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Review

# The viral paradigm in type 1 diabetes: Who are the main suspects?

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ABSTRACT

Type 1 diabetes (T1D) is an autoimmune disease characterized by the loss of pancreatic beta cells in the islets of Langerhans. Although genetic predisposition plays an important role in T1D development, studies of identical twins suggest that environmental factors such as viruses and other pathogens may be critical triggers either through direct cytolytic effect and gradual beta cell destruction, or by bystander activation of the immune system. In addition, viruses may circumvent the host immune response and have the capacity to establish chronic lifelong infections. The association of various viral infections with the induction of T1D has been extensively studied at the serological and epidemiological level. However, there is still little evidence from studies of human pancreas to confirm their presence or a causal role in disease pathogenesis. In this review, we identify possible suspects for viral triggers of disease and explain their potential roles in the "viral paradigm" of T1D.



**Contents**



## 1. Introduction

*Virus: An infective agent that typically consists of a nucleic acid molecule in a protein coat, is too small to be seen by light microscopy, and is able to multiply only within the living cells of a host* [1].

Although they are structurally and functionally simple, interactions of viruses with humans are quite complex. Infectious diseases have been reported throughout human history and viruses have colonized our ancestors in the past, as they do in the present day [2]. Most of the slowly evolving DNA viruses are ancient and have coevolved in close association with their hosts [3], while many RNA viruses appeared more recently, possessing extraordinary capacity for change [4]. Viral infections have stimulated the development of the human immune response and promoted the diversity of the major histocompatibility complex (MHC), T cell receptors and B cell antibody production [5]. Many battles between the immune system and viruses have been played along this love–hate relationship, and some of them, like the role of viruses in autoimmunity, are neither resolved nor well understood. In type 1 diabetes (T1D), viruses more often play the role of perpetrators of disease, either sitting on the sidelines or in the spotlight playing a major role. In this review, we navigate through the recent literature regarding viruses and the development of T1D in order to identify the key suspects and their roles in this "viral paradigm".

### 1.1. Enteroviruses

Enteroviruses (EV) are non-enveloped positive-sense, single-stranded RNA viruses, which belong to the *Picornavirus* family. Based on molecular and serological characteristics, EV are subgrouped into EV-A, which contains EV-71 and several Coxsackievirus group A (CVA)

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viruses; EV-B (Coxsackievirus group B (CVB) viruses and echoviruses); EV-C (polioviruses 1–3 and several CVA viruses); EV-D (EV-68 and -70); and Rhinovirus [6]. EV infections are very common and most frequently occur in children under the age of ten. They can induce a diverse array of clinical features, but usually lead to asymptomatic illness. However, EV infections have also been associated with more severe diseases such as myocarditis and T1D. Coxsackieviruses, and specifically CBV4, were one of the first EV to be associated with T1D. A strain of CBV4 was isolated from the pancreas of a patient with recent-onset T1D and was later shown to induce T1D in mice [7]. This made a strong case for the implication of EV in the pathogenesis of T1D as a possible trigger for the disease. Over the years scientists have compiled further indirect evidence to support this association, but have so far been unable to prove causality beyond reasonable doubt [8].

The need for definitive proof of an association between EV and T1D has pushed the development of a growing number of prospective studies in which at-risk children are recurrently tested for EV presence in their blood and/or their stool before and after seroconversion. The aim of these studies is to demonstrate the link between EV infection, the development of islet autoimmunity, and T1D. In the Norwegian Environmental Triggers of T1D (MIDIA) and the Diabetes and Autoimmunity Study in the Young (DAISY) studies, the frequency of confirmed EV RNA in fecal samples was not predictive of progression to T1D. Conversely, in blood samples, although a significant association between the presence of EV RNA and progression from islet autoimmunity to T1D was not consistently found throughout the study, EV RNA was detected more frequently at the stage of seroconversion [9–11]. Lastly, in the T1D Prediction and Prevention Project (DIPP) study, CVB1 was found to be associated with an increased risk of beta cell autoimmunity whereas CVB3 and CVB6 were associated with a reduced risk and a potential cross-protection against CVB1 [12].

These studies have improved our understanding of the possible role of EVs as environmental triggers of T1D, but larger cohorts need to be established in order to clarify whether the link between T1D and EV implies causality or may instead be merely a contributing factor to beta cell demise. Currently, the Environmental Determinants of Diabetes in the Young (TEDDY) study, which has examined more than 7000 patients across six clinical centers worldwide, is testing the hypothesis that specific viruses may trigger or reduce the risk of islet autoimmunity and/or T1D. So far, there is no evidence for EV viremia around the time of seroconversion in these patients with rapid-onset T1D but future results from this study may shed more light on the role of EV in the disease [13].

