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OTHER FORMS OF DIABETES AND ITS COMPLICATIONS (JJ NOLAN AND H THABIT, SECTION EDITORS)

# Enteroviral Infections as a Trigger for Type 1 Diabetes

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**Abstract**

**Purpose of Review** To provide an overview of studies that have detected enteroviruses (EV) in samples from people with type 1 diabetes (T1D), the techniques they have used, and which challenges they have encountered.

**Recent Findings** Recent studies have detected EVs in serum, blood, stools, nasal swabs, and pancreas of people with T1D before or around clinical onset of disease, indicating that an association between EV infections and T1D exists. However, definitive evidence for its role as disease triggers is lacking. Recent access to human samples is starting to provide the necessary tools to define their role in disease pathogenesis. Emerging evidence suggests that chronic infections take place in the pancreas of diabetic donors. However, the development of sensitive techniques able to detect low amounts of viral protein and RNA still constitute a major challenge for the field.

**Summary** New evidence at the protein, RNA, and host immune response level suggests a role for EV infections in the development of autoimmunity. In the upcoming years, new technologies, collaborative efforts, and therapeutic interventions are likely to find a definitive answer for their role in disease pathogenesis.

**Keywords** Enteroviruses · Type 1 diabetes · IFN-response · Viral protein · Viral RNA

**Abbreviations**

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| T1D | Type 1 diabetes |
| EV | Enterovirus |
| EVs | Enteroviruses |
| AAb+ | Autoantibody positive. |
| HLA-I | Human leukocyte antigen class I |
| ICI | Insulin containing islet |
| IDI | Insulin deficient islet |
| nPOD | Network for Pancreatic Organ Donors with Diabetes |
| ISH | In situ hybridization |
| RT-PCR | Reverse transcription polymerase chain reaction |
| CVB | Coxsackievirus |
| IFN | Interferon |

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## Introduction

Enteroviruses (EV) are single stranded RNA viruses that belong to the *Picornaviridae* family. It is estimated that they cause 10–15 million symptomatic infections per year in the USA alone [1]. The genus Enterovirus includes more than 100 serotypes; among them are important human pathogens like polioviruses, coxsackieviruses A and B, echoviruses, and others [2]. These viruses are transmitted mainly via the respiratory or the fecal-oral route and can be detected year-round but tend to increase in the summer [3]. The seasonality of type 1 diabetes (T1D) and its possible temporal association with EV infections has also been analyzed. Temporal clustering of T1D incidence over periods of a few months suggests that an infectious agent might contribute to disease development in susceptible individuals [4]. Several studies report peaks of T1D incidence in October to January and thorough June to August for centers in the northern hemisphere [5, 6].

However, the temporal association of EVs and T1D has been hard to determine mainly due to the difficulties on sample collection at short and frequent intervals following an acute infection, and the uncertainty about the establishment of a chronic infection in the pancreas that could lead to the development of T1D. In addition, the conditions that favor a chronic EV infection in the pancreas and could infer enough damage to kill beta cells and/or activate the anti-viral immune response are not known. Recent studies have found evidence for the presence of EVs in the pancreas of newly diagnosed individuals [7••], but the number of studied samples is still too small to draw strong conclusions. The inaccessibility of the target organ usually directs researchers to more accessible samples like blood, stools, and even isolated islets. In this review, we will describe clinical samples in which EVs have been detected, the techniques used, and the recent evidence pointing to an exacerbated host response to EV infection leading to the development of T1D.

## The Primary EV Replication Sites and Their Association with T1D

Based on their transmission route and entry in the host, EVs have two main replication sites, the gastrointestinal tract and the respiratory tract [8], neither of which have been extensively investigated in the context of T1D. In an epidemiological study on data recovered between 2000 and 2013, EV incidence among those younger than 1 year was 34%. EVs were detected most frequently in cerebrospinal fluid (55%), throat swab (29%), and stool (15%) samples [3] suggesting that these samples would be the most appropriate to detect an active EV infection. There are contradictory studies regarding the presence of EVs in the gut mucosa of people with T1D. Oikarinen et al. analyzed small bowel mucosal biopsies from 120 subjects, of which 39 were from people with T1D (age: 18–63 years; diabetes duration: 0–38 years) and 41 from controls (age: 23–76 years) [9•]. EV RNA positivity was found in 74% of T1D donors compared to 29% of controls by in situ hybridization (ISH). It was mainly detected in the epithelial cells of the villi and crypts but staining of the lamina propria was occasionally seen. The presence of viral RNA was confirmed by RT-PCR in 19% of the people with T1D and in 10% of the controls. In addition, the majority of subjects that were positive for virus RNA by ISH were negative for the presence of the viral protein VP1 by immunohistochemistry. Overall, VP1 protein was found in 22% of people with T1D and in 22% of the controls, and it was observed in the epithelial cells of the crypts. Conversely, Mercalli et al. did not detect EV RNA by ISH or RT-PCR in any of the small intestine biopsies from 25 individuals at different stages of T1D and 21 controls [10]. Similarly, VP1 staining was only found in two controls and one person with T1D. The differences observed in these two studies are striking and reveal important heterogeneity between different cohorts and/or methods. The samples were from distinct geographical cohorts, with slight differences in their demographic characteristics. In addition, similar techniques but different methods of detection were used, which might have had different sensitivities.

