection May Initiate and/or Invigorate CD8 T Cell Autoimmunity Against Islets

Mechanistic dissection of the role of CD8 T cells has mainly been performed in two mouse strains, i.e., the spontaneous NOD and the virus-induced rat insulin promoter–lymphocytic choriomeningitis virus (RIP–LCMV) model. CD4⁺ T cells were long considered the primary effector subset involved in diabetes development in the NOD mouse. However, adoptive transfer studies have unequivocally demonstrated that CD8 T cells are required for diabetes development and have the ability to cause beta cell destruction at various stages (excellently reviewed in [30]). The question then remains what initially triggers activation and expansion of these cells. A prerequisite for the T cells to become activated is obviously

their survival beyond thymic selection. In normal individuals, thymic selection eliminates T cell clones that recognize self-antigens with high avidity. It was theorized that in T1D patients, deletion of autoreactive T cell populations of relatively high avidity is incomplete, which gives rise to the release of potentially diabetogenic precursors into the periphery. High-avidity islet-specific CD4 T cells have been detected in peripheral blood from T1D patients, and their frequency increases as disease progresses [31, 32]. Similar rules appear to apply in the case of CD8 T cells, although data in human subjects are currently lacking. Unlike B cells, T cells do not have the capacity to optimize their antigenic receptor affinity by somatic mutation. Amrani and coworkers explained the gradual accumulation of high-avidity, diabetogenic CD8 T cells as disease progresses in the NOD mouse by the selective survival and expansion of a small set of high-avidity clones [33]. This population may subsequently become the driver population and cause the initial wave of beta cell destruction. Whereas this hypothesis is plausible in the spontaneous NOD model, it does not take into account the environmental component that is believed to trigger clinical disease in humans. Viral infections have, in that respect, garnered the majority of attention, and there is now convincing, albeit indirect, evidence that enteroviruses have the potential to initiate and/or accelerate diabetes progression [34–38]. Cytotoxic T lymphocytes are the principal adaptive lymphocyte subset involved in efficient clearance of viral infections, and a link between viruses and diabetes progression would likely imply the expansion and subsequent diabetogenicity of anti-viral CD8 T cells. The mechanisms connecting viral infection to diabetes development are poorly understood, but two main hypotheses have emerged. The first involves the specific chronic infection of pancreatic islets by a pancreotropic viral strain. In their landmark 1987 paper, Foulis and coworkers demonstrated that pancreatic islets in recent-onset T1D patients are characterized by the specific hyperexpression of MHC class I and IFN- α [39]. Both molecules are typically produced by cells that are infected with virus and mediate clearance by attraction of and antigen presentation to CD8 T cells. Direct evidence for such islet cell-specific chronicity is scarce but is supported by a recent study on the same samples used in the 1987 study. In over 60% of T1D cases, enteroviral capsid protein was detected in pancreatic islets as compared to 6% of control tissues [38]. The capacity of enteroviruses such as coxsackie B to infect human islet cells in vitro had been known for quite some time but an in vivo correlate was missing [40]. Studies in the NOD mouse did show that coxsackie B viruses (CVB) can efficiently replicate in pancreatic islets and that these cells express the CVB receptor CAR [41]. Moreover, the replication efficiency and inoculation dose determine a given CVB strain's diabetogenic potential [42]. Further proof of concept has been obtained in the NOD mouse, where CVB infection late in the prediabetic phase rapidly induces disease onset [41, 43]. According to the latter studies, pre-existing insulinitis is required to enable the virus to efficiently infect pancreatic islets and accelerate beta cell decay. This would be a potential explanation of a recent finding in patients that enteroviral infection is associated with progression from autoantibody positivity (prediabetic phase) to full-blown clinical disease [35]. In another cohort, a relationship was identified between enterovirus infection and autoantibody development during the early prediabetic phase [34]. Both scenarios are not mutually exclusive but rather show the importance of timing of infection in terms of clinical outcome in genetically distinct patients. Indeed, early infection of young NOD mice protects rather than promotes diabetes development owing to the induction of regulatory mechanisms [44]. The assumption that

