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Viral infections in type 1 diabetes mellitus — why the β cells?

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Abstract

Type 1 diabetes mellitus (T1DM) is caused by progressive autoimmune-mediated loss of pancreatic β-cell mass via apoptosis. The onset of T1DM depends on environmental factors that interact with predisposing genes to induce an autoimmune assault against β cells. Epidemiological, clinical and pathology studies in humans support viral infection — particularly by enteroviruses (for example, coxsackievirus) — as an environmental trigger for the development of T1DM. Many candidate genes for T1DM, such as *MDA5*, *PTPN2* and *TYK2*, regulate antiviral responses in both β cells and the immune system. Cellular permissiveness to viral infection is modulated by innate antiviral responses that vary among different tissues or cell types. Some data indicate that pancreatic islet α cells trigger a more efficient antiviral response to infection with diabetogenic viruses than do β cells, and so are able to eradicate viral infections without undergoing apoptosis. This difference could account for the varying ability of islet-cell subtypes to clear viral infections and explain why chronically infected pancreatic β cells, but not α cells, are targeted by an autoimmune response and killed during the development of T1DM. These issues and attempts to target viral infection as a preventive therapy for T1DM are discussed in the present Review.

Type 1 diabetes mellitus (T1DM) arises when the pancreatic β cells undergo long-term autoimmune attack, killing the majority of the β-cell population while the neighbouring α cells and δ cells are spared[1]. Destruction of the β cells manifests as a failure to produce insulin; consequently, patients with T1DM remain insulin-dependent for their lifespan.

In most cases, T1DM is characterized by pancreatic islet inflammation (insulitis) and progressive β-cell loss by apoptosis[1,2]. Histological analysis has demonstrated the presence of increased β-cell apoptosis among both patients with new-onset T1DM and those with

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Competing interests statement

The authors declare no competing interests.

Review criteria

Relevant publications were identified by searching the PubMed database using combinations for the following search terms: “diabetes”, “type 1 diabetes”, “pancreatic β cells”, “pancreatic islets”, “virus”, ”coxsackievirus”, “diabetes candidate genes”, “MDA5”, “RIG-I”, “TLR3”, “PTPN2”, “TYK2”, “GLIS3”, and “STAT1”. A manual search of some references cited in these papers, or in relevant articles related to the pathogenesis of diabetes mellitus, was also performed. All selected papers were English-language, full-text articles. Many of the references identified could not be included owing to space restrictions.

T1DM of long duration[3,4]. The defective insulin release characteristic of T1DM reflects progressive β-cell destruction in the range of 60–100%, depending on disease duration[4–7], as well as functional defects (for example, defective glucose-induced insulin release and delayed conversion of proinsulin to insulin[2,8]) that are probably caused by local release of proinflammatory mediators by infiltrating immune cells[2,9,10]. Pancreatic pathology among patients with T1DM is heterogeneous, with varying degrees of insulitis and β-cell loss observed in different lobes of the pancreas[1,10].

The genetic basis of T1DM is well established, with >50 candidate genes identified to date that explain ~80% of disease heritability[11,12]. Nevertheless, the estimated 1.5% annual rise in the incidence of T1DM in high-income countries[1,13], the observation that migration changes the risk of T1DM according to the country of residence[14], and differences in the penetrance rate between genetically similar populations, including monozygotic twins[15], all point to the contribution of nongenetic variables in the pathogenesis of this disease. The triggering of T1DM, therefore, probably depends on environmental factors that interact with predisposing genes to induce an auto-immune assault against the pancreatic β cells[2,12]. Among the potential environmental factors, epidemiological, clinical and pathology studies in humans support a role for viral infections, particularly by enteroviruses (for example, coxsackievirus), as triggers for the development of T1DM[16] (BOX 1).

In this Review, we will discuss potential mechanisms by which enteroviruses could contribute to the specific destruction of pancreatic β cells in T1DM, focusing on data obtained in clinical studies and human samples. Emphasis is given to the role of enteroviruses in the induction of insulitis and T1DM; how candidate genes for T1DM modulate the host response to the viral infection; and why pancreatic β cells are particularly susceptible to these infections. This issue is timely given that major advances in this area have occurred in the past 5 years, providing support for the role of viruses in the pathogenesis of T1DM.

Viruses as environmental triggers

The growing evidence for the role of viruses in T1DM has been examined in detail elsewhere[16–20]; therefore, this aspect will be only briefly discussed here.

The most striking evidence comes from cases of fulminant T1DM, which are particularly prevalent in Japan. In contrast to the slow progression and auto-antibody positivity usually observed in classic T1DM, fulminant T1DM is characterized by an abrupt onset of insulin-deficient hyperglycaemia and ketoacidosis among individuals without detectable autoantibodies[21]. The high prevalence (>70%) of preceding common-cold-like and gastrointestinal symptoms strongly suggests an infectious origin for fulminant T1DM[21]. Indeed, the onset of fulminant T1DM has been reported during acute infection with mumps, parainfluenza, human herpes virus 6 and enteroviruses[21]. The intense inflammation in the injured pancreas in response to the presence of viruses that is observed among patients with fulminant T1DM strongly suggests that pancreatic viral replication is linked to a devastating immune response, destroying not only β cells but also the surrounding exocrine pancreatic tissue[22].

