Abstract: Beyond acute clinical conditions, the role of enteroviruses (EVs) in chronic human diseases has been described. Although they are considered as highly cytolytic viruses, EVs can persist in various tissues. The persistence is believed to play a major role in the pathogenesis of EV related chronic diseases such as type 1 diabetes (T1D). T1D is characterized by an autoimmune destruction of pancreatic beta cells, and results from interplay between a genetic predisposition, the immune system, and environmental factors. EVs and especially group B coxsackieviruses (CVB) have been the most incriminated as exogenous agents involved in the development of T1D. Enteroviral persistence is the result of a virus-host coevolution combining a cell resistance to lysis through mutations or down-regulation of viral receptor, and a decrease of the viral replication by genomic modifications or the production of a stable double-stranded RNA form. CVB can persist in pancreatic cells and therefore could trigger, in genetically predisposed individuals, the autoimmune destruction of beta cells mainly through an activation of inflammation. The persistence of the virus in other tissues such as intestine, blood cells, and thymus has been described, and could also contribute to some extent to the enteroviral pathogenesis of T1D. The molecular and cellular mechanisms of CVB persistence and the link with the development of T1D should be investigated further. [Discovery Medicine 18(100):n-n, November 2014]

Introduction

Human enteroviruses (HEVs) include many major human pathogens such as poliovirus, rhinovirus, enterovirus 71, coxsackievirus, and echovirus. These small non-enveloped RNA viruses belong to the Picornaviridae family, and the genus Enterovirus currently encompasses 7 species involved in human diseases (Human enterovirus A-D and Human rhinovirus A-C) (Knowles et al., 2012; Tapparel et al., 2013). Non-polio enteroviruses are ubiquitous pathogens and can infect a wide range of tissues (Harvala et al., 2002). They can be involved in many severe acute clinical features such as meningitis, encephalitis, myocarditis, pancreatitis, hepatitis, or fulminant sepsis in newborns (Romero, 2008; Tapparel et al., 2013).

Enteroviruses (EVs), especially the group B coxackieviruses (CVB1-6), have also been associated with the development of chronic diseases like type 1 diabetes (T1D). T1D is characterized by a defect of insulin production as a result of an autoimmune destruction/dysfunction of pancreatic β cells in genetically predisposed individuals with an impaired immune regulation (Roep and Tree, 2014); but the role of exogenous factors in the initiation and progression of this disorder seems obvious, since only a small proportion of genetically susceptible individuals progress to clinical disease (Knip and Simell, 2012). Enteroviruses and especially CVB have been the most incriminated as environmental factors, and a relationship between these viruses and the development of T1D has been reported (Hober and Alidjinou, 2013; Hober and Sauter, 2010; Morgan and Richardson, 2014).

Although EVs are cytolytic viruses, they can establish persistent infections in vitro as well as in vivo (Pinkert et al., 2011), and viral persistence has been suggested as a major mechanism in the enteroviral pathogenesis of T1D (Jaidane and Hober, 2008; Jaidane et al., 2010).
Epidemiological studies have found, in T1D patients, a more frequent detection of enteroviral (EV) components in blood, in the intestine, and in pancreas (Yeung et al., 2011), most often beyond the stage of acute infection.

The persistence of EVs has already been associated in humans to other syndromes, including post-polio syndrome (Julien et al., 1999; Leparc-Goffart et al., 1996) and chronic fatigue syndrome (Chia et al., 2010). Furthermore, CVB persistence was shown to contribute significantly to the occurrence of chronic myocarditis and dilated cardiomyopathy through direct effects of viral replication as well as induction of inflammation in the heart (Chapman and Kim, 2008).

EVs are transmitted mainly by fecal-oral route and their primary replication occurs in the intestine mucosa. From the gut, a systemic infection can lead to dissemination of the virus to other target organs such as pancreas. Although the presence or the persistence of the virus in the pancreas is believed to be a major component of the enteroviral pathogenesis of T1D, the virus can also persist in other sites such as intestine or blood cells that could act as reservoir and contribute to the circulation of the virus and the maintenance of pancreatic cells infection.

In addition, CVB can infect the thymus, whose most important role is the induction of central tolerance, i.e., the ability of T cells to discriminate ‘self’ from ‘non-self.’ A persistent CVB infection of thymic cells could lead to the disturbance of immune tolerance and contribute to the autoimmune process in T1D, by loss of central self-tolerance to insulin-secreting pancreatic β cells (Jaïdane et al., 2012a).

