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## Enteroviruses and Type 1 Diabetes: Multiple Mechanisms and Factors?

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### Abstract

Type 1 diabetes (T1D) is a chronic autoimmune disease characterized by insulin deficiency and resultant hyperglycemia. Complex interactions of genetic and environmental factors trigger the onset of autoimmune mechanisms responsible for development of autoimmunity to  $\beta$  cell antigens and subsequent development of T1D. A potential role of virus infections has long been hypothesized, and growing evidence continues to implicate enteroviruses as the most probable triggering viruses. Recent studies have strengthened the association between enteroviruses and development of autoimmunity in T1D patients, potentially through persistent infections. Enterovirus infections may contribute to different stages of disease development. We review data from both human cohort studies and experimental research exploring the potential roles and molecular mechanisms by which enterovirus infections can impact disease outcome.

### Keywords

type 1 diabetes; enterovirus; islet autoimmunity; persistent infection

## INTRODUCTION

Type 1 diabetes (T1D) emerges after pancreatic islet  $\beta$  cells sustain prolonged autoimmune attack, leading to the loss of the majority of the  $\beta$  cell population. This results in a gradual loss of insulin production and disease onset (Figure 1). While genetic susceptibility has been well established in T1D, studies with monozygotic twins show only a 30–50% concordance rate, suggesting that genetics alone cannot cause disease and that environmental factors likely contribute to disease induction (1). Virus infection may contribute to the different stages of the disease (2) (Figure 1). In the actual triggering of  $\beta$  cell autoimmunity (defined by development of at least one islet autoantibody (IA), it is conceivable that viruses may be an etiological factor that precedes the appearance of a first islet autoantibody, the current

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others. Overall, more than 60 nonpolio enteroviruses cause disease in humans, and several of these are implicated in T1D, particularly the six serotypes of CVB viruses (2).

The link between enterovirus infections and development of T1D (formerly known as juvenile diabetes or insulin-dependent diabetes) has been studied since the 1960s, when Gamble et al. (11) reported a significant correlation between newly diagnosed diabetes patients and the annual prevalence data for Coxsackie type B4 (CVB4) virus and antibodies against CVB4 virus were found more often in diabetes patients (12). Since those early findings, significant efforts have been made to elucidate the specificity and temporality of this link. The current staging of T1D was not known at the time, nor was it understood that T1D might have been triggered years before clinical onset. In retrospect, it is not surprising that earlier serological studies inconsistently reported enterovirus antibodies to be more prevalent in T1D patients than in controls (13). However, many of the serological studies were limited by small sample sizes, enrollment of patients who were not matched for HLA alleles, or failure to differentiate between enterovirus serotypes. It was only after large prospective studies like the ones detailed below that serological evidence could be more carefully parsed in the context of the multiple variables that characterize the etiology and pathogenesis of T1D.

Enteroviruses have been found in the blood, gut, and pancreas of T1D patients in several studies, and they have also been associated with increased risk of T1D in prospective studies (14-17). Patients with T1D harbor enterovirus RNA in their peripheral blood mononuclear cells (PBMCs), and enteroviral RNA is more frequently found in the blood of patients newly diagnosed with T1D than in healthy individuals (18, 19). Studies have found that the presence of CVB RNA is associated with elevated levels of interferon  $\alpha$  (IFN- $\alpha$ ) in the blood of patients at various stages of T1D but not in individuals without the disease (20).

In addition to virome analyses detailed below, a few studies have found virus in gut tissue. For example, enterovirus was detected in small-intestine biopsy samples from patients with T1D, much more often than in samples from healthy individuals (21). These findings were confirmed in stools of a larger sample group by the same authors (22).

Although most data indicate associations between enterovirus and T1D outcomes, many other viruses have been implicated in T1D. Most of these older studies related findings to the time of clinical onset of the disease, not taking into account the prodrome of islet autoimmunity or the etiology of autoimmune islet disease. In addition, recent reports suggested a role for rotavirus, since introduction of routine rotavirus vaccination into the infant population was associated with a drop in the incidence of T1D (23,24). However, another meta-analysis of many other childhood vaccinations found no evidence of association between routine vaccinations and childhood T1D (25).

