Immunology in the clinic review series; focus on type 1 diabetes and viruses: how viral infections modulate beta cell function

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Summary

Type 1 diabetes mellitus (T1DM) 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 also documented the constant rise in the incidence of T1DM worldwide, with viral infections representing one of the candidate environmental risk factors identified by several independent studies. In fact, epidemiological data showed that T1DM incidence increases after epidemics due to enteroviruses and that enteroviral RNA can be detected in the blood of >50% of T1DM patients at the time of disease onset. 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.

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Introduction

Type 1 diabetes mellitus (T1DM) 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 also documented the constant rise in the incidence of T1DM worldwide, with viral infections representing one of the candidate environmental risk factors identified by several independent studies [1,2]. In fact, epidemiological data showed that T1DM incidence increases after epidemics due to enteroviruses and that enteroviral RNA can be detected in the blood of >50% of T1DM patients at the time of disease onset [3]. 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.

Several mechanisms have been proposed for viruses in triggering autoimmune beta cell destruction or functional beta cell impairment. In this study we review the scientific evidence on the effects of virus infections, with particular reference to enteroviruses, on beta cell-functional activities. The present review will analyse data obtained separately in three different settings: (a) cell lines; (b) experimental animal models; and (c) man.

In-vitro studies

Although the majority of experimental data about human or mouse pancreatic islet response upon viral infections derive from ex-vivo studies performed with different virus strains, many of the efforts to understand the mechanism of beta cell molecular changes secondary to virus entry have been performed on established beta cell lines or purified and cultured rodent beta cells, using double-stranded RNA (dsRNA).

dsRNA is generated during the life cycle of most viruses, and accumulates in and around infected cells during viral infection. These products can impair beta cell functions and lead to apoptosis.

One of the first attempts made to simulate the viral effects on beta cell functions is reported in the work by Rasschaert et al. [4,5], which described a global gene expression profiling of fluorescence activated cell sorter (FACS)-purified rat beta cells treated with both dsRNA, tested in the form of polynosinic–polycytidylic acid (PIC) and interferon (IFN)-γ, for an established culture period in order to simulate not only the viral infection of beta cells but also the cytokine milieu during insulinis. The authors observed that approximately 10% of genes analysed by microarray were modulated upon PIC and IFN-γ treatment (alone or in combination). The modulated genes encoded for proteins...
involved in a broad range of cell functions. Specifically, it was found that beta cells exposed to PIC and IFN-γ showed a functional inhibition that may precede cell death. Glut-2, insulin, PC-1 (pro-convertase-1) and genes belonging to glucose oxidation or to incretin (GIP and CCK) receptors were found to be decreased significantly upon treatment for 6 and/or 24 h.

The major proportion of the above-mentioned effects may indeed be addressed to a synergistic action of dsRNAs and IFN-γ at an early stage of beta cell infection, and appears as time-dependent. It has been observed that the percentage of genes related to beta cell metabolism, the expression of which has been found to be decreased after 6 h incubation with dsRNA alone, doubled in the case of a combined treatment with PIC + IFN-γ. Such a percentage of genes with decreased expression increased up to threefold after 24 h of incubation with PIC + IFN-γ. Of note, decreased insulin1 gene expression upon PIC + IFN-γ 24-h treatment did not correlate with a reduced expression or activity of Pdx-1, demonstrating that alternative pathways may be activated upon viral infection [5].

Moreover, PIC + IFN-γ treatment induced re-expression of genes of the Notch signalling pathway, contributing both to the loss of the differentiated beta cell phenotype and the growth/differentiation of newly generated beta cells [6].

The combination of PIC with IFN-γ seems to activate different pathways compared to the association with other cytokines. In the INS1 cell line, as well as in other rodent beta cell lines (e.g. MIN6, RINm5F), the combination of PIC and IFN-γ failed to activate inducible nitric oxide synthase (iNOS) expression and consequently NO production, while the combination of dsRNA with interleukin (IL)-1ß activated such expression strongly. Even if the authors observed a strong effect on iNOS expression with IL-1ß treatment alone, the combination with PIC potentiated this effect, demonstrating an important role for PIC in beta cells [7]. It has been reported that NO production has also important effects on beta cell function: it prevents electron transfer by altering adenosine triphosphate (ATP) synthesis and consequently the ATP/adenosine diphosphate (ADP) ratio, which partially controls insulin secretion. Moreover, it has been demonstrated that iNOS expression underlies the activation of nuclear factor kappa B, whose final effects have also been shown [13]. Interestingly, the expression of other beta cell proteins, such as glutamic acid decarboxylase (GAD) autoantigen, was increased by CV-B4, suggesting a potential role for CV-B4 in the induction and/or the potentiation of the autoimmune response candidate islet autoantigens [11].

Several studies reported functional beta cell alterations linked to Coxsackievirus B (CV-B) infections; in mice that developed hyperglycaemia 6–8 weeks after CV-B4 infection, a decrease in the rate of pre–proinsulin I and II transcripts associated with the viral genome persistence in beta cells was shown [13]. Interestingly, the expression of other beta cell proteins, such as glutamic acid decarboxylase (GAD) autoantigen, was increased by CV-B4, suggesting a potential role for CV-B4 in the induction and/or the potentiation of the autoimmune response candidate islet cell autoantigens [11].

