

Group B coxsackieviruses and autoimmunity: focus on Type 1 diabetes

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Group B coxsackieviruses (CVB) and/or their components have been found in the blood and pancreas of patients with Type 1 diabetes (T1D). CVB infections lead to the activation of the innate and adaptive immune systems, which can result in the induction or aggravation of autoimmune processes. Persistent and/or repeated infections of pancreas islet β cells with CVB and the resulting production of IFN- α and inflammatory mediators, combined with a predisposed genetic background, may induce bystander activation of autoimmune effector T cells and an autoreactive response to islet self-antigens through molecular mimicry. Moreover, the antibody-dependent enhancement of CVB infection of monocytes, as well as infection of the thymus can intervene in the pathogenesis of T1D. In contrast with the deleterious effect of CVB, it has been shown that these viruses can protect against the development of T1D under certain experimental conditions. The role of CVB in autoimmunity is complex, and therefore a better understanding of the inducer versus protective effects of these viruses in T1D will help to design new strategies to treat and prevent the disease.

KEYWORDS: autoimmunity • Group B coxsackieviruses • islet β cells • Type 1 diabetes

The immune system has the potential to make barriers that sense and neutralize any non-self-component entering the body. All individuals are tolerant of their own potentially antigenic substances. The loss of recognition of its own constituents as self is the fundamental cause of autoimmunity. Although the etiology of the breakdown of immune tolerance remains largely unknown, both environmental and inherited components participate in the development of autoimmunity [1]. Among environmental factors, the association between viral infections and autoimmune diseases has often been referred to. Viral infections generally induce a host defense reaction. However, in some cases, the host immune response may develop into a pathogenic process and lead to autoimmune diseases. Studies carried out in humans and in animal models strongly suggest that infectious agents can induce autoimmune diseases [2,3]. Group B coxsackieviruses (CVB) are associated with acute diseases of various organs, and their role in chronic autoimmune diseases, such as Type 1 diabetes (T1D) and myocarditis, is highly suspected. In this article, the association between CVB and T1D and the mechanisms by which CVB

infections can intervene in the pathogenesis of the disease have been analyzed. Before in-depth analysis of the relationship between CVB and autoimmunity, it is important to state that the involvement of a virus in the etiology of an autoimmune disease can be difficult to establish, particularly with regards to ubiquitous viruses such as enteroviruses. On the other hand, investigations to establish the etiology and mechanisms of human disease generally depend upon the availability of animal models. However, despite their indispensability in the study of mechanisms underlying human diseases, experimental models have limitations in disease investigations.

Group B coxsackieviruses

Group B coxsackieviruses are small nonenveloped positive ssRNA viruses belonging to the human enterovirus B species of the *Enterovirus* genus of the *Picornaviridae* family. CVB have a small genome of approximately 7500 bases that comprises a unique large, open-reading frame coding for a massive polyprotein of 2200 amino acids and leading, after successive cleavages, to the production of 11 mature proteins, of which four are structural and seven are nonstructural [4].

There are six different CVB serotypes (CVB1–6) [5,6]. The genome is flanked by two noncoding regions at both the 5' and 3' ends, which are essential for viral RNA synthesis and translation initiation. Virus replication occurs in the cytoplasm of the host cell harboring specific enterovirus receptors, with the most prominent of these receptors being the coxsackievirus and adenovirus receptor. As they are mostly located in the heart and pancreas, coxsackievirus and adenovirus receptors play an important role in the pathogenesis and CVB tropism of these target organs [7].

Infections with CVB, similarly to those with other enteroviruses, are asymptomatic or subclinical with only mild common cold-like symptoms. In some cases, CVB infections have been associated with acute manifestations both in humans and in mice: aseptic meningitis, myocarditis, encephalitis, pericarditis and pancreatitis [8]. In addition, CVB infections have also been associated with chronic diseases such as dilated cardiomyopathy, meningoencephalitis, chronic myocarditis and T1D [9–12]. They can establish a persistent infection *in vitro*, especially in human pancreatic β cells [13]. Persistence of these viruses in resting or quiescent cells has also been reported [14].