The COVID-19 global pandemic also raised questions concerning coronavirus involvement in syndromes involving extrapulmonary sites. Two reports examined human pancreas, islet single-cell RNA sequencing data sets, and COVID-19 autopsy material and found that expression of the viral ACE2 (angiotensin converting enzyme-2) receptor and TMPRSS2 (transmembrane protease serine-2) cofactor was absent in  $\beta$  cells and present in only some

ductal cells (26, 27). This indicated that direct infection of pancreatic  $\beta$  cells was unlikely due to lack of viral entry factors on  $\beta$  cells. It is possible that any interactions of diabetes and COVID-19 may be due to systemic inflammation or metabolic changes in other organs.

## PROSPECTIVE COHORT STUDIES

Over the last 20 years, several prospective studies have addressed the potential association between enteroviruses and T1D. Some, but not all, of the longitudinal efforts have found associations; the longitudinal studies (reviewed in 28) include DiMe, DIPP, TRIGR, BABYDIAB, DAISY, and MIDIA. Comparing these studies indicated a large range in the number of subjects followed, sample intervals used, types of virus assays, and types of study endpoints for autoimmunity seroconversion or clinical T1D (28, 29). Because of this variance, and perhaps also due to the small numbers of subjects and how they were selected at birth or later in life, the results have been controversial. More importantly, the frequency of sample collection to detect virus RNA has varied. Virus RNA is short-lived in plasma and found in stools for only 1–2 weeks after infections start in most children. A critical review of longitudinal studies completed before 2011 found inconsistent associations of enteroviruses with  $\beta$  cell or islet autoimmunity (defined by the appearance of one or several islet autoantibodies) or T1D and called for larger studies using frequent sampling intervals and the collection of multiple types of specimens to more accurately search for viruses (29).

The endpoints chosen to evaluate viral associations with disease outcomes are critical because there is growing recognition that both T1D and T2D may include variant forms of diabetes along a spectrum of etiologically based classification (30). These variants, often referred to as endotypes, may be distinguished on the basis of genetic risk factors, biomarker features such as autoantibodies or other plasma proteins, or distinct responder characteristics following treatment. The large TEDDY (The Environmental Determinants of Diabetes in the Young) study collected data from >8,500 newborns in a cohort followed for up to 15 years. TEDDY combined genotypic SNPs and phenotypic patient characteristics to define different T1D development pathways in children that were distinguished by the initiation of islet autoimmunity and subsequent risk of developing clinical diabetes (31). Development of islet autoantibodies (IA) usually involves one or more of several autoantibodies that commonly develop against cytoplasmic proteins in the beta cell: insulin autoantibodies (IAA), autoantibody against glutamic acid decarboxylase (GADA), autoantibody against protein tyrosine phosphatase (IA-2A), and zinc transporter 8 (ZnT8). Indeed, the TEDDY study discovered two endotypes of which autoantibody was first to be detected. In one- to four-year-olds and decreasing thereafter, IAA appeared first and primarily in children with HLA-DR4-DQ8. GADA appeared as the first autoantibody, primarily in HLA-DR3-DQ2 children, at 3–4 years of age but did not decrease with increasing age (31, 32). A key finding was distinct factors were associated with evidence of islet autoimmunity that were different for each respective autoantibody. Moreover, these factors did not necessarily correlate with those that predicted the progression from autoimmunity to diabetes. An underlying hypothesis is now emerging that seroconversion to either GADA or IAA as the first islet autoantibody seroconversion may follow independent and unique exposures to different viruses, and conversion from either of the two autoantibodies to T1D also may be independent.



are required to further elucidate temporal associations between virus exposure in mothers and IA or T1D in their offspring. These novel approaches should prove useful to further understand the overall contribution of enterovirus and perhaps other viruses to T1D.