A frequent association has been observed between classic T1DM and enterovirus infections. Epidemiological studies identified an increased incidence of T1DM following enterovirus epidemics[23]. Furthermore, enteroviral RNA has been detected in the blood of patients with newly diagnosed T1DM[24] and serological analysis confirmed a link between enteroviral infection and T1DM[25], particularly for the coxsackievirus B1 (CVB1) serotype[26]. The presence of anti-CVB1 antibodies was also associated with an increased risk of β-cell autoimmunity[27]. A meta-analysis of 24 studies that included a total of 4,448 participants reported a clinically significant association between the presence of molecular markers of enteroviral infections, autoimmunity related to diabetes mellitus and T1DM[28]. Of particular relevance, enteroviral RNA was detected by *in situ* hybridization in the islets of four patients with new-onset T1DM[29] and expression of the viral capsid protein VP1 was detected by immunohistochemistry in the islets of >60% of brain-dead organ donors with T1DM versus 8% among individuals without T1DM[30–33]. Only insulin-containing islets were positive for VP1, which indicates that coxsackievirus is able to infect β cells and to persist in the pancreatic islets of patients with T1DM. Coxsackieviruses isolated from pancreatic biopsy samples taken from six living patients with newly diagnosed T1DM failed to efficiently amplify *in vitro*, which suggests that despite persistent infection, the virus was poorly replicative[33].

A naturally occurring deletion at the 5′ terminus of the coxsackievirus genome enables chronic infection in mouse and human myocardium and in the pancreata of nonobese diabetic (NOD) mice, a model of spontaneous autoimmune diabetes mellitus[34,35]. Viruses harbouring this deletion are less replicative than wild-type viruses and are noncytopathic[35]. Whether this deletion is present in the VP1-positive β cells of patients with T1DM remains unclear. This 5′ terminus deletion could explain the long-term noncytopathic persistence of coxsackie-virus in the pancreatic islets, contributing to the β-cell autoimmunity characteristic of T1DM.

Finally, the absence of insulitis in 60% of insulin-containing VP1-positive islets[36], and in pancreatic tissue from an autoantibody-positive nondiabetic child[37], suggests that viral infection precedes the onset of insulitis. The link between coxsackievirus infection and T1DM is thus more likely to be causal than opportunistic.

Autoimmunity and β-cell death

Loss of β cells and triggering of insulitis after viral infection could result from several nonexclusive mechanisms. During the acute phase of infection, these effects might be a direct consequence of viral amplification and an excessive antiviral immune response to destroy infected cells among genetically susceptible individuals. This putative cell destruction is particularly deleterious because of the limited capacity of human β cells to proliferate[38] and thus compensate for their loss. Later on, progressive β-cell loss might be secondary to the activation of autoreactive[39,40] and bystander $CD8^+$ T cells[41,42], leading to progressive destruction of insulin-producing cells in the course of a chronic autoimmune assault. Conversely, viruses such as lymphocytic choriomeningitis virus or the coxsackievirus B3 (CVB3) serotype seem to protect against T1DM in animal models by either reducing or abrogating the autoimmune process[43,44]. Lymphocytic choriomeningitis

virus and CVB3 do not inflict any damage on β cells and protect against T1DM by triggering immunoregulatory mechanisms by at least two pathways. The first pathway involves upregulation of programmed cell death-1 ligand 1 on lymphoid cells, which prevents the expansion of diabetogenic $CD8^+$ T cells expressing programmed cell death-1. The second pathway enhances the number of $CD4^+CD25^+Foxp3^+$ regulatory T cells that produce TGF-β, which is crucial for the maintenance of immune tolerance in the periphery. The mechanisms leading to virus-induced autoimmunity and β-cell death are discussed in detail in the following sections.

Epitope spreading

During the acute phase of infection, viruses that specifically target pancreatic β cells replicate in these cells, directly leading to β-cell destruction and induction of a cytotoxic immune response[45,46]. With the exception of fulminant T1DM, the degree of β-cell loss is probably moderate during this phase, ending when the immune response arrests viral amplification[30]. Data obtained in mouse models suggest that β-cell loss might be alleviated to some extent (probably on a transitory basis) by conversion of α cells or precursor islet cells into β cells[47]. This effect might be secondary to *de novo* expression of key transcription factors for β-cell function, such as PDX1, PAX4 and NKX6.1 (REF. 47). During infection, the release of sequestered islet antigens could lead to presentation of β-cell antigens in the draining lymph nodes. If peripheral regulatory mechanisms fail, such antigen presentation will lead to epitope spreading[48]. In line with the relevance of virally induced islet-cell damage and consequent epitope spreading and development of autoimmunity, an epidemio logical analysis found a correlation between the pathogenicity of viral strains, the extent of the antiviral response and the incidence of autoimmunity[49].

The presence of viral markers in the islets of patients with T1DM, up to several years after disease onset, indicates that coxsackieviruses establish a persistent infection in the β cells[30–33]. This chronic infection is associated with low levels of viral replication, as indicated by the observations that only 5% of the endocrine cells per islet are VP1-positive[31]; the percentage of VP1-positive islets ranges from 2%[33] to 28%[31]; and the viral load obtained from pancreatic biopsy samples grown in culture is low[33]. Despite low levels of viral production, overexpression of the major histocompatability complex (MHC) class I protein is detected in both infected and noninfected β cells[31], which suggests that the presence of the virus affects all of the β cells within the infected islets. This overexpression of MHC class I is probably a consequence of local production of type I interferons[50] and consequent activation of the kinase TYK2 (REF. 51) (the product of a candidate gene for T1DM) and other downstream signals. MHC class 1 expression and presentation of β-cell derived peptides have a key role in islet-specific homing of $CD8^+$ T cells, as demonstrated in NOD mice[41]. Long-term overexpression of MHC class I proteins could lead to continuous presentation of β-cell epitopes to the immune system, increasing the risk of autoimmunity. Interestingly, several candidate genes for T1DM regulate key steps of this process (outlined in subsequent sections).

Bystander damage

Viral infection promotes the recruitment of natural killer cells and T cells to the islets[30] and the local production of inflammatory cytokines, particularly INF-α, INF-β, IFN-γ, tumour necrosis factor (TNF) and IL-1β[52]. The essential role of these cytokines in β-cell destruction has been demonstrated in NOD mice and rat models of diabetes mellitus[53–57]. The molecular mechanisms have been extensively studied and involve the induction of endoplasmic reticulum stress and activation of the intrinsic pathway of apoptosis in islets obtained from both patients with T1DM and rodent models of the disease[2,58]. Local production of cytokines thus contributes to β-cell destruction and the spreading of β-cell epitopes.