CVB & innate & adaptive immunity

After crossing the epithelial barrier which, in mammals, separates the host from the external environment, the infectious agent encounters a wide range of specialized cells and proteins that constitute the immune system responsible for defending the body against external attacks. The host response to viral infection can be divided into two main categories: the innate immune system, which can react quickly in order to stop or limit virus replication and spread; and the pathogen-specific adaptive immune system, which is activated following the reaction of the innate system and which strongly contributes to clearance of the infection [15].

Infection and recognition of CVB by pattern-recognition receptors such as TLR-3, RIG-1 and IFIH-1 activate the production of cytokines (i.e., chemokines, interleukins and interferons). Interleukins stimulate and activate cells (i.e., dendritic cells, monocytes and natural killer cells), while interferons, in addition to an immunomodulating effect, can act by upregulating proteins that can limit the permissiveness of the cell to virus infection [16–19].

The product of the *IFIH-1* gene, located in the cytosol, allows infected cells to detect CVB viruses [20]. This results in the production of type 1 interferon, which initially sharply limits viral replication and prevents damage to infected cells. Moreover, interferons act by enhancing overexpression of class 1 MHC molecules, resulting in increased exposure of infected cells to the immune system. The effect of interferons, combined with those of other inflammatory factors (e.g., TNF- α), directly contributes to damage of β cells. The *IFIH-1* gene (also known as *MDA-5*) is located in a region associated with T1D by genome-wide association studies. Alleles carried by the majority of the population predispose to the disease. Single nucleotide polymorphism studies of *IFIH-1* variants have demonstrated the existence of rare alleles that encoded for each of the known *IFIH-1* polymorphisms, which are known to have a strong protective effect against T1D [21].

Inflammation is a result of tissue infection or injury which, if deregulated, can contribute to the induction of autoimmune and inflammatory disorders such as T1D and myocarditis. Innate immunity and inflammatory mediators might play an important role in disease onset by contributing not only to the induction and amplification of the immune attack against β cells, but also to stabilizing and maintaining local pancreatic-islet inflammation. Activation of pattern-recognition receptor ligands might result in insulinitis and progressive β -cell destruction [22]. RNA-sensing Toll-like receptors have been implicated in the detection and recognition of CVB in human cardiac and plasmacytoid dendritic cells (TLR-7 and -8), and in monocytes (TLR-3) [23–25]. Intra- and extra-cellular virus dsRNA, generated in the course of CVB replication, activates pattern-recognition receptors and induce production of IFN- α and IFN- β , infected cell apoptosis and the production of chemokines. These processes, initiated to contain the infection, can be deleterious and involved in the development of autoimmunity associated with these infections [26]. It has been demonstrated that CVB3 infection downregulates cell surface expression of natural killer cell receptor ligands on infected cells and induces the production of IFN- γ by natural killer cells, thereby contributing to the host anti-CVB3 immune response [27].

IFIH-1 could be the link between how viruses, a patient's genetic background and their innate immune system can contribute towards the development of diseases (FIGURE 1) [28]. It has recently been demonstrated in mice that the product of the *IFIH-1* gene, a cellular receptor involved in the recognition of CVB3, is essential for preventing early virus replication. Indeed, *MDA-5* deficiency leads to faster virus replication, suggesting that *MDA-5* may play a critical role in CVB3-enhancing inflammation and tissue damage [29].

Several studies have demonstrated that a strong adaptive immune system is necessary for clearance of infecting virus and long-term immunity. Gammaglobulins, secreted by B lymphocytes, play a crucial role in CVB3 clearance and in preventing persistent virus infection in mice [30]. The potent role that antibodies could play in limiting the scope and severity of enterovirus infections is illustrated by the case of X-linked agammaglobulinemia. In this antibody deficiency syndrome, which affects B lymphocytes, the serum level of immunoglobulins is very low. Patients affected by this disease are particularly susceptible to infections such as chronic enteroviral meningoencephalitis [31,32]. In CVB3-induced myocarditis and pancreatitis, it has been demonstrated that T lymphocytes can limit virus replication in mice [33]. In response to *in vitro* challenge with CVB4 antigens, patients with T1D showed reduced activation and cytokine production by T lymphocytes, suggesting that T1D patients might be more susceptible to CVB4 infections and related complications than healthy subjects [34].