Because enterovirus is only transiently detected in stool, studies have moved to examination of multiple sequential stool samples to have a better chance of detecting virus infections in children. The DIPP study used polymerase chain reaction (PCR) combined with sequencing analysis of 4,781 longitudinal stool samples from 411 children and determined that cases had more enterovirus infections than controls (0.8 versus 0.6 infections per child) and that this excess of infections in cases occurred more than one year before the first detection of islet autoantibodies. Interestingly, the most frequent viruses were EV-A serotypes (22).

The largest multicenter TEDDY cohort study examined 8,654 sequential stools from 383 children in an age-matched cohort with metagenomic sequencing, using IA seroconversion as the outcome. The results confirmed that EV-B infections were associated with islet autoimmunity (41). More significantly, the type of enterovirus infection was important. Prolonged infection, typified by finding the same virus in sequential stools over months, was strongly associated with islet autoimmunity, whereas short EV-B infections without prolonged shedding of virus in stools were not associated (41). The stool sample observations are important to future investigations to relate prolonged infection to serum or plasma, perhaps PBMCs, viremia, nasal swabs, and possibly saliva to detail the appearance of neutralizing virus antibodies to IA seroconversion.

### **Evidence for Enterovirus Infection in Human Pancreas**

Although in vitro infection of human islets with CVBs has been reported many times, access to human pancreas samples is very limited and dependent on pancreas tissue collected through procurement organizations such as the Network for Pancreatic Organ Donors with Diabetes (nPOD) and the Nordic Network for Islet Transplantation. However, multiple lines of evidence using these tissue sources indicate that direct infections of the pancreas by enteroviruses are not rare. Examination of human pancreatic specimens detected cells immunostained for enterovirus VP1 in multiple islets of almost 60% of 72 young patients with recent-onset of T1D compared with weak signals in a few islets in 6% of 50 individuals without the disease (42). In addition, enterovirus RNA has been detected by in situ hybridization in multiple postmortem pancreas samples from patients with T1D but not in samples from individuals without the disease (16). Viral particles and enterovirus VP1 capsid protein detected by immunocytochemistry were reported in  $\beta$  cells in pancreatic tissue from three of six organ donors with T1D but not in the pancreatic tissue of 26 organ donors without T1D (43). However, viable enterovirus has only rarely been recovered from pancreas samples (43), which may also result from persistence of replication-impaired virus discussed below.

The Diabetes Virus Detection (DiViD) study in Norway enrolled six adults with recent-onset T1D who agreed to undergo voluntary surgery to allow pancreatic biopsy of tissue for study (44). Examination of this explanted tissue revealed low-grade enterovirus infection in the islets in four of six patients through detection of both VP1 capsid antigen and viral RNA. Infected islets were rare; only ~2% of islets contained VP1 (45). In addition, all six patients



had insulinitis, but the majority of T cells were associated with peri-insulinitis rather than within the islet parenchyma. Also, many IFN-stimulated genes (ISGs) were overexpressed by at least fivefold in islets affected by insulinitis in the DiViD patients compared with islets from nondiabetic organ donors. Most of these overexpressed ISGs, including *GBP1*, *TLR3*, *OAS1*, *EIF2AK2*, *HLA-E*, *IFI6*, and *STAT1*, were overexpressed in the islet core rather than in the peri-islet area containing immune cells (46). In contrast, the T cell attractant chemokine *CXCL10* was overexpressed in the peri-islet area instead of the islet, partly explaining T cell recruitment to this region. In summary, insulinitic islets from the six recent-onset T1D subjects showed overexpression of ISGs, with expression patterns similar to that of islets from mice infected with virus or exposed to interferons (46-48).

Finally, both in DiViD and in other human pancreatic tissue banks, HLA class I antigens were found to be hyperexpressed throughout the islets containing at least one infected cell. This was determined by HLA detection at the protein and RNA levels, which is also associated with an increased STAT1 expression. Taken together, HLA class I and STAT1 expression may be interpreted as contributors to the pathogenesis of T1D (49). In addition, viral RNA was detected by in situ hybridization in islet cells from T1D patients from tissue banks (50). It is not likely that additional patients with newly diagnosed T1D will be asked to volunteer a pancreatic biopsy study in the future because of medical risks. Future investigations will therefore depend on pancreas tissue collected through procurement organizations such as nPOD and the Nordic Network for Islet Transplantation, whose activities are critically important for further progress in understanding virus infection of islets.