Molecular mimicry

The molecular mimicry hypothesis reflects potential crossreactivity between viral protein epitopes that share homology at the amino acid sequence level with host islet proteins targeted by autoimmune T lymphocytes. Homologies have been predicted between pancreatic autoantigens and viral proteins, including those expressed by coxsackievirus[59–61]. Nevertheless, attempts to detect crossreactivity between autoimmune antibodies or T-cell clones and coxsackievirus epitopes have failed[48,62,63], arguing against epitope mimicry as a crucial factor in coxsackievirus-induced T1DM. By contrast, crossreactivity is observed with cytomegalo virus[61], but no strong epidemiological evidence exists to support a role for cytomegalovirus infection in T1DM. Interestingly, crossreactivity between viral epitopes and self-epitopes can augment (but not initiate) autoimmune disease in the context of repeated viral infections in a transgenic mouse model that expresses a viral antigen in the pancreatic β cells and thymus[64]. This finding suggests that epitope mimicry induced by recurrent viral infections might contribute to late events that lead to T1DM; namely, acceleration of the disease once autoimmunity (as evaluated by the development of islet autoantibodies) is already present. However, it remains to be clarified whether this mechanism is relevant for humans.

Bystander activation

Bystander activation is characterized by T-cell activation that does not involve specific recognition of a peptide presented to the T-cell receptor. During infection of cells adjacent to the β cells, secretion of proinflamma-tory cytokines by dendritic cells could initiate bystander activation among circulating naive islet-specific T cells in pancreatic islets or lymph nodes, thus accelerating β-cell destruction[48,65–67]. Potential adjacent cells include exocrine, endothelial, neuronal and islet α cells[68]. In line with this possible role of immune and adjacent cells, infection of the islets of NOD mice with the CVB1, CVB3 or CVB4 serotypes of coxsackievirus accelerates development of diabetes mellitus in this model[45,69,70]; however, this effect depends on the presence of a threshold number of autoreactive T cells in the islets[71].

The important role of IFN-α production has been underlined among patients infected with hepatitis C virus who were treated with IFN-α for up to 1 year. This long-term treatment with IFN-α increases the risk of developing T1DM by 10-fold to 18-fold[72–74]. Onset of T1DM is abrupt and irreversible for most patients (98%), indicating a rapid and complete

loss of β cells. Whether systemic treatment with IFN-α stimulates epitope presentation in islets and/or bystander activation of β-cell-specific T cells or alternative deleterious mechanisms remains to be determined.

Candidate genes

In the course of an infection, damage to the host can be a consequence of the invading microorganism, the host response to infection, or both. This observation suggests that the host–microorganism interaction must be examined to fully understand diseases with an infectious component. For example, pulmonary tuberculosis will develop in only 10% of all individuals infected by *Mycobacterium tuberculosis*; in these individuals, an excessive inflammatory response damages the lung tissue[75].

This situation is particularly relevant when considering an autoimmune disease such as T1DM. In most cases, the pathogenic role of the virus during the development of T1DM does not require massive lytic replication in the islet cells. Instead, T1DM occurs in the presence of a persistent low-grade infection that triggers different degrees of inflammatory response and consequently different degrees of β-cell damage and antigen release. Not understanding this nuanced context has led, in our view, to a misguided focus on identifying specific viruses that are present among patients with T1DM but not normoglycaemic individuals. One characteristic that can modulate an individual's response to viral infection is the genetic background. Here, we discuss candidate genes for T1DM.

Genome-wide association studies

Genome-wide association studies (GWAS) have identified >50 naturally occurring genetic variants (risk-conferring single-nucleotide polymorphisms (SNPs)) that are linked to T1DM susceptibility and explain ~80% of the heritability[11,76].

The MHC complex displays the highest odds ratio (>6.5) for T1DM; however, most of the genetic loci identified by GWAS confer only a modest risk of developing this condition (odds ratio <2.0)[76]. MHC-related genes are predominantly associated with the risk of developing autoimmunity. By contrast, the other T1DM risk alleles probably regulate the anatomical location of the autoimmune attack (for example, targeting the β cells in T1DM or the joints in rheumatoid arthritis), as well as evolution from autoimmunity to the disease state and the speed of this process (the time between the appearance of islet autoantibodies and the onset of symptomatic T1DM)[77]. Of note, families predisposed to T1DM exhibit an increased innate inflammatory state, which might reflect genetic variants that potentiate immune pathways independent of autoantibodies or HLA status[78]. Many of these polymorphisms are shared with other autoimmune diseases, particularly those associated with the development of autoantibodies[79], but not with type 2 diabetes mellitus.

The general assumption is that candidate genes for T1DM modify disease risk by acting at the level of the immune system[80]. However, data published in the past 4 years suggest that human pancreatic β cells express mRNA for >80% of the T1DM candidate genes[12,51,81,82], which suggests a role for these genes in both the immune system and the target β cells. In line with this hypothesis, comparison of SNP locations against chromatin maps for different

cell types indicates a primary signature of T1DM-related SNPs in the promoter regions of candidate genes in both T cells and pancreatic islets[83].

Functions of candidate genes

Candidate genes for T1DM might contribute to disease by regulating antiviral responses, innate immunity, activation of apoptosis or the β-cell phenotype[12,51,84–89] (FIG. 1). The potential roles of T1DM candidate genes at the levels of the immune system and the β cell have been reviewed elsewhere[12,80]. Here, we focus on emerging information on genetically regulated pathways that modulate antiviral responses in pancreatic β cells.