CVBs are associated with T1D

Type 1 diabetes is an autoimmune disease characterized by hyperglycemia that results from destruction of insulin-producing β cells. Although the cause of T1D is still unknown, it is nevertheless understood that inflammation plays a predominant role in the onset of the disease [22] and it is strongly suspected that T1D is a

result of complex interactions of immunological, genetic and environmental factors, particularly viruses [35]. The detection of enteroviruses in various tissues, blood, gut and the pancreas of patients with T1D suggests an association between these viruses and the disease [9,36–38]. It is highly suspected that enteroviruses, particularly CVB, are involved in the pathogenesis of the disease [35,39]. The initiation and progression of the autoimmune process can last several years and the subclinical phase is characterized by circulating autoantibodies targeting islet β -cell autoantigens. It has been demonstrated in nonobese diabetic (NOD) mice that T cells are involved in the impairment of β cells and other studies have shown that most patients develop both autoreactive T and B cells that recognize β -cell antigens [40,41].

Some retrospective studies have found no association between enterovirus infections and T1D. It should be noted, however, that all those studies were based on the detection of anti-enterovirus antibodies, and only a few enteroviruses were tested. Second, some of these studies included a relatively small number of cases and raise other questions concerning the procedure for selection of control subjects [42,43].

Nevertheless, enteroviruses have been suggested to be important etiological and/or aggravating factors in the development of T1D and the most commonly implicated in the disease process are CVB. It was first demonstrated that anti-CVB antibodies are more prevalent in T1D patients than in control subjects and that a strain of CVB serotype 4, isolated from the pancreas of a 10-year-old boy who died of diabetic ketoacidosis, could induce the disease in mice [44,45]. More recently, several retrospective and prospective studies conducted in humans have provided evidence to reinforce this hypothesis of the involvement of enteroviruses in the development of T1D. Some of these studies have demonstrated that enteroviruses are more frequently found in biological samples from diabetic patients (children and adults) than in healthy controls and, when identified, the most often involved enteroviruses are CVB (TABLE 1) [13,46–53]. CVB RNA was found in blood and in autopsy pancreatic samples of diabetic patients. In addition, many other studies have also revealed the presence of anti-enterovirus antibodies and/or enterovirus RNA, more frequently in prediabetic and diabetic patients than in healthy controls [10,12,54–56].

It could be argued that the viral genome was not identified thoroughly in some studies, since CVB genotype cannot reliably be deduced from sequencing of the 5' end of viral RNA, but only from sequencing VP1. However, the identified sequences belonging to the human enterovirus-B species were most probably CVB. Furthermore, immunohistochemical findings, not combined with

