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http://www.jrdiabet.ro
Rom J Diabetes Nutr Metab Dis. **19**(3):323-329
doi: 10.2478/v10255-012-0038-9

THE ASSOCIATION BETWEEN ENTEROVIRUSES AND TYPE 1 DIABETES

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received: June 03, 2012 *accepted*: September 02, 2012
available online: September 30, 2012

Abstract

The hypothesis that under some circumstances enteroviral infections can lead to type 1 diabetes (T1D) was proposed several decades ago, based initially on evidence from animal studies and sero-epidemiology. The mechanisms leading to the disease involve complex interactions between the virus, host target tissue (pancreas) and the immune system. The following article is intended as a review of several recent information of the topic based on human studies that try to establish a connection between a viral infection and Type 1 diabetes. Through understanding better this association and it's implications in the onset of T1D potential new ways of prevention and treatment may emerge.

key words: *Type 1 diabetes, etiology, enterovirus, beta cells, autoimmunity*

Introduction

Type 1 diabetes mellitus (T1D) is a multi-factorial immune-mediated disease characterized by the autoimmune destruction of insulin-producing pancreatic islet beta cells in genetically susceptible individuals. Epidemiological evidence has documented the constant rise in the incidence of T1D worldwide, with viral infections representing one of the candidate environmental risk factors identified by several independent studies. In fact, epidemiological data showed that T1D incidence increases after epidemics due to enteroviruses and that enteroviral RNA can be detected in the blood of >50% of T1D patients at the time of disease onset [1]. Furthermore, both *in-vitro* and *ex-vivo* studies have shown that viruses can infect pancreatic beta cells with consequent effects ranging from functional damage to cell death. More profound insights into the intricate relationship between viruses and their autoimmunity-prone host may lead ultimately to opportunities for early intervention through immune modulation or vaccination [1-3].

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Enteroviruses as possible determinants in T1D

Enteroviruses have been the primary candidate since Gamble reported high titers of neutralizing antibodies to Coxsackie B virus in recent-onset T1D patients [1], and Yoon isolated a Coxsackie B4 virus from a child with T1D and established several lines of evidence for causality [1]. Despite a number of impressive investigations using different approaches, the nature of the relationship between enteroviruses and T1D remains controversial. In most cases, diagnosis of T1D follows a long period of preclinical islet autoimmunity. The latter is essential for interpretation of etiological studies, as viruses present at diagnosis may have infected the host late in the disease process, or etiological infections may have been cleared at the time of diagnosis [1-3].

Enteroviruses are ubiquitous, single-stranded non-enveloped RNA viruses, transmitted generally through the fecal-oral route, and replicating primarily in the gut. There are more than 100 defined human enterovirus serotypes (http://www.picornaviridae.com), and many more strains have been defined by sequencing. Most enterovirus infections are asymptomatic, but some serotypes are associated with severe clinical symptoms in a small proportion of those infected. While most children encounter an enterovirus by the age of 2 years, infection with a given serotype is obviously not as common. Co-infections with two serotypes may occur. Epidemic outbreaks often follow natural circulation of the same serotype, and it is not entirely clear why outbreaks of enterovirus-associated disease sometimes occur. As spontaneous mutations and recombination are common among enteroviruses, it is speculated that virulent strains may emerge spontaneously during an infection. Prospective studies with frequent sampling of biological specimens and a sufficient number of cases with endpoint are necessary to document statistically significant associations between infections and later risk of islet autoimmunity or T1D. The available longitudinal studies investigating the potential link between serial postnatal measures of enterovirus infections and islet autoimmunity (or T1D) include three Finnish studies: DIPP (Diabetes Prediction and Prevention Study), DiMe (Childhood Diabetes In Finland) and TRIGR (Trial to Reduce IDDM in the Genetically at Risk); one USA study – DAISY (Diabetes and Autoimmunity Study in the Young) in Colorado; MIDIA in Norway (Environmental Triggers of Type 1 Diabetes) and the German BABYDIAB and Babydiet studies. Preliminary data from a study in Australia called Viral Etiology of type 1 Diabetes (VIGR) have been presented only in abstract form at the time of this manuscript writing [3], and the results were shown in the review by Yeung *et al*. [4], but details on methodology have not yet been published in full. All these studies include children with increased risk of T1D, defined by a first-degree family history, HLA susceptibility genes or both. Seven studies have published data from a total of 176 cases of islet autoimmunity, and one study (DiMe) followed subjects with islet autoimmunity for T1D as end-point. Sample frequency and method of detection varied between these studies [1,3].

We are giving in Table 1 the main arguments for or against the viral etiology of T1D.

Table 1. Pros and Cons in the viral hypothesis of T1D etiology. (Adapted after [3]).