As shown in FIG. 1, the cellular response to viral infection starts by recognition of the whole virus particle or some of its components (for example, double-stranded RNA (dsRNA)) by pattern recognition receptors, such as TLR3, RIG-I or MDA5, followed by local release of type I interferons. Secreted interferons bind to cell-surface receptors (IFNAR) in an autocrine and paracrine manner and activate specific kinases (TYK2 and JAK). These enzymes phosphorylate and activate key transcription factors (STATs), thereby inducing signal transduction pathways that establish an antiviral response in target cells via activation of interferon-stimulated genes[90].

Type I interferons—A role for type I interferons in human T1DM is evidenced by the fact that these cytokines and their downstream genes are expressed in pancreatic islets isolated from patients with T1DM[16,91], and that a type I interferon signature is present in the peripheral blood of both children genetically at risk of T1DM[92] and individuals with T1DM[93]. Transient blockade of either the type I interferon receptor or expression of Ifn-α by dendritic cells before the onset of insulitis markedly decreases the incidence of diabetes mellitus in NOD mice[94,95]. By contrast, NOD mice lacking a functional type I interferon receptor develop diabetes mellitus at the same rate as wild-type NOD mice[96]. A possible interpretation of these apparently contradictory findings is that viral infection and the subsequent production of type I interferons favours autoimmunity (and eventually T1DM) if the expression of interferons is induced at early and critical points during the development of this condition.

MDA5—A clear association exists between risk alleles for T1DM, viral recognition and interferon signalling pathways[12] (FIG. 1). SNPs leading to decreased expression of *IFIH1* (or *MDA5*) protect against T1DM[97,98]. Conversely, risk alleles in *MDA5* promote rapid progression to T1DM compared with protective *MDA5* alleles (31% and 11% within 5 years, respectively)[99]. *MDA5* risk alleles are also associated with the development of autoantibodies targeting β cells[100].

MDA5 is a cytoplasmic receptor for viral dsRNA. This receptor is expressed in human pancreatic islets and its mRNA expression levels are upregulated by enterovirus infection or exposure to a synthetic viral dsRNA molecule[85]. Inhibition of *MDA5* mRNA expression in human and rodent pancreatic β cells by specific small interfering RNAs (siRNAs) decreases the chemokine expression and release that is usually induced in response to synthetic viral dsRNA[85], potentially decreasing the homing of immune cells during insulitis. Mice with reduced levels of Mda5 expression exhibit a unique antiviral profile of type I interferons and

induce a regulatory, rather than an effector, T-cell response during viral infection, thereby preventing immune-mediated diabetes mellitus[101].

TYK2—SNPs in *TYK2* — the gene that encodes the kinase that acts downstream of type I interferon signalling — are associated with systemic lupus erythematosus, multi ple sclerosis, rheumatoid arthritis and T1DM[102,103]. The SNP rs2304256:C>A is located in exon 8 of *TYK2* on chromosome 19p13.2 and is thought to protect against T1DM. This variant causes a missense mutation in the TYK2 protein that decreases its interaction with IFNAR1 (REF. 104), as well as downstream signalling[51]. Inhibition of TYK2 by specific siRNAs in human β cells exposed to synthetic viral dsRNA decreases activation of the type I interferon pathway, lowering production of IFN-α and the chemokine CXCL10. These cells also exhibit decreased expression of MHC class I proteins, a hallmark of early β-cell inflammation in T1DM, and are less susceptible to apoptosis induced by synthetic viral dsRNA than are β cells treated with a control (inactive) siRNA[51]. By contrast, a spontaneous mutation that reduces Tyk2 expression in mice increases their susceptibility to virus-induced diabetes mellitus[105], and a *TYK2* promoter variant that reduces kinase activity in the protein increases the risk of fulminant T1DM among Japanese patients[106].

Taken together, the available information on MDA5 and TYK2 suggests that SNPs that decrease biological function protect against autoimmune T1DM, which indicates that an excessive inflammatory response to viral infections contributes to autoimmunity and eventual T1DM among susceptible individuals. In this scenario, triggering of autoimmunity is secondary to the long-lasting presence of a noncytopathic virus in the β cells and the consequent protracted and/or excessive innate immune or inflammatory response. Conversely, in situations where the β cells are directly destroyed by the viral infection, as seems to be the case for fulminant T1DM[21,22], decreased activity of these early antiviral responses might be deleterious and so accelerate disease[106].

PTPN2—The gene encoding a phosphatase, *PTPN2*, is another candidate gene for T1DM[107,108]. The rs45450798 SNP accelerates progression to T1DM after the appearance of β-cell autoantibodies[100]. PTPN2 has an important role in the modulation of interferon signalling in β cells[84–86]. Inhibition of *PTPN2* expression in β cells increases activation of STATs and augments apoptosis induced by IFN-α, IFN-β and IFN-γ via activation of the BH3-only protein BIM, particularly in its phosphorylated form (P-BIM), and subsequent triggering of the mitochondrial cell death pathway[84–86]. These observations suggest that SNPs that decrease *PTPN2* expression sensitize β cells to apoptosis secondary to local inter-feron production in response to viral infection, and that the T1DM-associated risk allele of *PTPN2* reduces the levels of its mRNA expression[109].

Candidate gene networks—Some evidence indicates that *BACH2,* another candidate gene for T1DM, regulates expression of *PTPN2* in β cells[89]. This finding reinforces the hypothesis that the risk of triggering T1DM during a viral infection depends on the presence of susceptibility variants in multiple genes interacting in pathways that leave individuals over-responsive to β-cell viral infections or to other danger signals[12,85,110]. In line with this possibility, an integrated analysis of gene networks and DNA sequence variations in T1DM identified the inter-feron regulatory factor 7 (IRF7)-regulated gene network (also known as

IDIN, for ‘IRF7-driven inflammatory network’) as a major contributor to T1DM risk[111]. MDA5 initiates virus-induced chemokine production via activation of the transcription factors IRF3, IRF7 and NF-κB[112], whereas PTPN2 and USP18 (a member of the IDIN network) provide negative feedback by inhibiting activation of the STAT signal transduction pathway and preventing β-cell apoptosis induced by interferons[84–86,113].