In countries with good sanitary conditions, one would expect the respiratory route to be the main infection mode. The respiratory tract is largely unexplored in the context of T1D and most studies have focused on the detection of EVs in serum or blood samples and their possible association with respiratory infections. Similarly to the gastrointestinal tract, studies in prospective cohorts have reported discordant results. While parent-reported early childhood respiratory infections in the Diabetes Autoimmunity Study in the Young (DAISY) showed no association with islet autoimmunity [11], respiratory infections were positively associated in two European studies, the environmental triggers for type 1 diabetes study (MIDIA) [12] and in a dietary intervention in children at increased risk for type 1 diabetes (BABYDIET) [13]. In addition, data based on statutorily insured patients in Bavaria, Germany, reported that respiratory tract infections occurred in a similar percentage of children that developed T1D and children who did not develop it (38.5 and 34.2%, respectively) [14]. However, T1D risk was increased in children who had a respiratory tract infection compared with children who had no respiratory tract infections in the interval that goes from birth to 2.9 months and between 3 and 5.9 months of age. Similarly, The Environmental Determinants of Diabetes in the Young (TEDDY) study reported that among children (3 months to 4 years of age), the number of respiratory infection episodes within any 9-month period was associated with the onset of islet autoimmunity within the 3 following months [15•]. In this 9-month period, parents reported common colds, influenza-like symptoms, sinusitis, and laryngitis/tracheitis [15•]. Moreover, the risk of islet autoimmunity was very high when the respiratory infection episodes occurred more often, and although the risk was detected during the whole year, it was more pronounced for winter infections [15•].

In light of these evidence, it would be extremely informative to determine the causative agent of each infection preceding islet autoimmunity, as it will provide information about possible single agents that create "multiple hit and run" scenarios or if there could be multiple viruses involved. Not all these viruses would be able to reach the pancreas or infect beta cells and most likely, not all of them would establish chronic infections in the pancreas (Fig. 1a). Further analysis of samples from the gastrointestinal and respiratory tracts like stools, nasal washes, or nasopharyngeal swabs, as well as tissue collection at the time of organ donation would be of interest for the T1D field and highlights a largely unexplored line of investigation.

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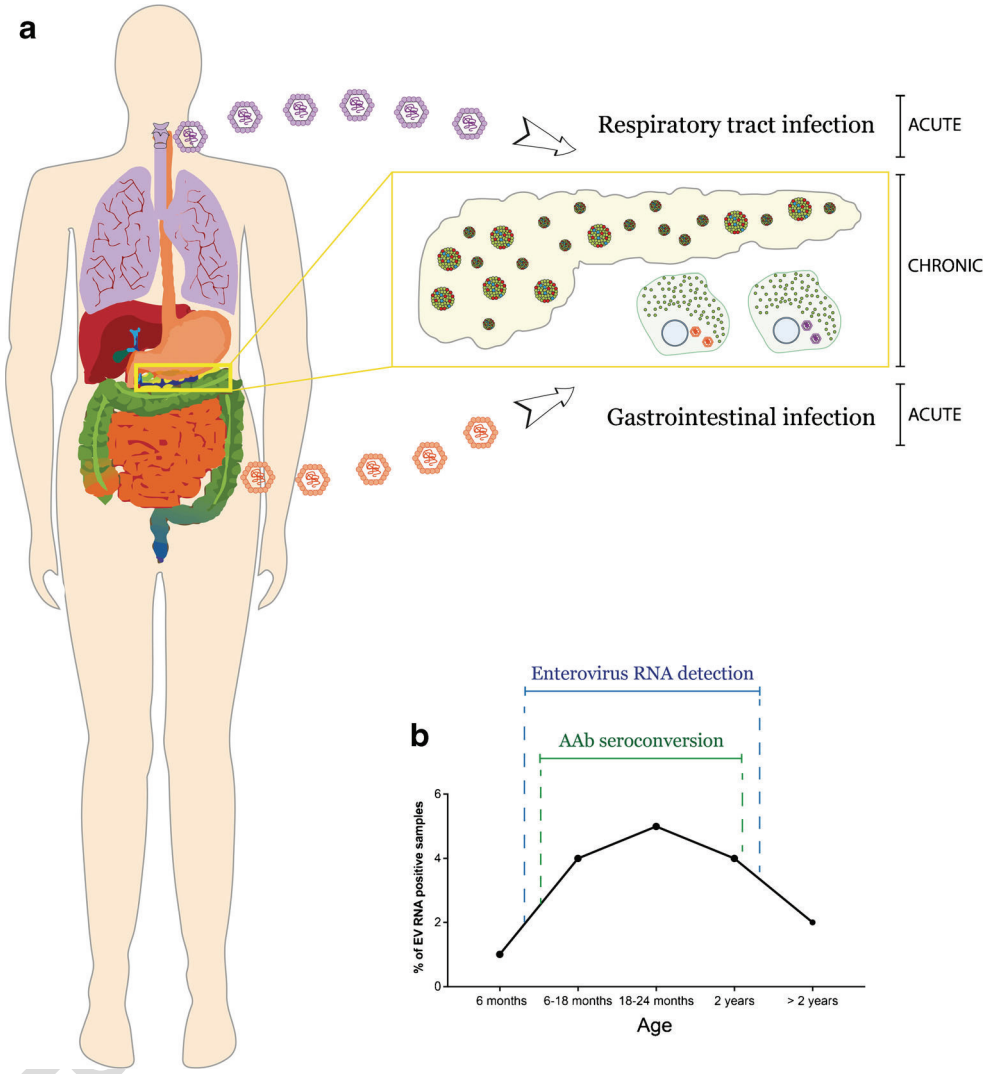


**Fig. 1** **a** EV spread from primary replication sites: EVs have two main replication sites, the gastrointestinal tract and the respiratory tract. After an acute infection in these tissues, EVs can spread systemically and reach the pancreas. Beta cells express CAR, which facilities EV entry. After a few beta cells are killed, the activation of an anti-viral response could contain the infection. The virus could then limit its replication in order to avoid immune surveillance and establish a chronic, low-grade infection in the pancreas. **b** Association of EV infection with seroconversion: the incidence of islet autoantibody seroconversion has a peak at 9 months to 2 years of age in children, which coincides with the peak of detection of EVs RNA [17••, 19••, 20•]