PRO	CON
Environmental factor involved	Risk is predominantly genetic and HLA-related
Seasonal fluctuations in T1D onset	Seasonality involves sun exposure and vitamin D deficiency
„Fulminant" T1D patients show flu like illness	„Fulminant" T1D lacks autoimmune component
Multiple studies show a connection between enterovirus infection and T1D onset/progression	Other studies, even some meta-analyses, fail to prove a definite connection
Enterovirus particles were found in pancreatic islets from T1D patients	Also found in T2D. Detection methods are flawed
Islets exhibit molecular markers potentially related to viral infection	Cause effect not demonstrated, marker of ongoing inflammation
Cytotoxic T Lymphocytes (CTL) are predominant in islet infiltrates	CTL are not initiators
Some viruses trigger or prevent autoimmune diabetes in mouse modes	Animal models are flawed, induction requires insulitis, protection in NOD mice is easy
Genetic defects could predispose to inappropriate anti-viral responses and T1D	Such mutations are very rare

Possible models of Enterovirus infection involved in T1D pathogenesis include:

1. The polio hypothesis

Some studies have suggested a relationship between perinatal infections and risk of T1D in childhood [5], while a number of others have not found any significant relationship. The analogy between poliomyelitis and the potential enterovirus – T1D link was pointed out a long time ago [6].

It is possible that declining proportions of pregnant women providing their infants with anti-enterovirus antibodies may explain some of the increasing incidence of T1D over time, although direct evidence for this in humans is lacking. Interestingly, diabetes induced with selected virus infections in LEW.1WR1 rat offspring could be prevented by infection with the same virus of the mothers prior to pregnancy, suggesting strongly that maternal antibodies could be involved [5]. Similar findings were observed for Coxsackievirus B3-induced diabetes in a transgenic NOD mouse model (P.G. Larsson and M. Flodström-Tullberg, results presented as conference abstracts in 2010 and 2011). The further possibility they discuss, as do Coppieters and colleagues [3], is that viral infection might be protective if encountered in early infancy, whereas delayed exposure is more likely to be diabetogenic. This, the polio hypothesis, actually does not work that well for polio, and has the further limitation that prospective studies from birth show that the first islet autoantibodies have typically appeared by the age of 2 years, and that multiple autoantibody positivity, the necessary prelude to most cases of childhood diabetes, is typically established by the age of 3 years, an observation which does not allow much opportunity for delayed exposure to the virus [5-8].

2. The hygiene theory

The question of hygiene and its role in reducing contact with fecal-oral transmitted microbes and viruses has been argued to be of potential importance when considering how human T1D comes about. A number of

potential mechanisms have been proposed to explain the so-called hygiene hypothesis for type 1 diabetes. Depending on the timing, enterovirus and other microbial agents may reduce the incidence of autoimmune diabetes in experimental animals. Induction of regulatory T cells is among the mechanisms involved, but it is unknown whether a similar phenomenon operates in humans [9].

3. The" hit-and-run" and „multiple-hits" hypothesis

Virus detected in patients at or after diagnosis may well have infected the host after disease onset, whether the virus is detected in tissues, blood or feces. Furthermore, lack of virus at diagnosis does not exclude a role of virus in the etiology, as 'hit-and-run'-type mechanisms may have been involved. Prospective studies with frequent sampling of biological specimens and a sufficient number of cases with hard endpoints are necessary to document statistically significant associations between infections and later risk of islet autoimmunity or T1D. The potential effect of enterovirus infections could, theoretically, be cumulative, reflecting multiple 'hits' over time. Alternatively, it could be argued that infections should occur just before islet autoimmunity to be implicated plausibly in the etiology [4,9,10].

From viral infection to autoimmunity (or T1D)

Viruses could potentially be involved at three stages in the pathogenesis of T1DM: they could play a primary role in initiating autoimmunity, a secondary role in promoting it or a tertiary role in precipitating the onset of disease. Various authors point out that viral infection might potentially influence the onset of diabetes at two key transitions: from genetic susceptibility to the onset of autoimmunity, and from established autoimmunity to the onset of hyperglycemia.

1. Genetic susceptibility to T1D

Genetic susceptibility to T1D is regulated primarily by the HLA gene locus. Genome-wide association studies have identified additional loci containing regions associated with altered risk for T1D development [5,6,9]. Some contain candidate genes with a role in the innate immune response, and it is possible that the T1D-associated non-synonymous single nucleotide polymorphisms (SNPs) alter the biological functions of the translated proteins. Considering the prominent role for the innate immune response during enterovirus infection, it is possible that some of these proteins contribute to the host defense against these viruses. In 2006 a novel T1D susceptibility locus was discovered, the identified gene was *mda5/ifih1*. Moreover, genetic variants of *mda5/ifih1* have been associated with other autoimmune conditions including psoriasis, autoimmune thyroid disease and systemic lupus erythematosus (SLE). The *ifih1/mda5* gene encodes the RLR (Retinoic acid Like Receptors) family member MDA5 (Melanoma Differentiation-Associated protein), known to recognize dsRNA (double stranded RNA) generated during the replication of certain picornaviruses (e.g. enteroviruses), as well as synthetic dsRNA (poly I : C). MDA5 is important for the induction of type I interferon (IFN) production by members of the picornavirus family but also by other types of viruses, such as West Nile, Dengue and measles virus. Using *mda5/ifih1*-/- mice, some authors have

demonstrated that MDA5 is crucial for host survival during CVB3 (Coxackie virus B 3) infection and that it plays an important role in regulating viral replication [2]. These observations strongly implicate MDA5 in the host response to enterovirus infections.