After a brief presentation of the currently known molecular mechanisms of EV persistence, the cumulative evidence in vitro and in vivo regarding EV persistence (with a focus on CVB) will be described in pancreatic cells and also in the other potential sites, and the link between the persistence and the pathological process leading to the development of T1D will be analyzed.

Factors Involved in the Persistence of EVs in Tissues

EVs are considered as cytolytic viruses; however, they can establish persistent infections in vitro as well as in vivo (Frisk, 2001; Pinkert et al., 2011). This suggests the role of a regulatory mechanism of viral replication under certain circumstances. Two major groups of persistent viral infections have been described: steady-state infections and carrier-state infections. The first group is characterized by infection of all cells (without lytic replication cycle), whereas in carrier-state culture systems, only a small proportion of cells are involved (with productive virus replication) (Frisk, 2001; Pinkert et al., 2011). EVs and especially CVB were shown to establish carrier-state persistent infections in vitro (Heim et al., 1992; 1995; Pinkert et al., 2011).

Most of the knowledge on viral persistence comes from in vitro systems, with some from in vivo models. Actually persistent infection by cytolytic viruses such as EVs is thought to result from a virus-host coevolution which combines a resistance developed by the cell, and an adaptation of the virulence of the viral strain (Pinkert et al., 2011). In this section, viral and cellular factors involved in the persistence of EVs are reviewed.

Viral factors are undoubtedly the most studied parameters during EV persistence. Since RNA-dependent RNA polymerases lack proofreading, the main mechanism reported is the selection of virus mutants that are less cytopathic for cells or that result in low-level viral replication. Some mutations were reported to affect the binding properties of the virus. A combination of mutations in the VP1 and VP2 capsid genes of poliovirus (PV) was shown to affect the cell binding and the receptor-mediated conformational changes necessary for viral penetration and uncoating. This modification has been suggested as the mechanism by which PV is able to establish persistent infections in HEp-2 cell cultures (Duncan and Colbère-Garapin, 1999; Duncan et al., 1998; Pelletier et al., 1998). Some amino-acid substitutions described in CVB3 strain emerging during viral persistence were associated with a weak interaction with the coxsackie and adenovirus receptor (CAR) but strong binding to the decay accelerating factor (DAF), as compared to the parental virus (Schmidtke et al., 2000).

Other genomic alterations have been reported in the EV highly conserved 5′NTR region. This region was shown to harbor the genomic determinants of EV replication (Bedard and Semler, 2004). Chapman and colleagues have demonstrated that in vivo CVB3 persistent infection of mouse or human heart, as well as in vitro infection of cardiomyocytes, was associated with a deletion in the 5′ end of the RNA. These ‘terminally deleted’ viruses have a lower replication rate and can persist in host cells over a prolonged period (Chapman et al., 2008; Kim et al., 2005; 2008). Recently, this deletion was also reported in a murine model during CVB persistence in the pancreas (Tracy et al., 2014).

The genomic modifications during EV persistence could explain at least partially the low detection rate of EV RNA by RT-PCR in samples from patients with EV
associated chronic diseases. However, an alternative viral persistence mechanism is possible especially in vivo. Indeed, it has been described that EV persistence in muscle and probably in other nondividing cells was not associated with the selection of mutant virus, but with the presence of a stable and atypical double-stranded RNA genomic form. Myofibers can harbor this RNA form for extended times without a production of detectable levels of infectious virus (Cunningham et al., 1990; Klingel et al., 1992; Tam and Messner, 1999).

Few authors have focused on the cellular factors involved in EV persistence. Feuer et al. (2002; 2004) reported that the cell cycle status affects CVB3 replication and suggested that the persistence of CVB3 in vivo may rely on infection of quiescent cells in which viral replication is lowered or suppressed. Cellular activation may also play a role in the outcome of CVB infection (Feuer and Whitton, 2008). The role of receptor mutations or reduction of receptor expression has been reported for EV persistence. Specific mutations in the

**Figure 1.** Possible mechanisms involved in the persistence of coxsackievirus B. A cytolytic virus such as coxackievirus B (CVB) can establish under certain circumstances a persistent infection in susceptible cells. Changes in cell and virus characteristics leading to a decreased or suppressed viral replication can be observed when the infection is persistent.
domain 1 of poliovirus receptor (PVR) were associated with an increase of cell resistance to lysis (Pavio et al., 2000), and a decrease of PV-induced apoptosis (Gosselin et al., 2003). A down-regulation of CAR has been reported during CVB3 persistence (Pinkert et al., 2011), and a decrease of CAR expression was known to be associated with a decrease of CVB infection and cell lysis (Fechner et al., 2007; Werk et al., 2005). A heart-specific deletion of CAR in mice resulted in a resistance to CVB infection (Shi et al., 2009).