If it is considered that the clinical onset of T1D is the endpoint of a prodrome that has lasted for months to years, it is remarkable that enterovirus may be found in the pancreatic islets, the target organ for  $\beta$  cell destruction. The question is whether (a) the enterovirus presence represents a chronic infection and enterovirus has been dormant, inactivated, or slowly replicating since the time of an etiological infection, or (b) the enterovirus represents an accelerator of T1D pathogenesis promoting  $\beta$  cell killing, resulting in insulinitis and clinical onset of the disease.

## **LIFESTYLE AND LOCATION MATTER: WHERE DO ENTEROVIRUSES TRIGGER ISLET AUTOIMMUNITY OR T1D?**

### **Persistent Infections**

Classically, enteroviruses are thought to cause primarily acute infections, and their replication and spread are believed to require cell lysis. This view has been seriously challenged in the last 20 years with growing awareness that persistent or chronic enterovirus infections occur much more commonly than previously thought. One report indicated that persistent infections lasting up to 30 days could be established in isolated human pancreatic islets in vitro (51). However, molecular mechanisms for viral persistence were lacking until a seminal report indicated that CVB3 could naturally generate 5' terminal deletions ranging in size from 8 to 49 nucleotides that eliminate parts of a key RNA cloverleaf structure (52) that binds host key proteins supporting RNA replication (53). The deletion results in

noncytolytic, slowly replicating virus (52). Although CVB is recognized to cause acute myocarditis, evidence of persistent virus infections was also obtained by finding virus in heart tissue from patients with dilated cardiomyopathy (54, 55). CVB with 5' terminal cloverleaf deletion was first isolated from the heart in a fatal myocarditis case (56) and then in other myocarditis case isolates (45, 46). Terminal deletion virus populations were studied in persistent murine cardiac infections and characterized by low ratios of positive to negative-sense RNA strands, a low production of infectious particles, and low viral protein synthesis activity (52). Other studies also showed that CVB3 can persist in murine pancreas through deletion of 5' terminal genomic sequences (57).

In addition to a mechanism for persistence in cells, the virus must have a mechanism for spreading between cells after the development of neutralizing antibodies if persistence is to be maintained longer than a few weeks. Cell lysis releases nonenveloped enterovirus, but recent discoveries indicate that enteroviruses commonly escape from infected cells without lysis by hijacking the cellular vesicular trafficking mechanisms and becoming quasi-enveloped within exosomes. Neighboring cells are constantly invaginating microvesicles, providing an efficient vehicle for cell-to-cell spread of enteroviruses without requiring cell lysis and avoiding exposure to neutralizing antibodies. Further, multiple virus progeny can gain infectious access to neighboring cells or macrophages within a single vesicle (58). The same mechanism is now known to enhance infectivity of enteroviruses and rotaviruses and possibly fecal transmissibility (59), and it has also been shown to mediate nonlytic enterovirus spread in a  $\beta$  cell line (60). In summary, mechanisms are now known to exist for both reduced nonlytic levels of replication and remarkably efficient cell-to-cell spread of these low levels of virus via nonlytic mechanisms.

### **Can Persistent Infections Lead to Islet Autoimmunity and Later T1D?**

Recent observations in the DIPP and TEDDY studies indicate that the risk for a first appearing islet autoantibody was independently associated with polymorphisms in the Coxsackie and adenovirus receptor (CXADR). New scenarios may therefore be contemplated. One is that  $\beta$  cells are infected by enterovirus but replication is slow and nonlytic and can spread slowly between islet  $\beta$  cells by exosomes rather than by infectious virus particles. Although IAA was the first appearing islet autoantibody primarily in HLA-DR4-DQ8 subjects, the next step toward the clinical diagnosis of T1D is the appearance of a second autoantibody. In children with a first islet autoantibody, 60% developed a second autoantibody within one year. If IAA was first, GADA was the most common second autoantibody. If GADA was first, IAA was the most common second autoantibody (61). Interestingly enough, there was no indication that the second appearing autoantibody was associated with HLA. In children with two or more autoantibodies, 70% progressed to clinical onset of T1D within 10 years. In children with a persistent single autoantibody, only 15% progressed to T1D within 10 years. It will therefore be important in longitudinal follow-up studies to determine the presence and characteristics of virus antibodies over time (e.g., neutralizing virus antibody titers as well as antigen specificity and affinity) to test the hypothesis that the prolonged and persistent nonlytic virus infection is contributing to a slowly progressive loss of  $\beta$  cells.