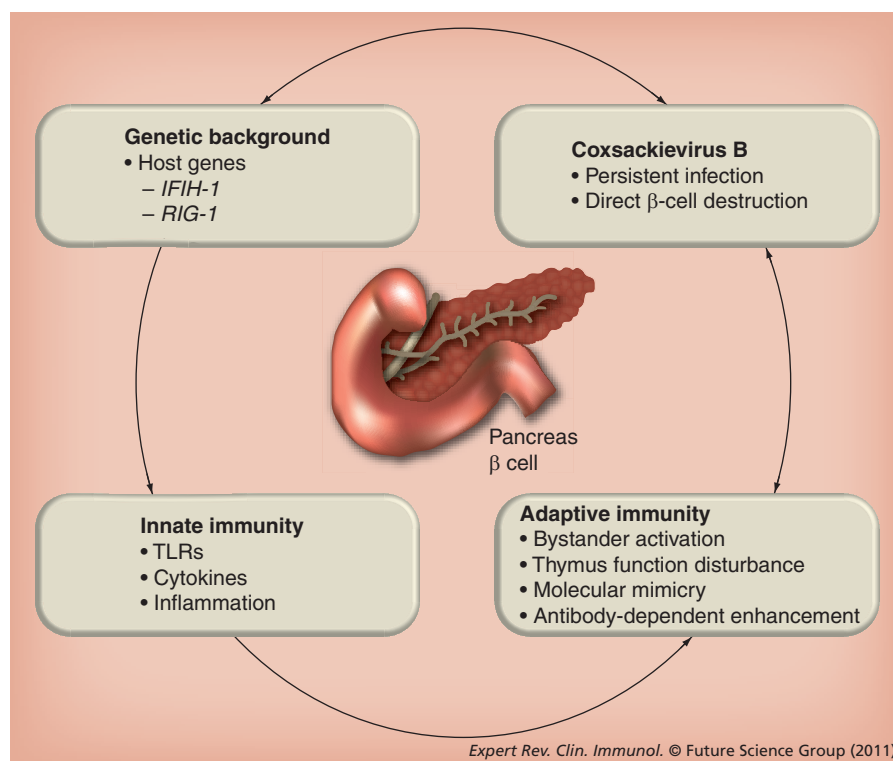


Figure 1. Through the interplay between the genetic background and the innate and adaptive immune systems, Group B coxsackieviruses can be involved in the development of an autoimmune attack against β cells that characterizes Type 1 diabetes.

genome and/or infectious virus detection could also be argued owing to the high sensitivity of the 5D8/1 antibody used in these studies, which may cause problems due to cross-reactions with cellular proteins [57,58].

With regard to the link between CVB or enteroviruses and T1D, it should be highlighted that some prospective studies have established a chronological relationship between enterovirus infections and the induction of β -cell autoimmunity or the development of T1D in children whose mothers had high levels of enterovirus antibodies during pregnancy compared with controls [59–61]. In a recent Finnish prospective birth cohort study, enterovirus RNA was found more frequently in case blood samples than in control samples and the risk for developing T1D was strongly linked with enteroviral RNA positivity during the 6-month period preceding autoantibody positivity [62].

The results of experimental studies support the hypothesis of a relationship between CVB and T1D. Studies in NOD mice that develop spontaneous autoimmune diabetes similar to human T1D have led to important observations concerning the susceptibility, diabetogenesis, tropism and mechanisms of pancreatic β -cell destruction in the context of CVB4 infection [63]. Our group has demonstrated that an infection of human islet cells can be obtained with CVB4 and CVB3 *in vitro*. In these experiments, CVB replicated and persisted in β and α cells, whereas expression of IFN- α was only observed in β cells [10]. These data support the hypothesis of a role of CVB in the high levels of type I interferons that have been detected in the pancreas or islet of patients with

Table 1. Association between group B coxsackieviruses and Type 1 diabetes.

Study (year)	Biological samples	Country	Cases/controls (n)	Positive cases/controls (n)	Method(s) of detection	Viruses	Ref.
Chehadeh <i>et al.</i> (2000)	Whole blood	France	56/37	21/0	RT-PCR	CVB2, CVB3, CVB4	[13]
Andréoletti <i>et al.</i> (1997)	Whole blood	France	24/27	6/0	RT-PCR	CVB3, CVB4	[46]
Schulte <i>et al.</i> (2010)	PBMCs	The Netherlands	10/20	4/0	RT-PCR	CVB	[47]
Toniolo <i>et al.</i> (2010)	PBMCs	Italy	112/58	92/2	RT-PCR	CVB ⁺	[48]
Yin <i>et al.</i> (2002)	PBMCs	Sweden	24/24	18/7	RT-PCR	CVB ⁺	[49]
Moya-Suri <i>et al.</i> (2005)	Serum	Germany	47/50	17/2	RT-PCR	CVB2, CVB4, CVB6	[50]
Kawashima <i>et al.</i> (2004)	Serum	Japan	61/58	23/2	RT-PCR	CVB2, CVB4, CVB5	[51]
Salminen <i>et al.</i> (2004)	Serum	Finland	12/53	10/22	RT-PCR	CVB ⁺	[52]
Dotta <i>et al.</i> (2007)	Autopsic pancreas	Italy	6/26	3/0	Immunostaining, cell culture, PCR, electronic microscopy	CVB4	[53]
Yoon <i>et al.</i> (1979)	Autopsic pancreas	USA	1/0	1/0	Cell culture, serology	CVB4	[45]

CVB and/or their components (e.g., RNA, protein) have been detected in biological samples from patients with Type 1 diabetes.

⁺CVB and other enteroviruses.

CVB: Group B coxsackievirus; PBMC: Peripheral blood mononuclear cell; RT-PCR: Retrotranscription PCR.