## Detection of EVs in Prospective Cohorts: Timing Might Be Everything

Virus detection in blood samples continues to be the most common method due to the larger accessibility to human samples and the possibility to study prospective cohorts. However, other samples like stools or nasal swabs are being incorporated into study designs with the aim of increasing the chances of detecting EV infections. In the DAISY study, the risk of progression to clinical T1D in the sample interval following detection of EV RNA in serum was significantly increased compared with that of intervals following a negative serum sample [16]. In the Finnish type 1 Diabetes Prediction and Prevention (DIPP) study, EV RNA was detected in 2.7% of the total number of serum samples [17••]. In case children, a total of 5.1% of the samples were enterovirus RNA positive compared with 1.9% in control children and it peaked during the 6-month period before the appearance of the first autoantibody. Interestingly, in children who were younger than 6 months of age, only 1% of the samples were positive while this proportion increased to 3.5% at 6–18 months and to 5% at age 18–24 months. Conversely, after the age of 2 years, the frequency decreased again to 4.3% and further to 2% in children older than 2 years [17••] (Fig. 1b). Moreover, case children had their first infection earlier than control children. Similarly, in a recent analysis of longitudinal stool samples collected from children who developed signs of autoimmunity and matched controls, EV infections were detected more frequently in case than in control children [18]. Children who developed autoantibodies had, in addition, a higher number of EV infections more than 12 months before islet autoimmunity seroconversion and most of these infections occurred before the age of 2 years. Collectively, these data bring forward two interesting hypotheses: (1) the incidence of islet autoantibody seroconversion has a peak at 9 months to 2 years of age in children, which coincides with the peak of detection of EVs RNA [19••, 20•] and (2) children that developed islet autoimmunity had more infectious episodes and their first infection occurred earlier than in children with no islet autoimmunity [17••, 18].

In an interesting and novel study, 44 longitudinally collected blood samples (from the DIPP study) from seven children carrying HLA-genetic risk for T1D and who were EV positive were analyzed with the aim of understanding the individual-level transcriptomic changes associated with EV infections and to characterize common features of EV responses in children [21••]. Three of the children had fever around the time of sample collection, were strongly EV positive by quantitative RT-PCR, and had a clear interferon (IFN) response [21••]. Peripheral blood transcript levels of genes involved in antiviral immune responses and especially IFN signaling were enriched, particularly genes like interferon induced with helicase C domain 1 (IF1H1), interferon regulatory factor 7 (IRF7), signal transducer and activator of transcription 1 (STAT1), STAT2, and myxovirus-resistance protein A (MxA). This signature was comparable to that of pancreatic islets and peripheral blood mononuclear cells (PBMCs) infected in vitro with Echovirus 9 or two different CVB1 wild-type strains [21••]. Of the four children with a strong IFN response, two remained autoantibody negative, one became positive for multiple autoantibodies afterwards, and the last one was AAb+ and later developed T1D [21••]. One important point of this study is that the associated changes were similar to those reported during an acute phase of virus infection in four of the children, while the rest had changes related to the activation of adaptive immune responses. This could reflect different stages of the immune response to the infection. Accordingly, previous studies have detected an increased IFN-I-inducible transcriptional signature in peripheral blood before the development of islet autoimmunity in samples taken before and after the first T1D clinical manifestation [22, 23]. Differences in the quantity and quality of the response might determine the outcome of the infection. Clearing the virus or favoring the establishment of chronic infections could decide the fate of beta cells and ultimately, the development of T1D. Therefore, this type of studies could be of tremendous importance to identify children at high risk of developing T1D following an acute EV infection and to define signatures associated to both acute and persistent viral infections.

## The EV Detection Challenge: Seeing Is Believing?

The studies reported above highlight one of the main problems in the field of T1D: the detection of potentially low amounts of virus protein or RNA in human samples. As of today, the most common techniques to detect EVs are still RT-PCR and immunohistochemistry. Indeed, the selection of appropriate techniques for the detection of low amounts of RNA or protein is not a simple matter, as the detection limit can vary considerably, even between different laboratories using similar techniques. Laiho et al. studied the sensitivity of different techniques for CVB1 detection. Human A549 cells were infected (multiplicity of infection: 10–15) and harvested at 1, 2, 4, and 5 h post-infection [24]. Then, dilution series that ranged from $10^{-1}$ to $10^{-8}$ were prepared. The semi-nested RT-PCR was the most sensitive technique, detecting even the highest dilution, while the real-time RT-PCR gave a positive signal at the $10^{-7}$ dilution. Proteomics was the next most sensitive technique, reaching the same dilution, followed by immunohistochemistry, which was able to detect viral protein up to the $10^{-6}$ dilution, with variability between different antibodies. Lastly, ISH detected virus dilutions of $10^{-4}$ [24]. Despite the good sensitivity of most of these techniques, one thing to keep in mind is that EVs are able to establish persistent infections, which are characterized (in vitro) by no evident cytopathic effect, expression of viral antigens in a low number of cells, production of viral particles at low titers, and secretion of cytokines and chemokines [25••]. An important consideration is that infected cell lines constitute an ideal testing sample and the sensitivity of these techniques on tissue samples, and especially pancreas, is expected to be lower. RNA degradation is also a major concern when analyzing pancreas samples and is likely to impact the results obtained by techniques like ISH, RT-PCR, and even sequencing. In a recent study, pancreas RNA quality was determined in 236 samples from organ donors collected through the Network for Pancreatic Organ Donors with Diabetes (nPOD) [26]. Variables like cause of death, length of hospitalization, lipase levels, tissue collection, and storage as well as different pancreatic regions influenced the quality of the RNA obtained from pancreas samples. RNA degradation might, in some cases, explain potential discordant results between the detection of viral protein and RNA. In the Diabetes Virus Detection (DiViD) study, pancreatic biopsies from living individuals with recent onset T1D were studied [7••]. The samples were collected under ideal conditions, eliminating some of the potential variables known to cause poor RNA quality. In these samples, viral protein was detected in the pancreas from all the people with T1D that participated in the study. However, viral RNA was not detected in two of these six people and the four positive cases had low virus titers. Partial EV sequences were identified but no specific genotype could be determined [7••]. This illustrates how challenging the detection of low abundance RNA and protein in the pancreas can be and how the use of optimized and standardize tissue collection and processing methods is key to obtain high-quality data [26].