viruses can directly infect islets and thus predispose them to CTL recruitment and attack may explain the “patchy” multifocal pattern of insulitis which shows a highly lobular distribution around onset [45]. It would be interesting to reveal whether this distribution is dependent on local viral infection, i.e., are the affected islets the ones that are infected at a given time. Finally, in the event of islet-specific viral infection, consequential immune recognition and apoptosis will lead to the release of cryptic islet molecules, which would in turn induce determinant spreading and a switch from viral immunity to islet-specific autoimmunity.

The second possibility that may link viruses to islet autoimmunity is that peripheral viral infection expands a T cell population that can subsequently initiate or contribute to ongoing insulitis. Further distinction can be made between so-called bystander scenarios and cross-reactivity through viral mimicry. The discussion below on the requirements for antigen-specificity in islet recruitment and extravasation of course pertains to either scenario. A model that has been intensively studied in this context is the virus-induced RIP-LCMV model [46, 47]. Diabetes in this model is provoked by a CD8 T cell population raised against the lymphocytic choriomeningitis virus (LCMV) that subsequently redirects against viral antigen that is transgenically expressed on the beta cells under the rat insulin promoter. Curiously, expression of neoantigen exclusively on beta cells (the transgene is not expressed in the thymus) does not by itself lead to islet autoimmunity but instead requires a potent peripheral stimulus. A similar approach in the susceptible NOD background confirmed this finding [48]. This type of experimental model serves as a prototypic example of molecular mimicry between antigenic trigger (the virus) and target (an islet-specific molecule). Whereas the immune pathology of this model has been described in great detail, it remains unclear as whether similar viral mimicry mechanisms are at play in humans. A number of reports in humans have described viral epitopes that may potentially lead to cross-reactivity with islet-specific antigens [49–51]. In view of the finding that many viruses seem to exhibit sequence similarity to islet antigens, these data could corroborate the idea that many types of viruses possess the ability to initiate or invigorate insulitis without directly infecting the target cells. The lack of chronicity and the latitude of viruses would act as confounding factors in detecting any strain responsible for the cross-reactive response and may clarify why most studies find that a certain virus is only associated with a portion of recent-onset cases. The alternative option, “bystander” contribution, also has its proponents and will be discussed below in conjunction with the requirements for T cell recruitment and retention in the islets.

Mechanisms Governing CD8 T Cell Recruitment to the Islets

The event of bystander activation entails secondary recruitment and activation of immune cells via non antigen-specific pathways such as through the influence of a local cytokine and chemokine milieu. A study by Horwitz et al. addressed this issue and concluded that in the context of diabetogenic coxsackie infection, islet-specific T cells can become activated in face of local immunity against the virus [52]. From the perspective of more recent studies indicating that islet antigen-specificity is required for islet recruitment, the results of this work remain plausible as the recruited population indeed recognized an islet antigen [53–56]. The latter studies, performed in spontaneous diabetes models based on the NOD mouse,