This and other scenarios need to be investigated, perhaps with more extensive PCR-based technologies, to follow chronic virus infections of innate cells such as monocytes or natural killer (NK) cells. As insulinitis does not seem to appear until T1D stage 2 (Figure 1), the final common pathway to  $\beta$  cell killing is likely preceded by other mechanisms. These mechanisms may include dormant enterovirus or other virus. Subsequent virus infections may accelerate the autoimmune process (29, 62). It cannot be excluded that biomarkers of infection may be more informative of these processes than biomarkers of inflammation.

### Enterovirus Infection of Antigen-Presenting Cells

Although considerable research focused on virus infection of  $\beta$  cells and its consequences, T1D is effectively an autoimmune disease resulting from loss of tolerance for self antigens. The development or acceleration of T1D depends on the balance between autoreactive effector T cells and regulatory T cells. This balance is particularly influenced by antigen-presenting cells (APCs) such as monocytes, macrophages, and dendritic cells (DCs). There has been comparatively little research on the consequences of enterovirus infection of APCs themselves. It was first shown that enteroviruses could replicate in primary human monocytes using poliovirus, an EV-C species. Virus yield was low and only 6% of primary monocytes could support infection, but when lymphocytes were co-cultivated, virus yields increased, suggesting higher replication in activated monocytes (62). This finding was extended to macrophages and DCs, where poliovirus was also found to replicate (63). When CVB was examined, early reports indicated that human monocytes were poorly infected (64), but human monocyte-derived macrophages can be infected by CVB4 in vitro, dependent on whether macrophages were derived with M-CSF or GM-CSF (macrophage- or granulocyte-macrophage colony stimulating factor). This finding indicated that specific gene expression profiles are required in macrophages to support virus replication (65). Another report indicated that echoviruses could infect human monocyte-derived DCs more productively than CVB strains tested. However, the investigators noted a substantial difference in replication between two serotypes of echovirus; EV7 was very cytolytic and produced no DC activation whereas EV1 produced lower virus replication with significant DC activation through production of ISGs, interleukin-6 (IL-6), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). CVB, in contrast, exhibited poor replication and poor activation (66).

The variable findings in the outcomes of infections of monocytes and DCs with the different enteroviruses in these studies may not be serotype dependent but rather strain dependent. This concept was illustrated when multiple clinical isolates of CVB1 were compared for replication and activation responses in human DCs and found to differ markedly in their capacity to induce innate immune responses in plasmacytoid DCs. Two of 18 strains produced strong IFN- $\alpha$  responses and accompanying cytokines (67).

Finally, in a different experimental concept, it was shown that phagocytosis of enterovirus-infected pancreatic  $\beta$  cells triggers innate immune responses and ISG induction in human DCs (68). ISG expression increased enough in DCs to produce an antiviral state. This was extended to an in vitro model for autoimmune T1D using enterovirus-infected  $\beta$  cells and human myeloid DC subsets. This report showed that enterovirus-infected  $\beta$  cells produce distinct response patterns in blood dendritic cell antigen (BDCA)1+ and BDCA3+ DCs;

however, they both promoted Th1 responses that could favor the induction or maintenance of  $\beta$  cell autoimmune reactivity. Much more work is needed in this area.