## Host Response to Infection: the IFN-Signature in T1D

As mentioned above, several studies have suggested that an antiviral signature linked to the activation of interferon-stimulated genes (ISGs) exists in T1D [22, 27–30]. To initiate

this response, components of the viral particle need to be recognized by pattern recognition receptors like melanoma differentiation-associated protein 5 (MDA5), retinoic acid-inducible gene I (RIG-I), or toll-like receptor 3 (TLR3). Their activation induces IFN-I production, which binds to the IFN receptor (IFNAR) and creates a positive feedback loop, inducing the production of more IFN-I [31]. It also activates specific enzymes and transcription factors like STATs in order to establish an antiviral response through the activation of ISGs [32]. Primary pancreatic islets and exocrine cells respond to Echovirus infection by upregulating the transcription of genes like MDA5 (recognition of viral genome), 2′-5′-oligoadenylate synthetase 1 (OAS1) and IFN-I (antiviral response), and the C-X-C motif chemokine 10 (CXCL10) and C-C chemokine ligand 5 (CCL5) (immune attraction) [33]. Human islets and EndoC-βH1 cells treated with IFN-α upregulate markers of inflammation like STATs, IRF9, CXCL10, MX1, and HLA-I, which contribute to ER stress and apoptosis [34]. Accordingly, PCR-array data from insulitic laser-captured islets from the people with T1D that participated in the DiViD study revealed the upregulation of genes like interferon-induced guanylate-binding protein 1 (GBP1), TLR3, OAS1, STAT1, CXCL10, CCL5, and caspase 1 (CASP1).

The existence of an exacerbated IFN-response in the islets of people with T1D could attract immune cells to the islets and could contribute to islet autoimmunity. In this context, any insult that triggers an IFN response could cause islet inflammation, and therefore, hypothetically, any virus (and not only EVs) could contribute to beta cell demise. However, not all viruses can infect beta cells. EVs are certainly able to do so through their binding to the Coxsackievirus and adenovirus receptor (CAR) [35] and decay accelerating factor (DAF) [36] receptors. CAR is a transmembrane protein that is expressed in both alpha and beta cells [37, 38•] while there is no evidence of the expression of DAF in human islets [39]. In addition, pliovirus receptor (PVR) and integrin αvβ3 can also be used for EV entry [40]. Accordingly, CBVs and Echoviruses are able to effectively infect human islets, and some strains are, in addition, able to replicate in the exocrine pancreas [33, 41]. In a recent study, the expression and distribution of CAR isoforms in human pancreas have been described [42•]. RNA and protein extracted from human islets and EndoC-BH1 cells revealed the expression of mainly two isoforms of CAR, CAR-SIV, and CAR-TVV, while the soluble CAR4/7 was less abundant and the CAR3/7 and CAR2/7 isoforms were barely present. CAR-SIV and CAR-TVV, which differ only on the sequence of their final aminoacids, retain the transmembrane domain while the soluble forms exit the cell [42•]. CAR-SIV expression was restricted to beta cells in human pancreas and isolated islets. It was localized in the cytoplasm, colocalized with insulin, and was located in immature but mainly in mature secretory granules [42•]. As expected, it was not present in insulin deficient islets in the pancreas of T1D donors. However, its expression in insulin containing islets from T1D did not differ from that of non-diabetic or AAb+ [42•]. The absence of the CAR-SIV isoform in alpha cells might explain why they seem to be protected from EV infections and why beta cells might be their main target [43]. In a study of 72 recent onset T1D pancreas, 61% of the people with T1D had VP1-positive cells in some islets and these were all beta cells [44•]. VP1 positivity highly correlated with protein kinase R (PKR), which is upregulated in response to viral infections. In addition, VP1-positive islets hyperexpressed HLA-I molecules but so did many insulin containing islets (ICIs) without the presence of VP1. Whether HLA-I expression reflects an active islet EV infection or might be the consequence of a former infection is not well understood.

HLA-I hyperexpression is a defining feature of T1D [45••]. It is induced upon stimulation of human islets with IFN-α and its expression remains elevated for long time after the stimulus has disappeared [46•]. HLA-I hyperexpression is not present in the islets of non-diabetic donors and seems to appear early in the disease process, as it has been detected in non-diabetic donors with two autoantibodies [47]. Its expression is not restricted to beta cells and virtually all islet cells are able to hyperexpressed HLA-I. In donors with T1D, it correlates with the amount of remaining ICIs and therefore, with disease duration [44•] and it has been observed in individuals with T1D that retained ICIs up to 11 years after disease onset [45••]. This suggests that HLA-I hyperexpression is present as long as beta cells remain and tends to disappear once beta cell destruction is completed and islet inflammation has decreased. Although rare, HLA-I hyperexpression can be seen in insulin-deficient islets (IDIs) in the pancreas of donors with T1D. However, the islets are three-dimensional structures and the presence of remaining beta cells in other areas within the same islet cannot be excluded. Additionally, HLA-I hyperexpression correlates to a certain extent with CD8 T cell infiltration [47] but, islets with high HLA-I expression but no insulitis can be also found in the pancreas of T1D donors, creating an intricate scenario. It is tempting to speculate that an increase in HLA-I expression favors an islet environment of high antigen presentation. This, together with the secretion of pro-inflammatory molecules, creates a fertile field for immune cells, which are attracted to the islets. Autoreactive T cells might be present among these immune infiltrates, and the islet microenvironment has the perfect conditions for beta cell antigen presentation and recognition, activating these cells and ultimately leading to beta cell destruction. Whether islet HLA-I expression is the direct consequence of a viral infection and IFN-production needs further investigation.

