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Enterovirus Infection and Type 1 Diabetes: Closing in on a Link?



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The famous French writer Victor Hugo (1802–1885) once said: “Perseverance, secret of all triumphs!” (1). Due to the divergence seen in monozygotic twin cohorts, we know that both genetic and environmental factors must contribute to the development of human type 1 diabetes (T1D) (2,3). For many decades, we have searched for environmental triggers, but it has remained difficult to define the key players. In addition to viral infections, nutritional and hygiene conditions as well as sunlight exposure and geographical locations have all been investigated but often with inconclusive results (4). Now, we might be getting closer to identifying a key culprit, which could be enteroviral infections. In this issue of *Diabetes*, Krogvold et al. (5) document the presence of a low-grade enteroviral infection in pancreata from recently diagnosed patients. The importance of this study resides on the uniqueness of the six specimens examined, which were collected via partial pancreatectomy from volunteers with very recently diagnosed diabetes (3–9 weeks) as part of the Diabetes Virus Detection study (DiViD) (6). Interestingly, hyperexpression of major histocompatibility complex (MHC-I) molecules and the presence of the enteroviral capsid protein 1 (VP1) were detected in the islets from all the patients. Additional confirmation was obtained by sequencing techniques in four of the six cases. These findings indicate that infections with enteroviruses could, at least in some cases, be a precipitating key factor for the onset of T1D.

The natural history tells us that T1D is an inflammatory disease affecting specifically the pancreatic islets of Langerhans, where autoantibodies to various islet antigens in many cases precede clinical diagnosis and autoreactive CD8 T cells can be documented in inflamed islets (7). Viruses have been traditionally implicated in the etiopathogenesis of the disease with no definitive proof so far. Enteroviruses are one of the main candidates because traces of this viral infection have been found more frequently in patients with T1D than in individuals without diabetes (8). This does not

directly prove that enteroviruses cause T1D. They could also be mere secondary players in a complex immune process that ends with the destruction of β -cells. Supporting the concept of a potential viral presence that would contribute to the demise of β -cells, elevated levels of MHC-I have been found in donors with T1D, consistent with a possible virus-induced interferon-mediated signal facilitating antigen presentation to infiltrating cells (9). Some studies have found a stronger interferon signature in individuals at high risk of developing the disease (10). Upregulation of MHC-I could then explain ensuing events, such as the infiltration of autoreactive CD8 T lymphocytes (that are also increased in blood from patients with T1D) and the selective loss of β -cells (11). This pathogenetic concept is depicted in Fig. 1.

Why has it been so hard to provide definite proof for a causative role for enterovirus infections in T1D? Many previous studies have tried to address this hypothesis by serological, immunohistological, and molecular methods (12,13). However, a direct one-on-one relationship of a viral strain only present in patients with T1D has never been found—the reason could be that viruses trigger diabetes as a hit-and-run event and therefore their traces are often not detectable. Alternatively or additionally, multiple viral strains of the same genus or even different viruses that trigger common pathways might be involved. Furthermore, multiple viral hits might be required before β -cell function is sufficiently impaired, and these could potentially come from different viruses. Last, viral infections can also have protective roles from autoimmunity by “tuning” the immune system, a concept that would support the hygiene hypothesis (14). Interestingly, Krogvold et al. (5) found evidence of high MHC-I expression in the islets of all patients with T1D and in only one control subject, being present in all the insulin-containing islets. In addition, all six patients with T1D were positive for VP1, which in turn was only detectable in two out of nine control

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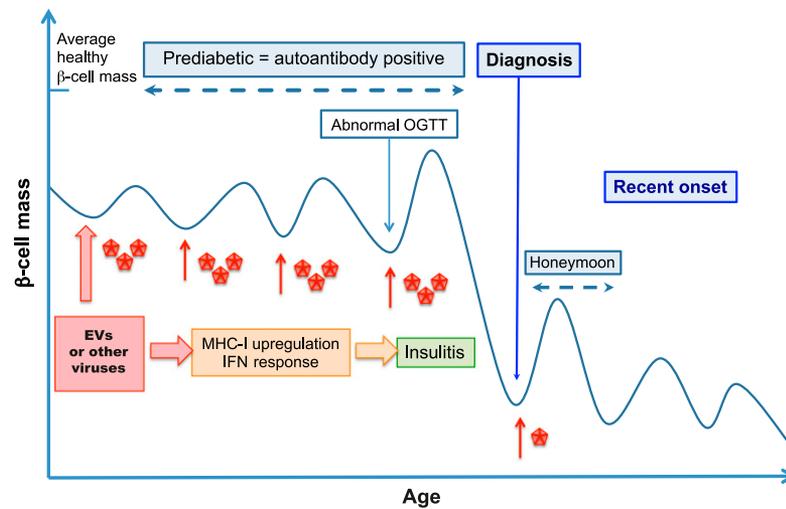