In summary, EV persistence depends strictly on the interactions within the virus-cell system. It probably combines many of the mechanisms described above, and others unknown. A better understanding of this phenomenon will provide a molecular basis to the pathogenesis of enterovirus-related chronic diseases like T1D.

**EV Persistence in Pancreatic Cells and Relationship with T1D**

The understanding of the pathogenesis of T1D requires undoubtedly focusing on pancreas. The pancreatic tropism of EVs both in animals and humans is well known. In humans, the evidence of enteroviral infection within pancreatic cells at the onset or during the progression of the disease has been difficult to obtain since this requires a biopsy that is invasive and often risky. Therefore, most of data available come from necropsies (Dotta et al., 2007; Richardson et al., 2009; Willcox et al., 2011; Ylipaasto et al., 2004). Pancreatic islets and especially β-cells, but not exocrine cells,

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**Figure 2.** Persistence of coxsackievirus B and relationship with type 1 diabetes. Coxsackievirus B (CVB) can persist in pancreas and trigger autoimmunity in predisposed individuals through the activation of inflammation, resulting in the destruction of β-cells by preexisting autoreactive T cells. The inhibition of trans-differentiation through persistent infection of ductal cells can contribute to the β-cell deficiency. Other tissues, such as intestine and blood, in which CVB can persist, may act as virus reservoir for pancreas infection or reinfection. The persistence of CVB in thymus can contribute to the onset of autoimmunity through the disturbance of self tolerance.
were found to be susceptible to enteroviral infection (Dotta et al., 2007; Richardson et al., 2009; 2013). Interestingly, the specific receptor of cosackieviruses, the CAR molecule, is expressed in the pancreas mainly by these β-cells (Oikarinen et al., 2008a; Spagnuolo et al., 2013).

CVB can effectively replicate in pancreatic cells and cause massive cell lysis (Anagandula et al., 2014; Elshebani et al., 2007; Hodik et al., 2013). In vivo, this extensive cell destruction upon CVB infection could lead to what is known as “fulminant diabetes” (Kobayashi et al., 2011; Tanaka et al., 2013), a particular and rare clinical feature especially described in Japanese patients (out of the scope of this review).

Things are different in CVB associated autoimmune T1D since a clinical disease occurs often many years after the appearance of islet specific autoantibodies which have been reported to be a result of enteroviral infection (Laitinen et al., 2014; Oikarinen et al., 2011). Such important damage is not observed in pancreatic cells of patients in which the virus components have been detected. The most likely scenario would be a persistent infection with probably a low grade viral replication.

In fact the outcome of CVB infection within pancreatic cells seems to depend on the serotype and even the strain of the virus (Elshebani et al., 2007; Frisk and Diderholm, 2000; Frisk et al., 2001; Hindersson et al., 2004; Roivainen et al., 2002; Tracy et al., 2000). In addition, the route of transmission was reported to impact the effect of CVB on pancreatic cells. Indeed, a study has compared intraperitoneal injection and oral administration in mice, and concluded that though both routes lead to systemic and pancreas infection, the oral administration that is the natural transmission route in humans, protects pancreas from damage (Bopegamage et al., 2005). This finding suggests that the viral titer reaching the pancreas after oral administration is lower, resulting in a non-highly cytopathic phenomenon.

It is well accepted that the selective destruction of beta cells in T1D patients is an autoimmune process (Roep and Tree, 2014). The main hypothesis addressing the relationship between CVB persistence and T1D is that non-cytopathic CVB infection triggers autoimmunity against beta cells through activation of inflammation.

Actually, pathological studies on pancreases from died T1D patients (Richardson et al., 2014) show a quasi-absence of beta cells and the presence of an inflammatory cell infiltrate (insulitis) composed mainly of CD8 cytotoxic T cells and at lesser extent CD4 T cells and macrophages, and sometimes NK cells were reported (Dotta et al., 2007; Willcox et al., 2009).