## New Strategies to Define the Role of EV in T1D

Ongoing efforts in the T1D research community are aiming to identify which specific EVs are infecting patients before

developing autoimmunity. Therefore, sequencing techniques are becoming increasingly important. An excellent example is the recent study by Honkanen et al., in which 66% of all EV-positive stool samples were genotyped as part of the DIPP study [48•]. This revealed that the most common EVs were coxsackie A viruses (CVA) with the A4 (28% of the samples), A2 (14%), and A16 (11%) as main genotypes. Conversely, 11 and 10% contained a CBV or Echovirus, respectively. This is in agreement with another birth cohort study that reported the predominance of CVAs [49] but differs from other studies in different geographical locations that reported a majority of CVBs [50, 51]. This might reflect important differences in the circulation of EVs in different populations or could be explained by the nature of the sample analyzed. Even the order of infections might decide the final outcome. Investigators in Finland reported that CVB1 conferred risk for developing autoantibodies only when it was the first serotype to infect its host, whereas when CVB3 or CVB6 infection occurred first, the risk of developing autoantibodies was lower [52]. Overall, this highlights the need to study large prospective cohorts in which EV presence is analyzed in the serum, blood, stool, and respiratory samples in order to identify EVs that preferentially infect the respiratory or the gastrointestinal tract, and where all the positive samples are genotyped with the aim of detecting which EV is infecting each patient [48•]. In addition, these studies should be conducted in different countries to account for different environments and potentially different viral species and susceptibility. Moving in this direction are new RT-PCR methods, which are able to target several regions of the EV genome. In a recent study by Genoni et al. [25••], primers directed to the virus 5′UTR, 2C and 3D regions were designed and a new, highly sensitive RT-PCR was tested. Plasma and blood leukocytes from healthy controls or people with T1D, post-polio syndrome (PSP) or chronic viral cardiomyopathy (CVC) were tested. In addition, leukocytes from these people were co-cultured with permissive cell lines for up to six passages [25••]. While the direct detection provided some positive results, the pre-culture step before RT-PCR yielded a significant increase in sample positivity indicating that the detection of low-replicating, persistent viruses might need amplification methods before direct detection is attempted on clinical samples. Similarly, monoclonal antibodies against CVBs showed positive signal in about 0.1–2% of the cells co-cultured with leukocytes from people with PPS, T1D, or CVC demonstrating the existence of a low-grade infection in people with these chronic disorders [25••]. In light of these findings and the potential presence of persistent infections in the pancreas of people with T1D, only the combination of highly sensitive molecular detection and identification together with the clinical, epidemiological, and histopathological findings is likely to provide a definitive answer about the role of EVs as potential triggers of T1D.

## Conclusions

Many years have passed since in 1969, Gamble and colleagues reported the presence of higher titers of CVB antibodies in people with T1D within 3 months of onset than in non-diabetic or people with T1D with longer disease duration [53]. However, since then, despite multiple studies and analyzed samples, there is still controversy in the field regarding its role as potential triggers of T1D. Perhaps, this has nothing to do with the true nature of their involvement in disease pathogenesis and it is based mostly on timing. As infections come and go, whether we are able to detect them or not is highly depending on the time of sample collection. Some viruses manage to stay longer and with sensitive techniques, we will be able to determine their presence in the immediate future. We have gathered evidence for the association of EVs and T1D but we are still missing some important parts of the story. Without them, it is unlikely that we will be able to determine if this association is causal or if viruses might only contribute to beta cell demise at the same level as other potential infectious or inflammatory insults. nPOD might provide some of the answers the field is eager to get through working groups in which collaborative studies involve different laboratories with unique expertise. The nPOD-virus group, created several years ago [54, 55], has studied the presence of EV protein, RNA, and the host anti-viral response in individuals with and without T1D and will publish interesting results soon. There is no doubt that it will open to field to new challenges. Only with optimal study design, cutting edge techniques and collaboration involving multiple laboratories and biobanks, we will be able to determine once and for all, if EVs are a trigger for T1D.

## Compliance with Ethical Standards

**Conflict of Interest** Teresa Rodriguez-Calvo declares that she has no conflict of interest.

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

## References

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. (CDC) CDC 23 July 2018. https://www.cdc.gov/non-polio-enterovirus/index.html.
2. Craig ME, Nair S, Stein H, Rawlinson WD. Viruses and type 1 diabetes: a new look at an old story. Pediatr Diabetes. 2013;14(3):149–58. https://doi.org/10.1111/pedi.12033.