T1D [64,65]. Furthermore, the persistence of CVB4 RNA up to 6 months postinfection in mice islets was associated with β -cell destruction and diabetes onset [66].

Pathogenesis of CVB-induced autoimmunity:

T1D model

Viral infections are regularly associated with autoimmunity, but the mechanisms underlying such associations remain to be explained. Various non-mutually exclusive mechanisms have been proposed to explain virus-induced autoimmunity. In the case of T1D, multiple mechanisms derived from experimental investigation, generally based on *in vitro* studies and on the use of animal models, have been proposed to explain the complex pathological processes leading to the onset of CVB-induced T1D [63]. Among these mechanisms, direct β -cell lysis, molecular mimicry, viral persistence, bystander activation, thymus function disturbance and antibody-dependent enhancement have all been suggested to play a crucial role in CVB-induced T1D (FIGURE 2) [35].

Persistent infections & direct β -cell destruction

Group B coxsackievirus can directly infect and replicate in human and mouse β cells, with subsequent IFN- α synthesis and islet cell destruction. Islet cell infection can activate the overexpression of various chemokines, which stimulates the expression of class 1 MHC molecules, and could initiate the autoimmune process against β cells [16]. Consequently, a persistent β -cell infection could induce an autoimmune response with insulinitis and anti-islet

autoantibodies [66]. These persistently infected β cells may be the target of anti-enterovirus cytotoxic T lymphocytes (CTLs), which can result in β -cell impairment [67]. Although persistent infection of islet cells *in vitro* by some CVB strains has been reported [13,68], most islet CVB virus or enterovirus infections are cytolytic and the characteristics of that lysis depend on the type and dose of the virus [69].

Previous findings from our laboratory have shown that an *in vitro* persistent infection of human islet cells by CVB3 and CVB4 is possible, with chronic IFN- α expression by β cells. Regardless of its antiviral role, IFN- α can be harmful by inducing overexpression of class 1 MHC antigens and by initiating autoimmunity towards β cells [10]. Indeed, it has been demonstrated that the expression of IFN- α by β cells in transgenic mice resulted in insulinitis and islet cell destruction through activation of T cells [70].

The dsRNA-activated protein kinase R and the VP1 double-staining immune-positivity in diabetic patients' islet cells could be considered as evidence of persistent infection [10].

Double-stranded RNA-activated protein kinase R is upregulated in response to enteroviral infection and it has been demonstrated in chronic inflammatory myopathy that CVB RNA persistency resulted in formation of dsRNA complexes [71].

Furthermore, a persistent CVB4 infection in human islets *in vitro* has been associated with a change of receptor usage, due to some amino acid substitutions occurring in the capsid protein VP1 [68]. Similar observations have been made in nervous system cell lines persistently infected with polioviruses [72].