The hygiene hypothesis

In addition to genetic background, early education of the immune system by microbial exposure during childhood can influence an individual's response to viral infection.

Accumulating data indicate that the progressive decrease in infections among high-income countries, which has occurred secondary to improved sanitation, socioeconomic status, vaccination and the use of antibiotics, might be an important contributory factor to the increased incidence of allergic and autoimmune diseases (for example, T1DM, coeliac disease and multiple sclerosis) reported in the past 50 years. This observation led to the hygiene hypothesis, which suggests that decreased microbial exposure in early childhood increases the risk of allergic and autoimmune diseases[114–116].

Definitive proof for the hygiene hypothesis based on intervention trials in humans is still lacking; however, epidemiological and experimental data from rodents support this theory[116–118]. For instance, infections with *Schistosoma mansoni, Trichinella spiralis* or *Mycobacterium bovis* prevent spontaneous diabetes mellitus in NOD mice[117]. Possible reasons why early decreases in microbial exposure increases the risk of autoimmune diseases include defective development of the regulatory T cells that produce TGF-β and IL-10 and have the capacity to prevent excessive innate and adaptive immune responses[116,118,119]; lack of microorganism-induced maturation of dendritic cells that favours the development of regulatory T cells[120]; and modifications in the gut microbiota[121]. Immunoregulatory mechanisms triggered by viral infections in NOD mice protect against diabetes mellitus through both increasing the number of regulatory T cells and preventing expansion of diabetogenic $CD8^+$ T cells[43,44].

The first indication of autoimmunity against β cells (the appearance of islet autoantibodies) occurs before 12 months of age among children who will eventually develop T1DM[122–124], which suggests that a putative protective effect of nonspecific infections and consequent immune system ‘education’ should take place before the first 6 months of life or even *in utero*[125].

We propose that the putative role for viral infections in triggering insulitis and T1DM must be understood in the context of genetic predisposition and/or defective immune system education secondary to decreased infections in early life. Indeed, if we take into account the variables involved — namely, the host–microorganism interactions determined by the nature of the virus invading the β cells; the genetically determined and immune-education-determined inflammatory responses by islet and immune cells; and a putative interaction between these factors (FIG. 2) — we can envisage different scenarios for the role of environmental pressure on the increasing incidence of T1DM. Thus, in a population with

high genetic risk of T1DM, removal of a protective factor, such as nonspecific infections in early life, might be sufficient to increase disease risk. Conversely, in populations with low genetic risk of T1DM, a combination of protection removal and increased infections by coxsackieviruses might be required for increased prevalence of T1DM. If this hypothesis is correct, one should not expect that similar environmental factors are driving the observed increase of T1DM incidence in different parts of the world.

Viral infection — why the β cells?

As discussed above, putative viral infection of the islet cells might induce local inflammation and potential presentation of β-cell antigens (native or modified) to the immune system. Such viral infection and antigen presentation could be the starting point of autoimmunity, eventually leading to T1DM[126]. A crucial unanswered question in this context is why only β cells become infected and then targeted by the immune system, whereas pancreatic α cells remain mostly unaffected by both viruses and autoimmunity[30–32].

The host defence against microorganisms in vertebrates is usually considered to be the task of specialized immune cells, a view that underestimates the capacity of nonimmune cells to activate self-defence or cell autonomous immune responses against infection. The required mechanisms are present at a basal level in many cell types but are upregulated upon viral infection[127,128]. These responses rely on detection of microbial signatures by pattern recognition receptors[127]. By comparing microarray and RNA sequencing data obtained in human islets[81,129] with known pattern recognition receptors and other antiviral or antibacterial factors[127,130], we found that human islets exposed to virus or cytokines express several antiviral proteins (TLR3, MDA5, RIG-1, APOBEC36, SAMHD1, TRIM22, CNP, TETHERIN and VIPERIN), whereas antibacterial factors (TLR4, NLRP1, CLEC6A and CLEC7A) are poorly expressed, which suggests that islet cells are under evolutionary pressure to counteract viral but not bacterial infections, probably because they are seldom confronted with bacteria[68].

Rat α cells and β cells purified using fluorescence- activated cell sorting[131] are equally sensitive to apoptosis induced by cytokines or the viral mimic dsRNA; however, these cells display differing permissiveness to replication of the coxsackievirus CVB4 and CVB5 serotypes[68], despite similar expression of the viral receptors Car (coxsackie–adenovirus receptor) and Daf. Accordingly, adenovirus tagged with green fluorescent protein (a tool that enables researchers to count the number of infected cells and measure expression levels based on the intensity of fluorescence) infects α cells and β cells via CAR with similar efficacy but leads to a reduced expression of green fluorescent protein in α cells[68]. This finding suggests that adenoviruses can enter both cell types but express viral genes and/or replicates less efficiently in α cells than in β cells owing to efficient blocking of translation in the former cell type. Consistent with productive entry of coxsackievirus in α cells, a clear increase in expression of the viral protein VP1 occurs 8 h after infection in both α cells and β cells from dispersed human islets (FIG. 3a,b) and in the pancreata of two of three children who died during the course of an acute and severe coxsackievirus infection[68]. Noncytopathogenic infection by three different coxsackievirus strains has been reported in

both primary human α cells and β cells[50]. These infections were long-lasting in both cell types but production of INF-α was only detected in the β cells[50]. A potential concern is that islet dispersion could modify the infection and its outcome. However, coxsackievirus infection of α cells was detected in rat primary α cells purified using fluorescence-activated cell sorting[68], dispersed human islets[50,68], whole human islets[50] and in pancreatic islets obtained from acutely infected patients[68].