3. Pons-Salort M, Oberste MS, Pallansch MA, Abedi GR, Takahashi S, Grenfell BT, et al. The seasonality of nonpolio enteroviruses in the United States: patterns and drivers. Proc Natl Acad Sci U S A. 2018;115(12):3078–83. https://doi.org/10.1073/pnas.1721159115.
4. Muirhead CR, Cheetham TD, Court S, Begon M, McNally RJ. How do childhood diagnoses of type 1 diabetes cluster in time? PLoS One. 2013;8(4):e60489. https://doi.org/10.1371/journal.pone.0060489.
5. Patterson CC, Gyurus E, Rosenbauer J, Cinek O, Neu A, Schober E, et al. Trends in childhood type 1 diabetes incidence in Europe during 1989-2008: evidence of non-uniformity over time in rates of increase. Diabetologia. 2012;55(8):2142–7. https://doi.org/10.1007/s00125-012-2571-8.
6. Szypowska A, Ramotowska A, Wysocka-Mincewicz M, Mazur A, Lisowicz L, Ben-Skowronek I, et al. Seasonal variation in month of diagnosis of polish children with type 1 diabetes - a multicenter study. Exp Clin Endocrinol Diabetes. 2018; https://doi.org/10.1055/s-0043-125321.
7.•• Krogvold L, Edwin B, Buanes T, Frisk G, Skog O, Anagandula M, et al. Detection of a low-grade enteroviral infection in the islets of langerhans of living patients newly diagnosed with type 1 diabetes. Diabetes. 2015;64(5):1682–7. https://doi.org/10.2337/db14-1370. **This study provides evidence of a low grade enterovirus infection in the pancreas of patients with recent onset T1D.**
8. Baggen J, Thibaut HJ, Strating J, van Kuppeveld FJM. The life cycle of non-polio enteroviruses and how to target it. Nat Rev Microbiol. 2018;16(6):368–81. https://doi.org/10.1038/s41579-018-0005-4.
9.• Oikarinen M, Tauriainen S, Oikarinen S, Honkanen T, Collin P, Rantala I, et al. Type 1 diabetes is associated with enterovirus infection in gut mucosa. Diabetes. 2012;61(3):687–91. https://doi.org/10.2337/db11-1157. **This study detected viral RNA and protein in the gut of patients with T1D.**
10. Mercalli A, Lampasona V, Klingel K, Albarello L, Lombardoni C, Ekstrom J, et al. No evidence of enteroviruses in the intestine of patients with type 1 diabetes. Diabetologia. 2012;55(9):2479–88. https://doi.org/10.1007/s00125-012-2591-4.
11. Snell-Bergeon JK, Smith J, Dong F, Baron AE, Barriga K, Norris JM, et al. Early childhood infections and the risk of islet autoimmunity: the diabetes autoimmunity study in the young (DAISY). Diabetes Care. 2012;35(12):2553–8. https://doi.org/10.2337/dc12-0423.
12. Rasmussen T, Witso E, Tapia G, Stene LC, Ronningen KS. Self-reported lower respiratory tract infections and development of islet autoimmunity in children with the type 1 diabetes high-risk HLA genotype: the MIDIA study. Diabetes Metab Res Rev. 2011;27(8):834–7. https://doi.org/10.1002/dmrr.1258.
13. Beyerlein A, Wehweck F, Ziegler AG, Pflueger M. Respiratory infections in early life and the development of islet autoimmunity in children at increased type 1 diabetes risk: evidence from the BABYDIET study. JAMA Pediatr. 2013;167(9):800–7. https://doi.org/10.1001/jamapediatrics.2013.158.
14. Beyerlein A, Donnachie E, Jergens S, Ziegler AG. Infections in early life and development of type 1 diabetes. JAMA. 2016;315(17):1899–901. https://doi.org/10.1001/jama.2016.2181.
15.• Lonnrot M, Lynch KF, Elding Larsson H, Lernmark A, Rewers MJ, Torn C, et al. Respiratory infections are temporally associated with initiation of type 1 diabetes autoimmunity: the TEDDY study. Diabetologia. 2017;60(10):1931–40. https://doi.org/10.1007/s00125-017-4365-5. **This article shows a positive correlation between recent respiratory infections in young children and an increased risk of islet autoimmunity.**
16. Stene LC, Oikarinen S, Hyoty H, Barriga KJ, Norris JM, Klingensmith G, et al. Enterovirus infection and progression from islet autoimmunity to type 1 diabetes: the diabetes and autoimmunity study in the young (DAISY). Diabetes. 2010;59(12):3174–80. https://doi.org/10.2337/db10-0866.
17.•• Oikarinen S, Martiskainen M, Tauriainen S, Huhtala H, Ilonen J, Veijola R, et al. Enterovirus RNA in blood is linked to the development of type 1 diabetes. Diabetes. 2011;60(1):276–9. https://doi.org/10.2337/db10-0186. **This study reported that EV RNA positive samples are more frequent in case children that progress to type 1 diabetes and that the strongest risk is related to EV positivity 6 months before the appearance of the first autoantibody.**
18. Mustonen N, Siljander H, Peet A, Tillmann V, Harkonen T, Ilonen J, et al. Early childhood infections precede development of beta-cell autoimmunity and type 1 diabetes in children with HLA-conferred disease risk. Pediatr Diabetes. 2018;19(2):293–9. https://doi.org/10.1111/pedi.12547.
19.•• Ziegler AG, Bonifacio E, Group B-BS. Age-related islet autoantibody incidence in offspring of patients with type 1 diabetes. Diabetologia. 2012;55(7):1937–43. https://doi.org/10.1007/s00125-012-2472-x. **This article reports that the age period 9 months to 2 years is associated with a high incidence of activation of type 1 diabetes associated autoimmunity in genetically at-risk children.**
20.• Krischer JP, Lynch KF, Schatz DA, Ilonen J, Lernmark A, Hagopian WA, et al. The 6 year incidence of diabetes-associated autoantibodies in genetically at-risk children: the TEDDY study. Diabetologia. 2015;58(5):980–7. https://doi.org/10.1007/s00125-015-3514-y. **Collaborative study that shows evidence for the appearance of autoantibodies very early in life The incidence of IAA peaks within the first year of life and declines over the following 5 years, but GADA only increases until the second year and remains relatively constant.**
21.•• Lietzen N, An LTT, Jaakkola MK, Kallionpaa H, Oikarinen S, Mykkanen J, et al. Enterovirus-associated changes in blood transcriptomic profiles of children with genetic susceptibility to type 1 diabetes. Diabetologia. 2018;61(2):381–8. https://doi.org/10.1007/s00125-017-4460-7. **Innovative study that used genome-wide transcriptomics data to characterise EV-associated changes in whole-blood samples from children with genetic susceptibility to type 1 diabetes.**
22. Ferreira RC, Guo H, Coulson RM, Smyth DJ, Pekalski ML, Burren OS, et al. A type I interferon transcriptional signature precedes autoimmunity in children genetically at risk for type 1 diabetes. Diabetes. 2014;63(7):2538–50. https://doi.org/10.2337/db13-1777.
23. Kallionpaa H, Elo LL, Laajala E, Mykkanen J, Ricano-Ponce I, Vaarma M, et al. Innate immune activity is detected prior to seroconversion in children with HLA-conferred type 1 diabetes susceptibility. Diabetes. 2014;63(7):2402–14. https://doi.org/10.2337/db13-1775.
24. Laiho JE, Oikarinen M, Richardson SJ, Frisk G, Nyalwidhe J, Burch TC, et al. Relative sensitivity of immunohistochemistry, multiple reaction monitoring mass spectrometry, in situ hybridization and PCR to detect Coxsackievirus B1 in A549 cells. J Clin Virol. 2016;77:21–8. https://doi.org/10.1016/j.jcv.2016.01.015.
25.•• Genoni A, Canducci F, Rossi A, Broccolo F, Chumakov K, Bono G, et al. Revealing enterovirus infection in chronic human disorders: an integrated diagnostic approach. Sci Rep. 2017;7(1):5013. https://doi.org/10.1038/s41598-017-04993-y. **Development of a new RT-PCR and in vitro amplification methods with the aim of increasing the sensitivity for the detection of EVs in clinical samples.**
26. Philips T, Kusmartseva I, Gerling IC, Campbell-Thompson M, Wasserfall C, Pugliese A, et al. Factors that influence the quality of RNA from the pancreas of organ donors. Pancreas. 2017;46(2):252–9. https://doi.org/10.1097/MPA.0000000000000717.
27. Huang X, Yuang J, Goddard A, Foulis A, James RF, Lernmark A, et al. Interferon expression in the pancreases of patients with type I diabetes. Diabetes. 1995;44(6):658–64.
28. Foulis AK, Farquharson MA, Meager A. Immunoreactive alpha-interferon in insulin-secreting beta cells in type 1 diabetes mellitus. Lancet. 1987;2(8573):1423–7.