2. Proinflammatory response to virus infection

A virus infection often results in the production of pro-inflammatory cytokines (e.g. IFNs) and the activation of APCs (Antigen Presenting Cells) such as DCs (Dendritic Cells). If this coincides with tissue damage, endogenous antigens might be taken up and presented by the APC. In an individual with a genetic predisposition, this may result in the aberrant activation of self-reactive T cells, so-called 'bystander' activation. Diabetes development in this hypothesis was dependent upon a preexisting insulitis, suggesting that the virus accelerates an already ongoing diabetogenic process rather than initiating it. Interestingly, recent data from the Diabetes and Autoimmunity Study in the Young (DAISY) suggests that progression from islet autoimmunity to clinical disease may increase after an enterovirus infection, and is thus in agreement with the animal model [5,6,9].

A weak innate immune response, for example by the inability to mount an efficient IFN response, is associated with unrestricted viral replication and increased viral spread. Tissue damage may follow as a direct effect of the infecting virus, and it is possible that the inefficient immune response favors viral persistence. An alternative scenario is that the innate immune response to infection is triggering the activation of self-reactive T cells. The stronger the response, the greater the risk that T cells become activated. The strong anti-viral activities of the IFNs are attributed to their ability to induce the transcription of hundreds of genes, many with distinct anti-viral activities such as protein kinase R (PKR) and 2′5′-oligoadenylate synthase (2′5′OAS) [11]. This results in the activation of a so-called anti-viral state in responding cells that aims to block viral replication and prevent uninfected cells from becoming infected. The IFNs also induce or up-regulate the expression of PRRs (Pattern Recognition Receptors) and related signaling molecules [e.g. MDA5, RIG-I (Retinioic acid Inducible Gene 1 like receptors) and signal transducers and activators of transcription (STATs)], allowing cells to recognize and respond more efficiently to infecting viruses**.** IFNs also modulate various cellular responses of the innate immune system including cytotoxicity of NK cells and maturation of antigen-presenting cells (APCs),

3. Role of antibodies enhancing the infection with coxackievirus-B

The mechanisms leading to the disease involve interactions between the virus, host target tissue (pancreas) and the immune system. The infection of target cells with viruses can be prevented by antibodies [1-3,12]. Conversely, the infection can be enhanced by antibodies. The antibody-dependent enhancement (ADE) of infection has been described with various viruses, especially Picornaviruses. In mice infected with CV-B3 this phenomenon resulted in an extended inflammatory reaction and myocarditis [2,3]. In humans, non-neutralizing antibodies can increase the infection of monocytes with CV-B4 and stimulate the production of interferon (IFN)-α by these cells

in vitro. CV-B4/immunoglobulin (Ig)G immune complexes interacted with a specific viral receptor [Coxsackievirus and adenovirus receptor (CAR)] and with IgG Fc fraction receptors (FcgRII and FcgRIII) on the surface of monocytes [3]. Such antibodies have been detected in patients with type 1 diabetes and they could be responsible for the presence of enteroviral RNA and IFN-α in peripheral blood mononuclear cells (PBMC) of these individuals [4,9]. The target of enhancing antibodies has been identified as the VP4 protein, which allowed the detection of these antibodies by enzyme-linked immunosorbent assay (ELISA). It cannot be excluded that antibodies enhancing the infection with CV-B may play a role in the pathogenesis of type 1 diabetes, induced or aggravated by these viruses. They can cause a viral escape from the immune response and may participate in the spreading of viruses to beta cells.

Conclusions

The hypothesis that a virus might in some way be involved in the causation of type 1 diabetes has a long history, but decades of research have failed to resolve the issue beyond reasonable doubt [1,4,7,10,12,13].

Numerous observations, ranging from clinical case reports to results emerging from large epidemiological studies, indicate that enterovirus infections play a role in T1D [14,15]. The virus has been isolated from patients at disease onset. It has been found more frequently in blood or serum from T1D patients compared to control individuals, the appearance of autoantibodies coincide with enterovirus infections in some individuals, and the virus has been found more often in the gut and pancreatic islets of T1D patients compared to control individuals. There are, however, studies that have failed to confirm these observations, opening the possibility that enterovirus infections contributes to some, but not all, cases of T1D.

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