Figure 1—Timeline of β -cell mass decline. An enterovirus (EV) or other viral infections cause or enhance inflammation in genetically predisposed individuals, triggering an interferon (IFN) response and the upregulation of MHC-I molecules in pancreatic endocrine cells. During the prediabetic phase, antibodies against β -cell antigens appear in the periphery as a consequence of immune activation and antigen presentation. The inflammatory environment in the pancreas facilitates the proliferation of autoreactive T cells that infiltrate the islets causing insulinitis. This phenomenon, although mild during the prediabetic phase, becomes more apparent closer to the onset of disease. Before complete β -cell demise, the first symptoms of diabetes become evident with the appearance of an abnormal oral glucose tolerance test (OGTT). The ability of the immune system to counteract the destruction of β -cells might depend on the number of encounters with the virus. Several infections might induce several waves of β -cell destruction before a critical mass is reached around the time of diagnosis. An enterovirus infection might occur right before onset, altering the delicate equilibrium of immune activation and regulation and precipitating the definitive loss of β -cells. A temporary remission might appear after clinical onset, termed the honeymoon phase. Overall, the fluctuation among destructive autoreactive responses, immune regulation, and β -cell proliferation creates a continuous relapsing-remitting β -cell mass profile that could be perpetuated by an enterovirus or other viral infections during the prediabetic phase and at disease onset.

subjects. In line with these findings, VP1 has previously been detected in recent-onset donors but rarely found in cases without diabetes (15). Thus, it appears now that in at least some diabetes cases, if one looks close enough around the time of diagnosis, enteroviral infections could have been a precipitator of disease onset. This goes along well with frequent clinical observations that patients have viral infections just prior to diabetes onset.

Despite this important finding, only 1.7% of the islets contained some of the overall 60 intensely VP1⁺ cells detected. Looking at these relatively low numbers, one might think that 60 VP1⁺ cells in 2,492 islets are not much. However, we should not underestimate the importance of this finding. It has recently been proven in NOD mice that enteroviruses and, more specifically, coxsackie viruses can persist in the pancreas as terminally deleted forms with slower replication and no evident cytopathic effect for up to 35 days after inoculation (16). If this were to be shown also for human pancreata, we might be able to detect persistent viral forms and in some cases prove that an enterovirus infection took place in the pancreas and islets before the onset of disease. The detection of low levels of viral protein in the biopsied samples could be a reflection of a previous acute infection, but many terminally deleted forms could be also present. Further insight could be gained by looking for enteroviral traces in samples from multiple autoantibody positive individuals

now available through the Network for Pancreatic Organ Donors with Diabetes (nPOD) (17). In addition, Krogvold et al. (5) tried to demonstrate the presence of an enterovirus infection on the genomic level. Enterovirus RNA was detected in islet culture supernatants by RT-PCR in half of the patients with T1D only at early days postculture (2,4). However, only one patient was found to be positive when whole frozen tissue was used. This demonstrates that the amount of virus present in these tissues is low and needs further amplification in order to be detected. More compelling is the evidence of enterovirus sequences in four of the six patients, despite the limitations on the amount of sequenced genes and the lack of a serotype identification. Efforts should be made in this regard as several coxsackie virus B strains have been implicated in the disease so far (18,19). Although it may be unlikely that a disease such as T1D is caused by only one enterovirus serotype, the identification of a specific strain would be beneficial and would facilitate the design of an enterovirus vaccine. Along these lines, Hyöty and colleagues (20) have proposed that coxsackie virus B1 is a strain mainly associated with T1D in their Finnish cohorts. Nevertheless, the study by Krogvold et al. (5) shows good concordance among laboratories using different protocols and techniques. This is even more convincing if one considers that only a small part of the pancreatic tail was resected and this region might not have been the predominantly infected area.

What will the future hold? Krogvold et al. (5) are the first to demonstrate the presence of enteroviruses in pancreatic tissue from living individuals very close to the time of diagnosis. Their findings support the notion that an enterovirus infection takes place in the pancreas before or around the time of diagnosis as a precipitating event but cannot prove a causal relationship between infection and disease. For example, it is possible that islets during the development of T1D undergo pathological changes for immunological and metabolic reasons and are then more susceptible to infections by enteroviruses because they upregulate the coxsackie and adenovirus receptor, a notion supported by F. Dotta, G. Frisk, and colleagues (unpublished observations) and Ylipaasto et al. (21). Still, the present findings make it more likely that enteroviral (and maybe other) infections are a critical trigger to push an already dysfunctional metabolic equilibrium over the brink, resulting in a rather rapid and cataclysmic loss of β -cells around the time of diagnosis. Future preventive measures could then focus on appropriate vaccines and other measures prior to the onset of diabetes.

Duality of Interest. M.G.v.H. is an employee of Novo Nordisk. No other potential conflicts of interest relevant to this article were reported.

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