These observations suggest that the viral cycle is initiated in both α cells and β cells but that α cells control viral amplification, which leads to an abortive infection. This difference might explain why VP1 labelling is not detected in the α cells of infected human islets after long infection times[132,133] or among patients with T1DM[30,32]. The resistance of α cells to viruses could be the consequence of both lowered competence of these cells to replicate coxsackievirus, compared with β cells, owing to shortage in crucial cellular factors used by the virus and/or of a more efficient antiviral response in α cells versus β cells. Despite low expression of VP1, α cells induce a more rapid and marked antiviral response than do β cells and, in α cells, viral expression declines after 8 h of infection, which suggests that α cells take the control of the viral amplification whereas β cells do not[68]. Furthermore, an unrelated DNA virus (adenovirus) is also translated less efficiently in α cells that in β cells, which argues for an efficient antiviral response in α cells. Supporting the latter hypothesis, antiviral factors (Rig-1, Mda5, Pkr, Mx1 and Viperin), chemokines (Cxcl-10 and Ccl2), cytokines (Ifn-α) and downstream transcription factors (Stat1) that are required to control viral infections are more highly expressed in rat α cells than in β cells[68] (FIG. 3c), similar to the findings observed when comparing virus-resistant granule neurons with virus-sensitive cortical neurons (cortical neurons are preferentially destroyed during West Nile virus encephalitis, compared with granule cell neurons)[134]. This potent antiviral response remains to be demonstrated in human α cells. Considering that viral markers are present in the α cells of acutely infected patients[68], but not during chronic infection[30–33], it might be suggested that human α cells are also more efficient at clearing infection than human β cells.

In agreement with these observations, human β cells are also more sensitive than α cells to coxsackievirus-induced infection and functional impairment[30,32,132]. Taken together, these converging data suggest that pancreatic α cells and β cells have different cell autonomous signatures. This divergence could explain their differential ability to clear viral infections and potentially explain why chronically infected pancreatic β cells, but not α cells, are targeted by an autoimmune response and killed during T1DM[68,135].

Prevention of T1DM

The identification of key viruses involved in the development of T1DM might enable a preventive approach by vaccination[18,136]. Similarly, evidence for the persistence of a particular virus in β cells acting as a crucial trigger for T1DM might offer the possibility of pharmacological approaches to clear viral infection and prevent disease onset.

Vaccination

As discussed above, an excessive inflammatory response seems deleterious to virally infected pancreatic β cells. Thus, a vaccine developed to boost the immune response could

aggravate the problem. Ideally, a vaccine against coxsackievirus should generate a neutralizing immune response that aims to avoid the primary infection of target cells. That is, the vaccine should contain non-infectious (inactivated) viruses and be administered early in life. In line with this approach, vaccination of NOD mice with formalin-fixed CVB1 leads to an efficient production of neutralizing antibodies, which prevent both viral replication and the acceleration of diabetes mellitus onset in these animals[70].

Antiviral drugs

At present, no licensed drugs for the treatment of enter-oviral infection exist. Nevertheless, antiviral drugs that act at different levels of the viral cycle (reviewed elsewhere[137]) can successfully reduce enterovirus amplification. Pleconaril and its analogues bind to the viral capsid, thereby inhibiting entry of the virus in the target cells. Antiviral drugs such as guanidine hydrochloride efficiently interfere with viral replication; peptide and non-peptide inhibitors block the viral proteases 3C and 2A, whereas hydantoin prevents viral assembly. The availability of drugs targeting different steps of the viral cycle enables combined treatment to avoid viral resistance to the antiviral therapy. Some compounds have shown efficacy to reduce coxsackievirus amplification and to protect pancreatic islets from coxsackievirus-induced cytopathogenic effects[138–141]; to eradicate the virus in infected pancreatic β cell lines[142]; and to prevent and/or reduce the incidence of virally-induced diabetes mellitus in mice[140,141]. Adapted antiviral treatment at the pre-diabetic stage could eliminate persistent infection, thus reducing inflammation and the risk of autoimmunity and T1DM.

The problem remains to identify the correct target(s). A growing body of evidence suggests that infection by coxsackievirus B serotypes is an early event in the development of T1DM among genetically susceptible individuals[17,18]. Both insulin-positive and MDA5-positive islet cells are detected in patients with T1DM. This observation, coupled with local expression of VP1 (REF. 32), supports the idea of chronic and persistent low-level viral infection in the β cells[35,68].

Conclusions

Several viral infectious events probably contribute to auto-immunity and the progressive β-cell death that leads to T1DM. This contribution could occur at various stages during disease evolution and the eventual disappearance of the pathogenic virus after the onset of autoimmunity might complicate the goal of defining a unique causal virus. Identifying the viruses associated with the development of T1DM and determining their contribution to pathogenesis must be done in the context of genetic background. Furthermore, cell autonomous responses and putative differences in the early education of the immune system should also be considered as factors that might affect the degree of β-cell damage and the transition (or not) from innate immunity to adaptive immunity (and autoimmunity). Future research in the field should take these points into careful account. Indeed, only by tackling the pathogenesis of T1DM at its true level of complexity will it eventually be possible to develop preventive and curative therapies for this disease.

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Key points

- Viral infections — particularly by enteroviruses (for example, coxsackievirus) — have been implicated in the development of type 1 diabetes mellitus (T1DM)
- Many candidate genes for T1DM regulate antiviral responses in pancreatic β cells
- Pancreatic islet α cells trigger a more efficient antiviral response than β cells following infection with diabetogenic viruses, thus enabling α cells to eradicate viral infections without undergoing apoptosis
- An inability to clear viral infections could explain why chronically infected β cells, but not α cells, are targeted by an autoimmune response and killed during development of T1DM
- The identification of key diabetogenic viruses and the downstream mechanisms leading to insulitis might enable a preventive approach to T1DM by vaccination

Coxsackieviruses

Coxsackieviruses belong to the genus enterovirus in the *Picornaviridae* family. These nonenveloped viruses have a linear positive-sense single-stranded RNA genome. They are present worldwide and cause infections that are predominantly asymptomatic or associated with mild respiratory or gastrointestinal symptoms. In some cases, however, infection can lead to severe myocarditis or, as presently discussed, contribute to triggering autoimmunity against pancreatic β cells[143,144]. Among the two subtypes (A and B), only coxsackieviruses of the B subtype have been associated with the development of diabetes mellitus[143]. Six different serotypes (CVB1 to CVB6) are able to replicate in pancreatic β cells. The serotypes enter the β cells through CAR[29], a component of the tight junction. At present, neither specific treatments nor vaccination are available to prevent or cure coxsackievirus infections.