As fecal or serological samples might not reflect an ongoing infection in the pancreas, other studies are aiming to detect EV proteins or isolate EV RNA directly from the target organ. The Diabetes Virus Detection Study (DiViD) collected pancreatic tissue from six living individuals 3 to 9 weeks after onset of T1D through pancreatic tail resection [75]. Interestingly, in all patients (and 2/9 controls), the presence of enteroviral capsid protein 1 (VP1) [14] along with hyperexpression of MHC-I accredited a viral signature. However, confirming previous findings from a UK cohort of patients with recent-onset T1D [15], less than 2% of the islets were positive for VP1, which indicates a low grade rather than an acute infection. This may explain why it has been difficult to find evidence of the presence of EV in patients with T1D. In addition, EV genomes were detected using PCR and sequencing techniques in four of the six patients. These results were recently confirmed in an elegant study on the same patients in which cell lines exposed to pancreas homogenates expressed cytoplasmic VP1. In addition, CVB-A or -B species were identified in all of the DiViD patients (Krogvold L., personal communication). Interestingly, Laiho and colleagues recently released an article in which they compared the relative sensitivity of several techniques to detect CVB1 in an infected cell line. Although all the techniques were able to detect CVB1, RT-PCR was the most sensitive method and potentially the most likely to expose a low-grade infection [76]. These results are another piece that has recently been added to the EV jigsaw puzzle, confirming the presence and potential involvement of EV before or around the time of diagnosis of T1D, but also highlighting the importance of developing a very sensitive method to detect ongoing chronic infections with a low level of replication.

The main question raised by the presence of EV in the pancreas is its role in the physiopathology of the disease and its relationship with beta cells. Human coxsackieviruses are known for their tropism for endocrine but not exocrine pancreatic tissue [16]. Moreover, in mice pancreata, 5′ terminally deleted coxsackieviruses were shown to persist up to a month after infection with a low replication rate and in the absence of cytopathic effect [77]. In addition, a recent study in rats showed that beta cells have a limited capacity to clear enteroviral infections compared to alpha cells [17]. Lastly, Gallagher et al developed a viral infection model in immunodeficient mice bearing human islet grafts and showed that CBV4 could induce T1D three weeks after infection. Most importantly, EV more often infected beta cells than other islet cell populations and insulin but not glucagon secretory capacity was decreased [18]. This may partially explain why pancreatic beta cells but not alpha cells are chronically infected, targeted by an autoimmune response and then killed during T1D.

In conclusion, EV remains the prime suspect for a hypothetical infectious trigger for T1D but there is still a lot of information that is required before we can reach a final consensus within the field. We need to focus both on the epidemiology of EV infection at the initiation of autoimmunity and the possible pathogenic role of EV at the onset of T1D.

### *1.2. Herpesviruses*

In addition to EV, the role of other viruses such as herpesviruses in the development of T1D has been explored. *Herpesviridae* is a large family of double-stranded DNA viruses. There are eight herpesvirus known to infect humans: herpes simplex viruses 1 (HSV-1) and 2 (HSV-2), varicella–zoster virus (VZV), Epstein–Barr virus (EBV), human cytomegalovirus (hCMV), human herpesvirus 6 (HHV-6), human herpesvirus 7 (HHV-7) and Kaposi's sarcoma-associated herpesvirus (KSHV). Following primary infection, which usually occurs in early childhood, herpesviruses remain in a latent lifelong state in the human host and become reactivated in the later stages of life. Although there are several reports regarding the association of human herpesvirus infections with diabetes, the *Herpesviridae* family has generally been less associated than EV, with the development of T1D.

HSV-1 preferentially induces disease in oral mucosa. In a study by Chen et al [19], HSV-1 was detected in the human pancreas; however, so far, no link between HSV-1 and the incidence of T1D has been reported. On the contrary, in a Chinese cohort of patients, the association between HSV-1 infection and type 2 diabetes has been demonstrated by ELISA [20]. The prevalence of HSV-1 infection (presence of antibodies in serum) was significantly higher in the diabetic than in the control group. Similarly to HSV-1, HSV-2 infection is predominantly characterized by lesions of mucosal tissues but is mainly localized to the genital mucosa. To date, there has been no report indicating the association of HSV-2 and T1D, and unlike HSV-1, this virus has not been detected in the human pancreas [19].

VZV infection occurs primarily in the oral mucosa and skin [21], whereas latent persistence of this virus is mainly detected in nerve sensory ganglia [22]. There are no reported links between VZV and diabetes. Although the involvement of VZV in the development of T1D has not been demonstrated; nonetheless, it has been suggested that patients with diabetes mellitus have significantly reduced cell-mediated immunity (CMI) to VZV [23]. It is thought that the increased risk for herpes zoster in diabetes mellitus patients could be attributed to this decrease in VZV-specific CMI.

EBV, together with CMV, is perhaps one of the most interesting viruses in this family regarding its possible association with T1D. Due to the sequence homology of HLA-DQ8 with BERF4-encoded EBNA3C protein of EBV [24], it has been suggested that EBV might be associated