29. Newby BN, Mathews CE. Type I interferon is a catastrophic feature of the diabetic islet microenvironment. Front Endocrinol (Lausanne). 2017;8:232. https://doi.org/10.3389/fendo.2017.00232.
30. Newby BN, Brusko TM, Zou B, Atkinson MA, Clare-Salzler M, Mathews CE. Type 1 Interferons potentiate human CD8(+) T-cell cytotoxicity through a STAT4- and Granzyme B-dependent pathway. Diabetes. 2017;66(12):3061–71. https://doi.org/10.2337/db17-0106.
31. Morse ZJ, Horwitz MS. Innate viral receptor signaling determines type 1 diabetes onset. Front Endocrinol (Lausanne). 2017;8:249. https://doi.org/10.3389/fendo.2017.00249.
32. Op de Beeck A, Eizirik DL. Viral infections in type 1 diabetes mellitus–why the beta cells? Nat Rev Endocrinol. 2016;12(5): 263–73. https://doi.org/10.1038/nrendo.2016.30.
33. Sarmiento L, Frisk G, Anagandula M, Hodik M, Barchetta I, Netanyah E, et al. Echovirus 6 infects human exocrine and endocrine pancreatic cells and induces pro-inflammatory innate immune response. Viruses. 2017;9(2) https://doi.org/10.3390/v9020025.
34. Marroqui L, Dos Santos RS, Op de Beeck A, Coomans de Brachene A, Marselli L, Marchetti P, et al. Interferon-alpha mediates human beta cell HLA class I overexpression, endoplasmic reticulum stress and apoptosis, three hallmarks of early human type 1 diabetes. Diabetologia. 2017;60(4):656–67. https://doi.org/10.1007/s00125-016-4201-3.
35. Freimuth P, Philipson L, Carson SD. The coxsackievirus and adenovirus receptor. Curr Top Microbiol Immunol. 2008;323:67–87.
36. Selinka HC, Wolde A, Sauter M, Kandolf R, Klingel K. Virus-receptor interactions of coxsackie B viruses and their putative influence on cardiotropism. Med Microbiol Immunol. 2004;193(2–3):127–31. https://doi.org/10.1007/s00430-003-0193-y.
37. Chehadeh W, Kerr-Conte J, Pattou F, Alm G, Lefebvre J, Wattre P, et al. Persistent infection of human pancreatic islets by coxsackievirus B is associated with alpha interferon synthesis in beta cells. J Virol. 2000;74(21):10153–64.
38.• Hodik M, Anagandula M, Fuxe J, Krogvold L, Dahl-Jorgensen K, Hyoty H, et al. Coxsackie-adenovirus receptor expression is enhanced in pancreas from patients with type 1 diabetes. BMJ Open Diabetes Res Care. 2016;4(1):e000219. https://doi.org/10.1136/bmjdrc-2016-000219. **This article shows that CAR is expressed in the pancreas and its expression is enhanced in T1D patients, favoring a potential EV infection.**
39. Ylipaasto P, Klingel K, Lindberg AM, Otonkoski T, Kandolf R, Hovi T, et al. Enterovirus infection in human pancreatic islet cells, islet tropism in vivo and receptor involvement in cultured islet beta cells. Diabetologia. 2004;47(2):225–39. https://doi.org/10.1007/s00125-003-1297-z.
40. Petzold A, Solimena M, Knoch KP. Mechanisms of Beta cell dysfunction associated with viral infection. Curr Diab Rep. 2015;15(10):73. https://doi.org/10.1007/s11892-015-0654-x.
41. Sarmiento L, Medina A, Aziz K, Anagandula M, Cabrera-Rode E, Fex M, et al. Differential effects of three echovirus strains on cell lysis and insulin secretion in beta cell derived lines. J Med Virol. 2016;88(6):971–8. https://doi.org/10.1002/jmv.24438.
42.• Ifie E, Russell MA, Dhayal S, Leete P, Sebastiani G, Nigi L, et al. Unexpected subcellular distribution of a specific isoform of the Coxsackie and adenovirus receptor, CAR-SIV, in human pancreatic beta cells. Diabetologia. 2018; https://doi.org/10.1007/s00125-018-4704-1. **This study characterizes the expression of CAR and concludes that CAR-SIV, the predominant isoform of CAR, is expressed in beta cells and is located in the cytoplasm, colocalizing with insulin.**
43. Marroqui L, Lopes M. dos Santos RS, Grieco FA, Roivainen M, Richardson SJ et al. differential cell autonomous responses determine the outcome of coxsackievirus infections in murine pancreatic alpha and beta cells. elife. 2015;4:e06990. https://doi.org/10.7554/eLife.06990.