Persistent CVB infection is thought to induce an inflammatory response (and especially IFNα production) in pancreatic endocrine cells. Yet, this response might depend on a genetic background since some polymorphisms of IFIH1 gene have been epidemiologically associated with an increased risk of T1D (Nejentsev et al., 2009; Smyth et al., 2006). This gene encodes for MDA5 protein which is a cytoplasmic innate immune sensor for CVB (Kato et al., 2006). The local inflammation could lead to a beta cell antigen presentation that is enhanced by the hyperexpression of class I major histocompatibility complex (MHC) by endocrine cells (Richardson et al., 2014). The result of this antigen presentation is a destruction of beta cells by CD8 cytotoxic T cells that interestingly were found to be antigen-specific (Coppieris and von Herrath, 2009). These T cells probably preexist in predisposed individuals and are recruited to islets, guided by antigen presentation and driven by chemokines (Roep et al., 2010; Sarkar et al., 2012).

In contrast to the non-obese diabetic (NOD) model, the insulitis seems to be moderate in humans, and only a limited number of infiltrating cells are observed (Carrero et al., 2013; Willcox et al., 2009). In vitro studies confirmed that pancreatic islets can support persistent CVB infection which results in a production of IFNα (Chehadeh et al., 2000a), and a disturbance in the function of beta cells (Yin et al., 2002a).

Other mechanisms involving persistent CVB infection in T1D could include molecular mimicry and an inhibition of the trans-differentiation of pancreatic ductal cells. The hypothesis of molecular mimicry is supported by the homology between a conserved sequence of the enteroviral 2C protein and glutamate decarboxylase (GAD), an autoantigen frequently detected in T1D patients (Hou et al., 1994; Kaufman et al., 1992). This possibility has not been investigated further, since CVB infections have been associated with T1D only in some patients, and this autoantigen was also reported to share some homologies with other viral peptides (Hiemstra et al., 2001; Honeyman et al., 2010).

The trans-differentiation of pancreatic ductal cells is thought to be a renewal process of beta cells following a loss of these cells in a context of T1D, for example. An inhibition of this phenomenon could contribute to a rapid development of T1D (Lysy et al., 2013; Sane et al., 2013). Interestingly, our team has established a persistent CVB infection in a pancreatic ductal cell line (Panc-1 cells), and found that the persistent infection
reduced the expression of Pdx-1, a transcriptional factor required for the differentiation of ductal cells (Sane et al., 2013).

**EV Persistence in Other Tissues and Relationship with T1D**

**EV persistence in the intestine**

After transmission most of time by oral route, CVB can replicate effectively in the gastrointestinal tract and especially in the intestine, and thereafter can spread from this site to the pancreas or other target organs. However, intestine is not just a crossing for the virus and there is some evidence that EV can establish a persistent infection in the intestine. Oikarinen et al. (2008b; 2012) have detected the presence of EV in the mucosa of small intestine of T1D patients but not in controls. Interestingly, patients remained EV positive 12 months after, and evidence of intense viral replication was not observed, suggesting a persistence of the virus in the gut of these patients (Oikarinen et al., 2012). In addition, enteroviral infection was associated to a chronic inflammation in the intestine (Oikarinen et al., 2012). This finding is compatible with previous reports which found an enhanced immune activation in the small intestine of T1D patients (Westerholm-Ormio et al., 2003). This environment could constitute a reservoir from which the virus spreads to the pancreas and triggers autoimmunity, since intestine is highly vascularized. Nevertheless, the hypothesis of the role of gut in the persistence of enteroviruses in patients with T1D should be investigated further, since data reported by Oikarinen et al. were not confirmed by those of another team (Mercalli et al., 2012). *In vitro*, the persistence of CVB in human intestinal cell line (Caco-2 cells) has been demonstrated (Harrath et al., 2004; Riabi et al., 2012). However, cells involved in the replication and the persistence of CVB *in vivo* in the intestine have not been precisely identified. In infected mice, the virus was reported to predominate in the lymphoid cells of the gut mucosa (Harrath et al., 2004).

**EV persistence in blood cells**

The blood is the main vehicle that spreads the virus in the whole body. The majority of epidemiological studies that investigated the relationship between EVs and T1D have focused on blood because it can be easily sampled by venipuncture. Thus, a large number of reports have found a more frequent detection of enteroviral RNA in the blood of T1D patients as compared to healthy individuals. EV RNA has been detected in the blood long before onset of clinical T1D and up to 6 months before the appearance of diabetes-associated autoantibodies (Oikarinen et al., 2011), and moreover the virus has been found both in recent and long-term diabetic patients (Yeung et al., 2011).