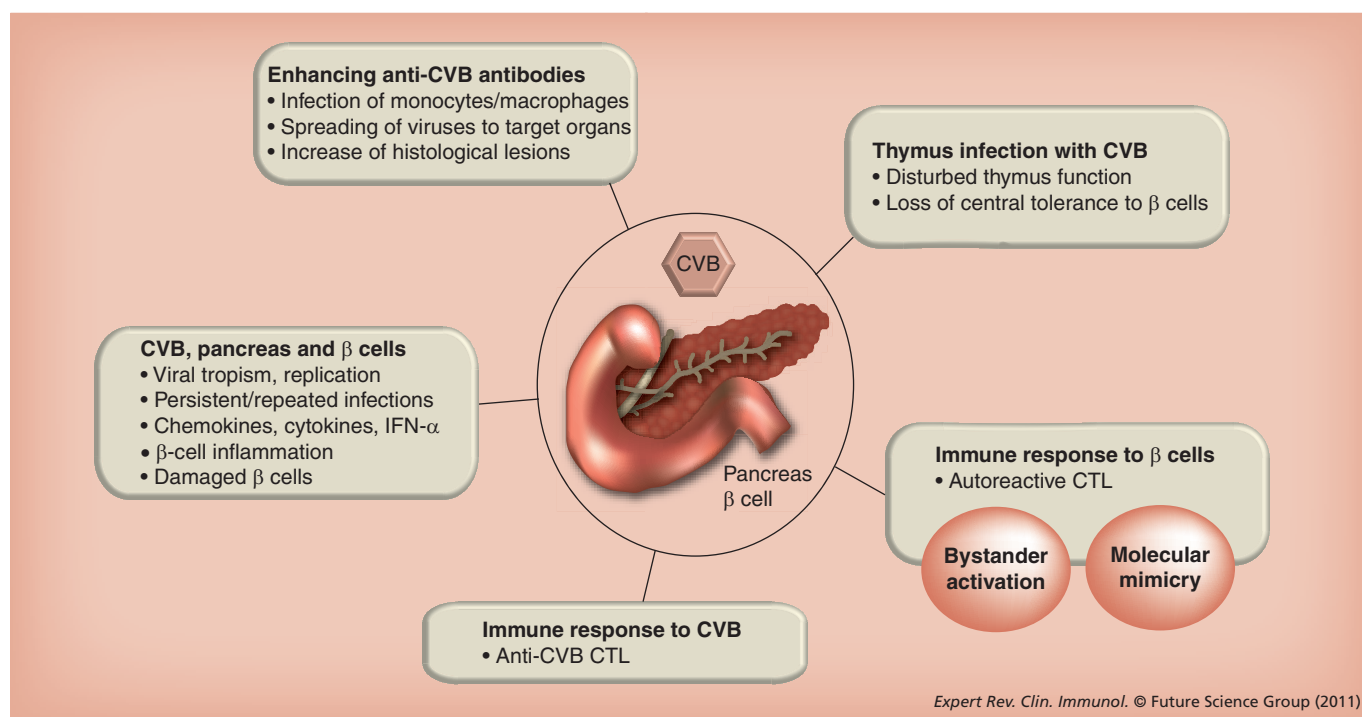


Figure 2. Group B coxsackieviruses can induce or aggravate the development of Type 1 diabetes through various mechanisms. Enhancing antibodies can increase the extent of CVB infection. The tropism of CVB for β cells results in inflammation and cell damage. The innate and adaptive immune systems are activated to fight the virus. The subsequent release of β -cell antigens can activate an autoimmune response through bystander activation and molecular mimicry. Infection of thymus with CVB can result in an abnormal function of that organ involved in the central tolerance of β cells. CTL: Cytotoxic T lymphocytes; CVB: Group B coxsackievirus.

Persistent infection and/or consecutive CVB infections may be relevant in the process leading to the disease, as it can result in a prolonged inflammatory state and activation of the immune system, which may be involved in recruitment of autoreactive CTLs. The ultimate consequence of this process could be the destruction of insulin-producing β cells.

Bystander activation & molecular mimicry

A persistent infection may induce an inflammatory reaction resulting in the recruitment of autoreactive CTLs to the islets. In a mouse model, it has been demonstrated that CVB4 provokes diabetes owing to the inflammation of the pancreas (with release of toxic mediators able to destroy β cells) that induces a bystander activation of pre-existing autoreactive CTLs [73]. CVB4 inoculation accelerates the development of diabetes in a NOD mouse model via a bystander effect when there is a sufficient number of pre-existing autoreactive T cells [74].

The infection of β cells causes inflammation, which leads to cell damage and release of sequestered antigens. Local inflammation results in recruitment of cells, encompassing autoreactive T lymphocytes directed against these self-antigens, which will be activated [75,76]. The autoimmune process particularly involves islet cell antigens such as glutamic acid decarboxylase (GAD65), resulting in insulin-producing β -cell destruction [77]. It has been demonstrated that the T-cell response to GAD65 is detectable in the spleen of prediabetic NOD mice and in the peripheral blood of humans

before the onset of the disease [78,79]. Thus, through bystander activation, T cells that recognize β -cell self-antigens and are reactive towards these antigens are present in the pancreas tissue, which can participate in the pathogenesis of the disease [80]. Initiating and sustaining autoimmunity through bystander processes might be closely linked with antigen-presenting cell activation. Indeed, the inflammatory response resulting from β -cell infection induces their phagocytosis by antigen-presenting cell and results in β -cell destruction and increased self-antigen presentation [81].