provide convincing arguments for the antigen-specific nature of CD4 and CD8 T cell recruitment and retention during diabetes progression. Data in the conventional NOD mouse favor this idea, as CD8 T cells specific for three islet antigens insulin, IGRP, and dystrophin myotonia kinase constitute over 60% of the inflammatory infiltrate associated with islets [57]. However, we argue that in the context of diabetogenic viral infection, anti-viral T cells can act as important “bystander” effectors without contradicting the necessity to recognize antigen at the islet site. First, in the event of pancreotropic virus, soluble cytokines that are produced by anti-viral cells clearing the (mostly exocrine) infection are likely to affect beta cell viability especially when a critical threshold of bona fide islet-reactive T cells is already present. This is conceivable because the effects of cytokines on beta cell apoptosis induction often surpass direct killing by perforin/granzyme-mediated pathways [58]. Second, provided that a population of relatively high-avidity islet-specific T cells aberrantly escapes thymic selection, peripheral viral infection can tip the balance from ignorance to autoimmunity. In support of this notion, it was demonstrated that LCMV infection can break ignorance of an OT-I-reactive CD8 T cell population in a non antigen-specific fashion and lead to the recruitment and diabetogenicity of these cells in RIP-OVA transgenic mice [59]. Finally, a profound effect of viral infection may not depend on a cellular contribution but can be solely mediated by factors produced by the immediate exocrine environment or the islets themselves. Potential mediators of CD8 T cell recruitment and activation induced by viruses include IFN- γ and CXCL10, which are both potently induced upon viral infection by pancreotropic viruses [60, 61]. In summary, we propose that non-islet-specific “bystander” T cells raised during anti-viral immunity can be recruited to the pancreas and will affect beta cell viability indirectly via soluble factors. The antigen-specific nature of extravasation still applies as these cells respond to viral antigen that is present in the pancreas (Fig. 1).

Beta Cell Damage by Direct Recognition and Cytokine Production

Since we know that beta cells have the capacity to upregulate MHC class I molecules on their surface and thus are able to present antigen directly to CD8 T cells, contact-dependent death mechanisms are likely to contribute to beta cell death in T1D. Data obtained from mice that specifically lack beta cell MHC class I gene expression suggest that hyperexpression is a pivotal mechanism for induction of full-blown beta cell death and ensuing hyperglycemia [62]. Contact-dependent apoptosis induction by CTL involves either of two mechanisms [63]. In the first pathway, a membrane-disrupting protein known as perforin together with serine proteases (granzymes) are secreted by exocytosis and induce apoptosis of the beta cell. The second pathway involves the engagement and aggregation of beta cell death receptors, such as Fas (CD95), by their cognate ligands, such as Fas ligand (FasL), on the CTL, which results in beta cell apoptosis. The importance of the first pathway has been exhaustively assessed in vivo and appears to be of major importance in the NOD model [64]. Perforin in fact turns out to be the only effector mechanism for which genetic deficiency by itself is protective from diabetes [65]. In the RIP–LCMV model, genetic ablation of the perforin gene has yielded conflicting results. In one study by Kagi and coworkers, it was found that perforin is required for the induction of full-blown diabetes, whereas we found that it is dispensable [58, 66]. The best interpretable data on the action of Fas signaling have come from experiments using NOD mice carrying a beta cell-specific

disruption of Fas signaling [67, 68]. These studies showed delayed spontaneous diabetes and thus indicate a partial, dispensable contribution of this pathway during beta cell decay. Taken together, contact-independent pathways play a major role during all phases of beta cell decay but appear to be complemented by contact-independent, cytokine-mediated signaling. We found that interferon- γ is a particularly important player in the RIP-LCMV model, as genetic disruption of this cytokine completely disrupted clinical diabetes development [69]. The effect of the local production of this cytokine in combination with IL-1 by insulinitic CD8 T cells can directly contribute to beta cell toxicity by, e.g., induction of iNOS and NO in the beta cells [70]. Indirect consequences of high local interferon levels are beta cell upregulation of MHC class I and Fas, followed by enhanced CD8 T cell recognition and contact-dependent apoptosis induction [58, 71, 72].

How all these mechanisms partake in eventual beta cell killing likely depends on which animal model is studied, but it seems reasonable to assume a cooperative action in a possibly time-dependent fashion.