Beyond in vitro data described above, importantly, enteroviral RNA was found in peripheral blood monocytes of 7 of 42 patients with T1D. Further, negative-strand enterovirus RNA was found in monocytes of 6 patients, indicating active replication. IFN- $\alpha$  mRNA was detected in blood and in monocytes in 12 of 42 patients with T1D but not in monocyte-depleted PBMCs of the same individuals. Significant plasma levels of IFN- $\alpha$  ( $> 5$  IU/mL) were found in 6 patients. This suggested that monocytes of T1D patients can harbor enterovirus RNA, which may be replicating and stimulating IFN production (69). Also, both enhancing and neutralizing anti-CVB activities were examined in children, which followed from in vitro data showing an antibody enhancement of CVB infectivity of human monocytes (64). Sustained anti-CVB enhancing activity was observed in consecutive serum samples in patients with T1D in this small matched cohort. The pattern of responses differed between children who developed T1D and control children. In T1D patients, the anti-Coxsackie virus enhancing activity was predominant or even exclusive over the neutralizing activity, whereas in controls the enhancing and neutralizing activities were more balanced or the neutralizing activity was predominant (70). In summary, the impact of enterovirus infection on APC functions in immune activation or regulation is not well understood and remains a crucial open question in the search for viral triggering mechanisms.

## POSSIBLE ROLES OF ENTEROVIRUS INFECTION IN $\beta$ CELL DAMAGE

### Molecular Mimicry

The infiltration of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, B cells, and macrophages within the pancreatic islets and the presence of autoreactive T cells are also thought to play a role in disease development (71). The molecular basis of autoimmunity in CVB infection is proposed to be through molecular mimicry (Figure 2): The 2C nonstructural CVB protein has a shared sequence with the glutamic acid decarboxylase 65 (GAD65) enzyme, which is predominantly expressed in pancreatic  $\beta$  cells (72). A recent report shows a potential cross-reactivity between the 2C of CVB4 and GAD247–266 at the T cell receptor level in memory CD4 T cells in one patient (73). This suggests that exposure to CVB4 could indeed activate GAD-specific CD4 T cells via molecular mimicry. However, despite numerous studies, there is still a lack of strong evidence for this hypothesis. Also, in the TEDDY study, prolonged shedding of EV-B was associated with IAA as the first appearing autoantibody in HLA-DR4-DQ8 children (41). Levels of the metabolite  $\gamma$ -aminobutyric acid (GABA) were increased in these children prior to seroconversion (74). In contrast, other virus infections were likely to contribute to trigger GADA as the first appearing autoantibody, which primarily occurred in HLA-DR3-DQ2 children. Other metabolites, but not GABA, preceded GADA as the first autoantibody. Another hypothesis is that molecular mimicry does not act as a disease trigger but rather contributes to disease pathogenesis. In this context, mouse studies indicate that the expansion of previously primed autoreactive T cell populations via heterologous virus infections and molecular mimicry could lead to the acceleration of the disease in already prediabetic hosts (75). This indicates that pre-existing inflammation of the islets may be needed for the contribution of virus infections to molecular mimicry or to

disease pathogenesis in general. In this case, a careful examination is needed to identify the multiple factors that may contribute to disease.

### **Coxsackie Adenovirus Receptor Expression Variants**

The Coxsackie adenovirus receptor (CAR) is required for CVB entry into cells (Figure 2). The CAR gene produces five alternatively spliced mRNAs resulting in five isoforms of CAR, but only two have a transmembrane domain and are retained in cell membranes (designated CAR-SIV and CAR-TVV) (76, 77). The soluble CAR isoforms can inhibit CVB3 infection of cells by preventing virus attachment to membrane-bound CAR-SIV or -TVV (77).

Human  $\beta$  cells express CAR-SIV dominantly, but CAR is not in other islet cell types (78). Interestingly, CAR expression in  $\beta$  cells is heavily skewed toward insulin secretory granules, suggesting mechanisms for the sensitivity of  $\beta$  cells to virus infection.

In another study, CAR staining of islets was detected more frequently in patients with T1D and autoantibody-positive individuals than in controls (79). Further, the expression level of CAR was increased in explanted islets exposed to proinflammatory cytokines/chemokines produced by infected islets. These data indicate that the likelihood of CVB infection may increase in individuals with IA or T1D due to the higher induction of CAR expression by cytokines.