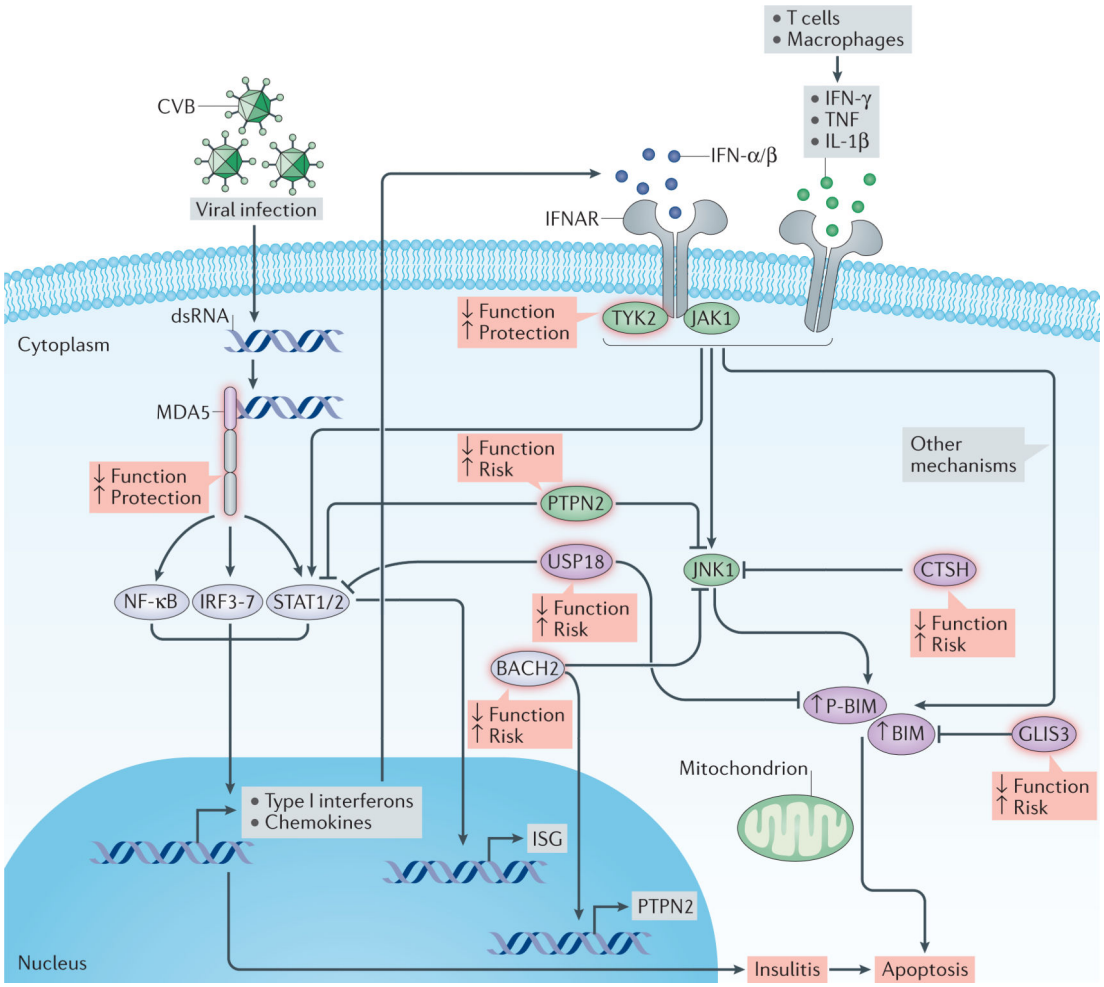

Figure 1. Regulation of key antiviral responses in pancreatic β cells

Following infection, replicating coxsackievirus subtype B (CVB) produce cytosolic double-stranded RNA (dsRNA), a nonphysiological form of mRNA recognized by the cytoplasmic receptor MDA5. Binding of MDA5 to the dsRNA activates the transcription factors NF-κB, IRFs and STATs, triggering production of type I interferons and chemokines, thus contributing to local inflammation (insulitis). Type I interferons (IFN-α and IFN-β), type II interferon (IFN-γ) and the cytokines TNF and IL-1β contribute to β-cell destruction among genetically susceptible individuals. Type I interferons bound to the IFN-α/β receptor (IFNAR) signal via TYK2 and JAK1 and induce activation of STATs and expression of interferon-stimulated genes (ISGs) with antiviral properties. Proinflammatory cytokines promote the activation of JNK1, which induces the intrinsic (mitochondrial) apoptotic pathway through the proapoptotic protein BIM and its phosphorylated form (P-BIM). PTPN2 modulates β-cell death induced by interferons by regulating activation of P-BIM via JNK1. BIM and/or JNK1 are downregulated by BACH2, GLIS3 and CTSH. PTPN2 also functions as a negative regulator of the STAT signalling pathway, whereas USP18 exerts negative feedback on interferon-induced STAT signalling and mitochondrial apoptotic pathways in β cells. Candidate-gene regulated factors implicated in type 1 diabetes mellitus are framed in red and the consequences of their modulated expression and/or activity on

biological function or type 1 diabetes mellitus risk indicated. Kinases and phosphatases are indicated by green ovals and transcription factors by grey ovals.