44.• Richardson SJ, Willcox A, Bone AJ, Foulis AK, Morgan NG. The prevalence of enteroviral capsid protein vp1 immunostaining in pancreatic islets in human type 1 diabetes. Diabetologia. 2009;52(6):1143–51. https://doi.org/10.1007/s00125-009-1276-0. **This study reports that VP1 is commonly found in the islets of recent-onset T1D patients compared to controls.**
45.•• Richardson SJ, Rodriguez-Calvo T, Gerling IC, Mathews CE, Kaddis JS, Russell MA, et al. Islet cell hyperexpression of HLA class I antigens: a defining feature in type 1 diabetes. Diabetologia. 2016;59(11):2448–58. https://doi.org/10.1007/s00125-016-4067-4. **This collaborative study analyzed samples from the EADB, nPOD and DiViD cohort. It describes hyperexpression of HLA class I molecules as a defining feature of T1D.**
46.• Coomans de Brachene A, Dos Santos RS, Marroqui L, Colli ML, Marselli L, Mirmira RG, et al. IFN-alpha induces a preferential long-lasting expression of MHC class I in human pancreatic beta cells. Diabetologia. 2018;61(3):636–40. https://doi.org/10.1007/s00125-017-4536-4. **This study shows that HLA-I overexpression can be maintained for long time after the inflammatory stimuli has dissapeared.**
47. Rodriguez-Calvo T, Suwandi JS, Amirian N, Zapardiel-Gonzalo J, Anquetil F, Sabouri S, et al. Heterogeneity and Lobularity of pancreatic pathology in type 1 diabetes during the Prediabetic phase. J Histochem Cytochem. 2015;63(8):626–36. https://doi.org/10.1369/0022155415576543.
48.• Honkanen H, Oikarinen S, Nurminen N, Laitinen OH, Huhtala H, Lehtonen J, et al. Detection of enteroviruses in stools precedes islet autoimmunity by several months: possible evidence for slowly operating mechanisms in virus-induced autoimmunity. Diabetologia. 2017;60(3):424–31. https://doi.org/10.1007/s00125-016-4177-z. **This article suggest that EV infections diagnosed by detecting viral RNA in stools are associated with the development of islet autoimmunity with a time lag of several months.**
49. Simonen-Tikka ML, Pflueger M, Klemola P, Savolainen-Kopra C, Smura T, Hummel S, et al. Human enterovirus infections in children at increased risk for type 1 diabetes: the Babydiet study. Diabetologia. 2011;54(12):2995–3002. https://doi.org/10.1007/s00125-011-2305-3.
50. van der Sanden SM, Koopmans MP, van der Avoort HG. Detection of human enteroviruses and parechoviruses as part of the national enterovirus surveillance in the Netherlands, 1996-2011. Eur J Clin Microbiol Infect Dis. 2013;32(12):1525–31. https://doi.org/10.1007/s10096-013-1906-9.
51. Tan CY, Ninove L, Gaudart J, Nougairede A, Zandotti C, Thirion-Perrier L, et al. A retrospective overview of enterovirus infection diagnosis and molecular epidemiology in the public hospitals of Marseille, France (1985-2005). PLoS One. 2011;6(3):e18022. https://doi.org/10.1371/journal.pone.0018022.
52. Laitinen OH, Honkanen H, Pakkanen O, Oikarinen S, Hankaniemi MM, Huhtala H, et al. Coxsackievirus B1 is associated with induction of beta-cell autoimmunity that portends type 1 diabetes. Diabetes. 2014;63(2):446–55. https://doi.org/10.2337/db13-0619.
53. Gamble DR, Kinsley ML, FitzGerald MG, Bolton R, Taylor KW. Viral antibodies in diabetes mellitus. Br Med J. 1969;3(5671):627–30.
54. Pugliese A, Yang M, Kusmarteva I, Heiple T, Vendrame F, Wasserfall C, et al. The Juvenile Diabetes Research Foundation network for pancreatic organ donors with diabetes (nPOD) program: goals, operational model and emerging findings. Pediatr Diabetes. 2014;15(1):1–9. https://doi.org/10.1111/pedi.12097.
55. Pugliese A, Vendrame F, Reijonen H, Atkinson MA, Campbell-Thompson M, Burke GW. New insight on human type 1 diabetes biology: nPOD and nPOD-transplantation. Curr Diab Rep. 2014;14(10):530. https://doi.org/10.1007/s11892-014-0530-0.