In vitro studies on isolated human islets are the only strategy to dissect the sensitivity of beta cells to the aforementioned death pathways. In line with the animal data, perforin/granzyme-mediated killing was found to be a dominant CTL death mechanism in vitro [73, 74]. Apoptosis triggered by Fas signaling requires prior exposure of the beta cells to cytokines in order to induce significant expression levels [75]. An interesting finding was that newly formed beta cells are more sensitive to the action of proinflammatory cytokines, which may be the reason why beta cell regeneration in T1D patients appears to be such an inefficient process [76]. Collectively, these in vitro data largely confirm the notion that multiple death pathways contribute to variable extent.

In T1D patients, one would expect high levels of beta cell apoptosis under conditions of insulinitis, especially in most of the available histological specimens from individual with recent-onset disease. Surprisingly, the rate of beta cell apoptosis appears to be very limited based on the currently available data. One group found no evidence of beta cell apoptosis in a set of 13 biopsies from recent-onset patients [77]. Other studies did find detectable levels of beta cell apoptosis, which were estimated at around 6% of all beta cells by Meier et al., approximately double those found in control patients [78]. The most recent paper from the same laboratory, however, showed lower levels of beta cell apoptosis, averaging 0.2 TUNEL-positive beta cells per islet as compared to virtually none in healthy controls [79]. A limited number of articles identified potential pathways to apoptosis in T1D patients, mostly focusing on the role for Fas–Fas ligand (CD95–CD95L) interactions. In support of this contact-dependent death mechanism, beta cells were shown to express Fas, most likely in response to cytokines produced by infiltrating immune cells [77, 80]. This would render them susceptible to apoptosis by way of interacting with FasL-expressing lymphocytes. The relative contribution of the Fas pathway to the total ratio of beta cell apoptosis in humans remains unknown.

An interesting yet unresolved question is how many CD8 T cells are required to induce a relevant degree of beta cell damage. In vivo imaging data from our lab indicate that in the RIP-LCMV model, high numbers of beta cell-specific CD8 T cells are required for

induction of beta cell apoptosis (*PLoS ONE* and unpublished data). When one performs a crude extrapolation of these data (box 1) to human prediabetic individuals this may suggest why, at the subtle rate of T cell infiltration typically seen in patients, clinical T1D generally takes years to develop.

Potential Therapeutic Implications

Most of the approaches that aim to achieve antigen-specific tolerization in T1D have concentrated on the induction and expansion of CD4⁺ regulatory T cell subsets. Expansion of natural (CD4⁺CD25⁺foxp3⁺) Tregs or promotion of adaptive Tr1 cells will in turn alter the effector function of local CD8 T cells through immunomodulatory cytokine production of antigen presenting cell (APC) killing [83]. Few studies, however, have attempted to target CD8 T cells directly to achieve antigen-specific tolerance in autoimmune diabetes. Much like CD4 T cells, CD8 T cells can be functionally manipulated by tolerogenic administration of cognate peptide ligands. Examples include the use of CTL epitopes derived from insulin and glial fibrillary acidic protein in protecting against autoimmune diabetes in the NOD mouse [84, 85]. Likewise, injection of LCMV MHC class I-restricted glycoprotein peptide prevents diabetes in the RIP-LCMV mouse [86].

CD8⁺ Tregs have always stood in the shadow of their CD4⁺ counterparts. Most studies in the NOD mouse point towards preferential induction of CD4⁺ Tregs after anti-CD3 therapy, in particular in combination with tolerizing doses of autoantigen [87]. Nevertheless, treatment of human T1D patients with non-Fc binding anti-CD3 antibody, a treatment modality currently in advanced clinical development, induced a population of functional CD8⁺CD25⁺ Tregs [88]. Recently, Tsai and colleagues showed that this population also exists in the NOD mouse and can be therapeutically targeted by MHC class I coated nanoparticles loaded with islet antigens [89]. This leads to the *in vivo* expansion of low-avidity, memory-like CD8 T cells. Their suggested *modus operandi* would be via APC killing and IFN- γ -mediated IDO induction in beta cells or DCs, which would render the latter tolerogenic.