Another proposed process that could explain CVB-induced autoimmunity in the T1D model is the cross-reactivity between CVB antigens and β -cell endogenous proteins; a mechanism known as molecular mimicry. It has been demonstrated that some β -cell self-antigens have a partial sequence homology with CVB peptides. Some T1D-associated autoantigens have been identified, such as tyrosine phosphatases (IA-2 and IAR), GAD65 and heat shock protein 60/65 (HSP60/65) [82,83]. The IA-2/IAR diabetogenic peptide, a major target self-antigen in T1D, can be recognized by CVB4-induced antisera. Thus, cross-reactivity of antibodies and T cells raised against CVB peptides with islet self-antigens was observed [84]. Interestingly, these findings highlighted the role that molecular mimicry might play in the process leading to autoimmune disease. It can lead to acceleration of autoimmune diabetes in prediabetic hosts but not to *de novo* induction of disease in naive mice. Thus, molecular mimicry itself would be unable to trigger autoimmune processes, but rather would accelerate or aggravate an ongoing disease state [85].

Thymus function disturbance

Type 1 diabetes is an autoimmune disease which results, among other factors, from the disturbance in immune tolerance. The central tolerance, the ability to discriminate 'self' from 'non-self', is acquired by T cells in the thymus where peripheral tissue antigens are expressed. Several studies have demonstrated that medullary thymic cells contain promiscuous genes that express various tissue-specific antigens, such as insulin-related genes *Ins*, *Igf1* and *Igf2* [86]. A defect in *Igf2* transcription in a diabetes-prone biobreeding rat (BBDP) thymus could strongly contribute to the loss of central self-tolerance to insulin-secreting pancreatic β cells [87]. Anomalies of Tregs are one of the main consequences of the loss of thymus tolerance [88]. Central tolerance is regulated by the thymus and the infection of this organ by CVB may lead to disturbance of thymus function which can initiate an autoimmune process. In CVB4-infected mice, it has been observed that T1D onset was preceded by anomalies in thymic, peripheral and splenic T lymphocytes as a consequence of impaired thymus T-cell ontogeny [89]. Studies have shown that CVB4 can infect, replicate and persist in human thymic epithelial cells in culture and an *in vivo* infection of thymus was also obtained in mice [89,90]. CVB4 RNA was detected in the thymus up to 70 days postinfection in mice infected via the oral route [91]. Human CD4⁺CD8⁺ thymocytes can be infected by CVB4, which is accompanied by an overexpression of MHC class I molecules and severe thymocyte depletion [92]. Furthermore, abnormal T-cell maturation and/or differentiation were obtained in thymus fragments of fetal mice infected with CVB4 *in vitro* [93]. Together, these data suggest that CVB can induce an abnormal thymocyte function, which may reduce the capacity to maintain tolerance and may participate in the autoimmune process of T1D.

CVB & antibody-dependent enhancement

Another mechanism that can intervene in the CVB-induced autoimmunity process is the antibody-dependent enhancement (ADE) of the infection with CVB. ADE of viral infections has been obtained *in vitro* with viruses belonging to various families [94] and may play a role in virus dissemination and in the pathogenic process of diseases induced by these viruses. The ADE of CVB infection concerns CVB3 and CVB4. It has been shown that CVB4 infection in the human system could be mediated by anti-CVB4 antibodies able to increase the replication of the virus in peripheral blood mononuclear cell-derived monocytes and to enhance CVB4-induced IFN- α synthesis by these cells [95–97]. The enhancing effect observed with CVB4 in the human system was higher with plasma from T1D patients compared with plasma from healthy subjects [98]. The target of enhancing antibodies is the VP4 protein and the region of the protein that binds the antibodies has been identified, which allows an assay to detect them in serum to be set up [99,100]. The prevalence and titers of anti-VP4 antibodies were higher in patients with T1D than in controls [100]. In the case of CVB3, where experiments were conducted in mice, it was observed that enhancing antibodies increased the viremia and the infection of secondary organs such as the heart, which resulted in an aggravation of myocarditis lesions compared with mice infected

with the virus in the absence of enhancing antibodies [101,102]. This mechanism, which increases the extent of infection, may participate in several models *in vivo* in which an increase of histological lesions and disease was observed. Thus the interplay between enhancing antibodies and recurrent and/or persistent CVB infections resulting in iterative production of IFN- α and worsening target organ lesions may be involved in CVB-induced autoimmunity [103].