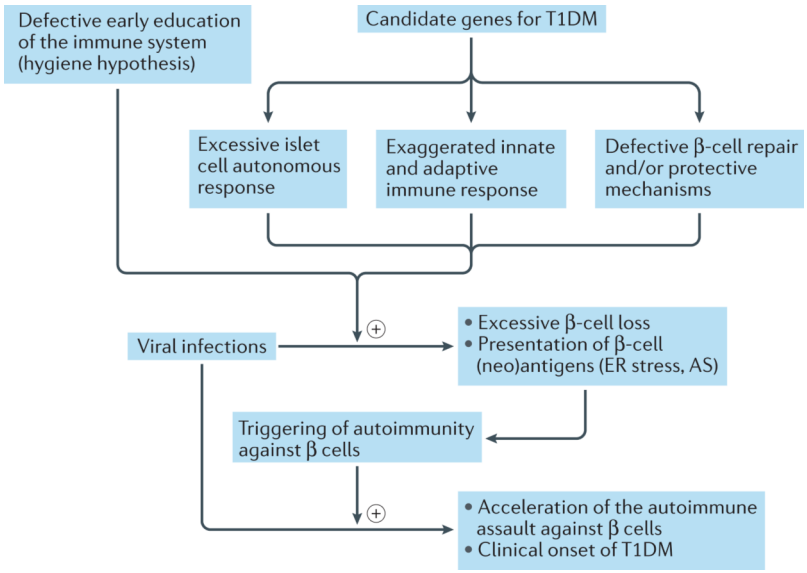

Figure 2. Crosstalk between viral infection, genetic background and early education of the immune system

Genetic background and early immune education, either alone or in combination, define the individual's capacity to modulate the cell autonomous response upon viral infection. These diverse responses to viral infection can lead to different outcomes, including excessive β-cell loss (with or without viral persistence) and triggering of an autoimmune response. Repeated viral infection might accelerate the ongoing autoimmune assault against β cells, culminating in clinical disease. AS, alternative splicing; ER, endoplasmic reticulum; T1DM, type 1 diabetes mellitus.

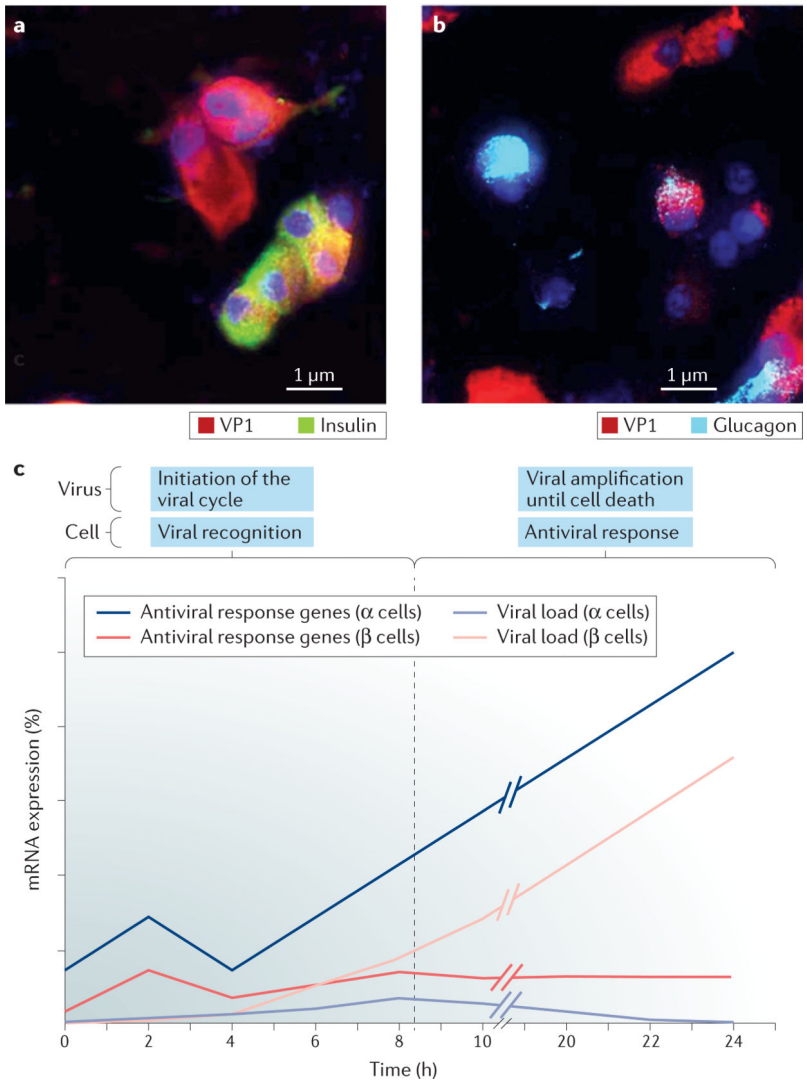

Figure 3. Differential autonomous antiviral response determines the outcome of infection in pancreatic α cells and β cells

a | and **b** | Viral protein expression in human islet cells infected by coxsackievirus B5 serotype (CVB5) for 8 h. Immunocytochemistry labelling of VP1 (red), insulin (green), glucagon (light blue) and nuclei (dark blue) indicates expression of the viral protein at an early time point of infection in both insulin-producing-β cells (part a) and glucagon-producing-α cells (part **b**). Scale bar, 1 μm. **c** | Time course of CVB5 proliferation versus expression of antiviral response genes in rat α cells and β cells[68]. Purified rat α cells and β cells were infected with CVB5 and examined at 1, 2, 4, 6, 8 and 24 h after infection. The mRNA expression levels of antiviral genes (*Stat1* and *Mx1*) and viral genes (*VP1*) are shown. Basal expression of antiviral genes is higher in α cells than in β cells and rapidly increases, which enables this cell type to eradicate the viral infection and survive. By contrast, β cells exhibit lower and less effective antiviral responses than α cells, which enables the viral load to increase and eventually kill the infected cells.