Another recently published approach used saporin-coupled MHC I tetramers to kill antigen-specific CD8 T cells while sparing irrelevant T cells [90]. Saporin is a type I ribosome-inactivating protein and, upon antigen-specific internalization by the target cells, induces cell death in 72 h. These studies thus provide support for the feasibility to target CD8 T cells in T1D by either skewing their functional phenotype or inducing the specific deletion of deleterious subsets.

Conclusions

CD8 T cells play a crucial role in the immunopathology of T1D in mice and humans. The potential association of viral infections with initiation and/or acceleration of islet autoimmunity has been hypothesized and may represent a clue to the predominance of CD8 T cells in human islet lesions around onset. Several scenarios could be envisioned to mechanistically link viral infection with the development of insulinitis and may act in concert to culminate in clinical disease. Likewise, complex mechanisms dictate the recruitment and

effector function of islet-specific CD8 T cells. Understanding their mode of activation and the mechanisms of CTL homing to the islets may lead to potent interventions that can prevent or cure T1D.

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Box 1**Hypothetical extrapolation of CD8 T cell-mediated beta cell killing rates from mouse to man**

The pancreas from a **B6 mouse** harbors approximately 1000 islets with 1000 total cells/islet of which 77% are beta cells or 770.000 beta cells per adult pancreas⁸¹. We assume equal distribution of CD8 T cells over all islets in the acute RIP-LCMV model, i.e. 250 per islet (as per 3D imaging *in vivo*) or 250.000 islet-associated CD8 T cells in total per affected pancreas. Death rate as determined by in vivo two-photon microscopy was one beta cell/islet per 30min (unpublished data) which means that killing 80% of the beta cell mass would take 13 days (770 beta cell/islet $\times$ 0.5 hours). This is approximately what is observed in the RIP-LCMV model with clinical onset generally around two weeks post infection.

Human pancreas contains approximately one million islets with 1000 total cells/islet of which 55% are beta cells or 550 million beta cells per (young) adult individual. Data on T cell counts per islet in prediabetic individuals are scarce but based on⁸, average 43 CD8 T cells in 6% of islets in 5 micron sections from 2/62 Ab+ cases at various stages of prediabetic development. Since the average islet is 100 micron in diameter, we overestimate at roughly 800 CD8 T cells per islet in 3D. This gives 800 CD8 T cells in 60.000 islets for 2/62 patients, or an average of 1.5 million CD8's in total per Ab+ pancreas. This also roughly corresponds to data obtained from biopsies within the Japanese population around onset⁸².

The extrapolated time window for development of clinical diabetes (defined here as 80% beta cell loss), assuming that in mice, there's a beta cell/CD8 T cell ratio of 3, in humans this would translate to 370 or a factor of 124. The time needed to reach hypoglycemia in humans is thus 13 days multiplied by 124, which equals 1612 days **or roughly 4.5 years on average to clinical diabetes.**

Fig. 1.

Potential pathways toward virus-induced beta cell death in T1D. **a** Chronic viral infection of the beta cells can induce local production of INF-α. Through paracrine signaling, all islet cells start hyperexpressing MHC class I and thus become vulnerable for recognition by anti-viral CTL. Determinant spreading will subsequently lead to the recruitment of islet-specific CTL species that have escaped thymic selection in susceptible individuals. **b** Viral infection of the pancreas may also be non-beta cell-specific and lead to the initial influx of anti-viral CTL that clear the virus. Such localized CTL response may, possibly in the presence of

ongoing islet autoimmunity, attract and activate islet-specific and/or non-specific “bystander” T cells. The beta cells themselves may be considered “bystanders” as well, as they upregulate MHC I expression in response to the local cytokine milieu. **c.** Viral mimicry would lead to the generation of an activated anti-viral CTL population that cross-reacts with islet-derived epitopes. After viral clearance, the CTL would redirect to the islets from susceptible individuals and directly cause beta cell death and possibly determinant spreading