Beneficial effects of CVB in autoimmunity

In NOD mice, the best experimental model of spontaneous T1D, it has been reported that CVB infections can provide significant protection against the development of the disease [104]. The mechanisms by which CVB infections could suppress or slow T1D pathogenesis have been investigated. It has been demonstrated that when no direct β -cell destruction is caused by infection, CVB can have a protective effect rather than inducing an autoimmune disease. The mechanism relies on the triggering of immunoregulatory processes that result in an increase in protective Tregs with TGF- β production, which are involved in long-term tolerance [105].

It has been observed that CVB infections are beneficial in young mice and deleterious in older mice, suggesting that CVB strain virulence and replication rate, and the stage of insulinitis of pancreas islet cells play an important role in the pathogenic process [106,107]. These observations are classified in the same category as the hypothesis that viral infections occurring early in childhood have a protective effect against the risk of developing T1D [108]. The protective effect of viral infections is in agreement with the hygiene hypothesis, which establishes a parallelism between increased hygiene levels and decreased enterovirus exposure rates, resulting in a higher risk of developing T1D [109].

The relationship between environmental factors, especially viruses, and autoimmune diseases is under investigation. The Environmental Determinants of Diabetes in the Young (TEDDY) study is an observational cohort study in which six centers of research across the world are following children with higher risk genes until they are 15 years of age [110]. The purpose of this current study is to identify the infectious agents, dietary factors and all other environmental triggers associated with an increased risk of autoimmunity and which cause children to develop diabetes or that protect against the disease.

Expert commentary

Retrospective and prospective epidemiological studies strongly suggest that there is an association between CVB infections and T1D. Previous studies were based on the detection of anti-CVB antibodies in serum of patients and more recently several groups discovered the presence of CVB RNA in the blood and pancreas of patients with T1D. The hypothesis of the role of CVB in autoimmune diseases, such as T1D, is strengthened by the results of experimental investigations both *in vitro* and in animal models. Several non-mutually exclusive mechanisms are supposed to be involved in the processes by which CVB may induce or aggravate the development of T1D: repeated and/or persistent infection of β cells resulting in inflammation and production of IFN- α , bystander activation of T cells directed towards β -cell antigens, autoreactive response to islet cell

self-antigens through molecular mimicry, antibodies enhancing the infection with CVB, and infection of the thymus.

The role of CVB in autoimmune disease is probably complex since it has been demonstrated that these viruses can protect against the development of T1D. Therefore, in order to develop new strategies for the treatment and prevention of disease, further studies are needed to understand the mechanisms of the protective effect of CVB and those behind the CVB-induced pathogenesis of autoimmune diseases such as T1D.

Five-year view

In the near future, studies regarding coxsackieviruses and autoimmunity focused on T1D are likely to be devoted to deepening the knowledge of the mechanisms and consequences of persistent infections with these viruses. The patterns of innate and adaptive immune responses to these infectious agents and their interactions with the host genetic background will be investigated. Furthermore, the development of sensitive and specific tests for detecting coxsackievirus infections is required to conduct a thorough reflection on potential preventive options such as vaccination against target viruses. Further studies are needed to understand the mechanisms of the protective effect of CVB.

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Key issues

- Type 1 diabetes (T1D) is an autoimmune disease characterized by hyperglycemia that results from an impairment or a destruction of the insulin-producing β cells.
- T1D results from complex interactions between immunological, genetic and environmental factors, especially viruses.
- Group B coxsackieviruses (CVB) and/or their components have been found in the blood and pancreas of patients with T1D.
- The *IFIH-1* gene could be the link between CVB and a patient's genetic background and innate immune system in the development of such diseases.
- Direct β -cell lysis, molecular mimicry, viral persistence, bystander activation, thymus function disturbance and antibody-dependent enhancement have been suggested to be among the mechanisms that play a crucial role in CVB-induced autoimmunity.
- Persistent infection and/or consecutive CVB infections may be relevant in the process leading to disease by inducing a prolonged inflammatory state resulting in the recruitment of autoreactive cytotoxic T lymphocytes and the destruction of insulin-producing β cells.
- Under certain conditions, CVB infections can have a protective effect rather than inducing an autoimmune disease.

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