Most of these investigations were performed using whole blood or serum, and few authors focused on the blood cells that could harbor the virus (Chehadeh et al., 2000b; Salvatoni et al., 2013; Schulte et al., 2010; Toniolo et al., 2010; Yin et al., 2002b). EV RNA has been detected in peripheral blood mononuclear cells (PBMCs) of T1D at a relatively higher rate than in plasma or serum (Schulte et al., 2010; Yin et al., 2002b). In addition, EV RNA was still detected in blood beyond the stage of acute infection, after the detection of EV RNA in throat and stool samples was negative (Schulte et al., 2010). These data suggest that EVs can be detected in PBMCs during and after the “viremic” stage.

Experiments performed in our laboratory have shown that among PBMCs of T1D patients, EV RNA was harbored mainly by monocytes, which also displayed an increase in susceptibility to enteroviral infection *in vitro* (Alidjinou et al., submitted). Although monocytes are poorly permissive to enteroviral infection *in vitro*, they can be efficiently infected under some circumstances, especially the presence of enhancing antibodies (Chehadeh et al., 2005; Hober et al., 2001). Moreover, our team has shown that CVB mixed with enhancing IgG can establish a persistent infection in a monocytic cell line (Goffard et al., 2013).

It can therefore be hypothesized that PBMCs and especially monocytes could constitute a reservoir contributing to the enteroviral infection or reinfection of target organs such as pancreas. Whether macrophages can play a role in the persistence of CVB deserves further investigations.

**EV persistence in the thymus**

The thymus, a primary lymphoid organ, is a major component of immune system and the site of initiation of self-tolerance. The self-antigens are expressed within the thymus, and self-tolerance is established during T-cell ontogeny by elimination of autoreactive T lymphocytes (negative selection). In addition, self-antigen-specific natural regulatory T cells (nTregs) are generated to inactivate periphery self-reactive T cells that have escaped negative selection (Klein et al., 2009). A disturbance of thymus function can initiate an autoimmune process, and since T1D is an autoimmune disease, it makes sense to explore the involvement of the thymus in its pathogenesis.

The thymus is a target for EV infection as supported by
reports in humans (Cavalcante et al., 2010) and in animal models (Jaidane et al., 2006). After inoculation by oral route in mice, CVB can infect the thymus and viral RNA is still detected until 70 days post-inoculation (Jaidane et al., 2006).

In vitro, human epithelial thymic cells can be infected by various strains of CVB4. The virus can replicate and persist in these cells, and induces the production of interleukin (IL)-6, leucocyte migration inhibition factor (LIF), and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Brilot et al., 2002). CVB4 can infect immature thymocytes in human fetal thymus, which results in an increased expression of class I MHC molecules and a severe depletion of thymocytes (Brilot et al., 2004).

The CVB infection of murine thymic cells in vitro was reported to disturb the T-cell maturation and differentiation processes (Brilot et al., 2008; Jaidane et al., 2012a).

Recently our team established a persistent CVB4 infection in murine thymic epithelial cell line. The infection led to a decrease in the production of type 2 insulin-like growth factor (Igf2), the dominant polypeptide of the insulin family, which has a tolerogenic effect towards insulin (Jaidane et al., 2012b). A defect of Igf2 expression in the thymus was suggested to play a role in the development of autoimmune diabetes in a BBDP rat model (Kecha-Kamoun et al., 2001).

These data suggest that it cannot be excluded that a persistent CVB infection of the thymus could disturb self-tolerance at the central level, and could then play a role in the pathogenesis of T1D.

Conclusion

Enteroviruses can be involved in acute and lytic infections, but they can also persist in tissues through an adaptation of characteristics of both virus and host cell. This persistence is thought to be the main mechanism in the pathogenesis of chronic enterovirus-related diseases. EVs and especially CVB can persist in pancreas, leading to, in predisposed individuals, a progressive and moderate inflammatory response that can activate the beta-cell autoimmune destruction process by preexisting cytotoxic T cells. In addition, a persistence of CVB can also occur in other sites such as intestine or blood cells that could serve as a reservoir for infection or reinfection of pancreas, and in thymus resulting in a defect of central self-tolerance that could lead to autoimmune diseases such as T1D.

Further in vivo and in vitro studies are still needed for a better understanding of the molecular mechanisms of enteroviral persistence in these tissues, and its contribution to the pathogenesis of T1D.

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References


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Hindersson M, Orn A, Harris RA, Frisk G. Strains of coxsackievirus B4 differed in their ability to induce acute pancreatitis and the responses were negatively correlated to glucose tolerance. *Arch Virol* 149:1985-2000, 2004.


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