### **MicroRNA Dysregulation by Virus**

MicroRNAs (miRNAs) have been investigated extensively in the context of T1D, particularly in PBMCs of T1D patients (80). In addition, miRNAs have proved important in  $\beta$  cell function, B cell dysfunction, and autoantigen generation, and they may have utility as biomarkers (80). Recent studies have examined the effect of enterovirus infection on miRNA expression in ductal-like PANC-1 cells and human islets. A total of 81 miRNAs were dysregulated in PANC-1 cells persistently infected with CVB4. Forty-nine of the 55 known T1D risk genes were predicted as putative targets of at least one of the dysregulated miRNAs (81). In another study, the expression of 754 miRNAs in CVB5-infected human pancreatic islets were analyzed (82). In total, 33 miRNAs were significantly dysregulated in the infected compared with control islets. These differentially expressed miRNAs were predicted to target mRNAs of 57 known T1D risk genes that collectively mediate various biological processes, including the regulation of cell proliferation, cytokine production, the innate immune response, and apoptosis (82).

### **Production of Novel Defective Ribosome Products**

Major histocompatibility complex class I peptides are products of endogenous cellular protein degradation. These peptides are bound in the cleft between the heavy chain and  $\beta$ 2-microglobulin and form the trimolecular complex that interacts with the T cell receptor on CD8<sup>+</sup> T cells. Following the degradation of their source proteins—be they self-proteins or virus proteins—the trimolecular complex is used by host cells to alert the immune system during pathogen infection. Another source of these proteins can be defective ribosome products (DRiPs), which include polypeptides produced by premature

translation termination and misfolding (Figure 2). The high demand for insulin may lead the  $\beta$  cells to produce aberrant translation products that generate diabetogenic epitopes (83). An alternative open reading frame within human insulin mRNA encodes a highly immunogenic polypeptide that is targeted by T cells in T1D patients. T1D patients can have cytotoxic CD8<sup>+</sup> T cells directed against the N-terminal peptide of this nonconventional product that can kill human  $\beta$  cells and thereby may be diabetogenic. Additionally, environmental or viral factors leading to endoplasmic reticulum (ER) stress may control expression of an alternative reading frame as described for other stress-induced proteins (84). Indeed, human  $\beta$  cells seem exceptionally sensitive to ER stress (85). In addition to an effect on translation initiation processes, environmental stress may also have an impact on the degradation of insulin byproducts. Viruses disrupt translation at multiple levels through cleavage of translation factors by viral proteinases or by increasing or altering DRiPs (86, 87).

### Inflammation-Induced Bystander Damage

Several lines of evidence suggest involvement of IFN signaling in T1D pathogenesis. Before T1D onset, a type I IFN transcriptional gene signature is detected in PBMCs (88). Blood transcriptome analysis, followed longitudinally in 400 TEDDY children and validated in Finnish DIPP children, revealed an NK signal that was present at the time of seroconversion and lasted through stage 1 and stage 2 of T1D onset until clinical diagnosis (89). T1D patients have increased IFN- $\alpha$  levels within the pancreata and enhanced IFN- $\alpha$  synthesis from peripheral blood plasmacytoid DCs compared to healthy patients (90, 91). On a cellular level, virus infection can promote the recruitment of NK cells and T cells to the islets (43) and the local production of inflammatory cytokines, particularly INF- $\alpha$ , INF- $\beta$ , IFN- $\gamma$ , TNF, and IL-1 $\beta$  (92). This exposure to IFN can have far-reaching consequences in the context of T1D. First, IFN directly triggers ER stress and apoptosis (92). Second, IFN can induce hyperexpression of HLA class I on  $\beta$  cells, which makes them more visible to cytotoxic T lymphocytes and more susceptible to destruction (93), and low-level virus infection promotes HLA hyperexpression in islets (49). Thus, infection of  $\beta$  cells itself may not even be needed for pathogenesis. In theory, even infection of cells adjacent to the  $\beta$  cells can lead to secretion of proinflammatory cytokines by DCs. It is envisioned that this could initiate bystander activation among circulating naive islet-specific T cells in pancreatic islets or lymph nodes, thus accelerating  $\beta$  cell destruction.

## ENTEROVIRUSES AND CELIAC DISEASE

Enteroviruses were also recently associated with development of celiac disease, which is an autoimmune disease that shares HLA risk associations with T1D and is dependent on autoimmunity versus an antigen, tissue transglutaminase. A case-control study within a Norwegian birth cohort analyzed sequential monthly stools in children from age 3 to 36 months carrying the HLA genotype conferring increased risk of celiac disease (94). Both EV-A and EV-B were significantly associated with celiac disease, and long-lasting infections or higher-titer stools had a higher odds ratio (94). In another study using virus seroconversion as a marker of infection, enterovirus infections were found more frequently in case children than in controls before the appearance of celiac disease-associated tissue transglutaminase autoantibodies (95). A third study from TEDDY indicated that cumulative

stool enteroviral exposures between 1 and 2 years of age in addition to higher gluten intake were associated with increased risk of seroconversion to celiac disease autoimmunity (96). Thus, emerging evidence indicates enteroviruses may be linked to autoantibody generation against antigens important in other autoimmune diseases, not exclusively those related to T1D.

## CONCLUSION

T1D is a complex and multifactorial disease. In recent years, there has been increasing recognition of the potential role of enteroviruses in initiating autoimmunity in predisposed individuals, eventually leading to subsequent destruction of islet  $\beta$  cells. There may be a combination of mechanisms through which enterovirus infection may contribute to pathology, including ER stress and inflammation as well as direct persistent infections of  $\beta$  cells and potentially APCs that alter antigen presentation. With recent clinical data and the development of novel tools and human model systems, the field is now poised to start exploring the viral mechanisms that may play a role in enteroviral persistence and their contribution to both the etiology through trigger mechanisms of islet autoimmunity and the subsequent pathogenesis eventually resulting in the clinical onset of T1D in predisposed individuals.

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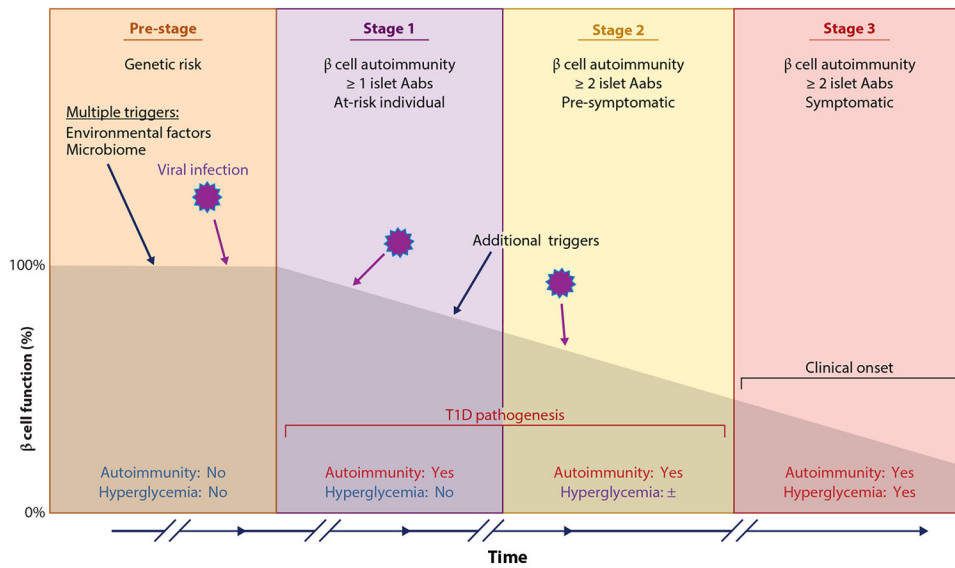


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**Figure 1.** Staging and progression of type 1 diabetes (T1D). Multiple stages are recognized in the development of T1D that are defined by the appearance of single or multiple autoantibodies (Aabs) and by dysglycemia stages. Virus infection triggers or other environmental triggers can occur in multiple stages, and multiple triggers may be commonly required to produce the clinical onset of T1D.