In order to test the ability of Coxsackieviruses to replicate in murine pancreatic beta cells, Frisk et al. [14] used five different strains of CV-B4 and one strain of echovirus 11 (Echo-11). These strains interfered with the physiological changes in cytoplasmic Ca\(^{2+}\) concentrations \([(Ca^{2+})_{i}]\) induced by glucose. In a control group, most beta cells responded to 11 mM glucose with large-amplitude oscillations of \((Ca^{2+})_{i}\), whereas these oscillations, essential for the glucose-lowering effect of the hormone, appeared only in a few beta cells after inoculation with the viral strains.
To evaluate viral replication, inflammation and glucose tolerance, infection with three CV-B4 strains (E2, V89 4557 and VD2921) and with one CV-B3 strain (Nancy) was performed in CB6f1 mice [15]. A glucose tolerance test, performed at several time-points post-infection, showed that both in control mice and infected mice with CV-E2 and CV-B3 there was normal glucose absorption from the blood. In contrast, the glucose clearance in mice infected with the CV-B4 strains V89 4557 and VD2921 were impaired significantly against both uninfected controls and mice infected with the other CV-B strains [15].

Toll-like receptor 3 (TLR-3) and cytoplasmic retinoic acid-inducible gene I-like (RIG-I-like) receptors (RLR) represent two types of sensors that sense dsRNA and promote the release of cytokines, which are secreted by the cells in order to limit viral-induced injury [16–18]. RLRs include melanoma differentiation-associated gene 5 (MDA5) and RIG-I. MDA5 is expressed in islets and in exocrine pancreas during viral infection and acts locally to protect beta cells from virus-induced damage, as demonstrated by the finding that MDA5−/− mice developed transient hyperglycaemia in response to encephalomyocarditis virus strain D infection [16]. The anti-viral activities induced by dsRNA are mimicked in animal models and in cultured cells by PIC, a synthetic dsRNA [19]. In vivo, PIC is able to induce hyperglycaemia in BioBreeding Diabetes Resistant (BBDR) rats and enhances the development of diabetes in diabetes-prone (DP) BB rats [20,21]. dsRNA or viral infections increased expression of mRNAs encoding for TLR-3 in rat beta cells [4]. Islet cells isolated from wild-type or TLR-3 knock-out mice were used to assess the detrimental effects induced by PIC on beta cell viability. Indeed, it has been observed that disruption of the TLR-3 pathway prevented the deleterious effects of external PIC (PICex) + IFN-γ [19]. To confirm the pivotal role of the TLR-3 pathway in beta cells injured by PIC, the authors silenced the TLR-3 intermediary transcriptional regulatory protein IRF-3. PIC treatment of beta cells derived from IRF-3−/− and from wild-type mice resulted in a reduced insulin content in wild-type mice compared to knock-out mice. However, glucose-stimulated insulin release was reduced both in wild-type and in IRF-3−/− mice when exposed to PICex + IFN-γ, while islet cells from IRF-3−/− mice appeared more resistant to the deleterious effects of internal PIC (PICin). PICin also induced a marked decrease in protein biosynthesis, as demonstrated by the phosphorylation of α-subunit of the eukaryotic initiation factor-2 (eIF2α) and endoplasmic reticulum (ER) stress response [19]. Protein kinase R (PKR), a protein kinase activated by dsRNA by a number of cytokines by growth factors and stress signals, led to inhibition of protein synthesis via the phosphorylation of eIF2α [22]. The inhibition of protein synthesis derived from sequestration of eIF2B and subsequent inhibition of GDP for GTP exchange [23]. It was also established that, in C57BL/6 mice, dsRNA induced islet cell apoptosis mediated by PKR and this was associated with normal islet insulin secretion. When dsRNA + IFN-γ were administered they induced islet production of nitric oxide, correlated with inhibition of insulin secretion and, finally, islet cell necrosis [23].

Studies on human islets

It is not simple to elucidate the exact relationship between virus infection and diabetes in humans because it is highly probable, at the time of diabetes diagnosis, that patients have been exposed to multiple viruses and the specific causative viral infection has been cleared. It has been established that human enteroviruses, particularly Coxsackieviruses, have major effects at the level of both exocrine and endocrine pancreas and beta cells leading to local inflammation [1,24,25]. Epidemiological studies performed worldwide have shown that anti-enterovirus antibodies as well as enterovirus RNA can be found more frequently in the blood of recent-onset type 1 diabetic patients than in healthy controls [3]. In addition, several research groups have observed that Coxsackieviruses are capable of infecting isolated human pancreas [26–29], and in-situ hybridization studies on post-mortem pancreatic tissue of type 1 diabetic patients showed enterovirus-positive cells exclusively in islet cells [30]. Using electron microscopy and immunohistochemical staining, our group demonstrated the presence of enteroviral VP1 capsid protein in beta cell specimens from three of the six type 1 diabetic organ donors, providing direct evidence that enteroviruses can infect beta cells in patients with type 1 diabetes; of note, VP-1 positivity was restricted to beta cells and was not detected in alpha cells (Fig. 1). In addition, in these three patients with signs of beta cell enteroviral infection, it was possible to show the presence of non-destructive insulitis with natural killer (NK) cell infiltration associated with functional impairment of pancreatic beta cells [31]. Indeed, isolated virus from infected type 1 diabetic islets was able to infect beta cells in vitro from non-diabetic multi-organ donors, causing beta cell dysfunction characterized by impaired glucose-stimulated insulin release (Fig. 2). In a more recent study, VP1-immunopositive cells were detected in multiple islets of 44 of 72 young recent-onset type 1 diabetic patients, compared with a total of only three islets in three of 50 neonatal and paediatric non-diabetic controls [32]. However, whether and how viruses influence beta cell function and which are the molecular mechanisms involved in viral-induced beta cell damage remains not fully elucidated.

The first step in a viral infection is the binding of the virus to its receptor, a molecular cell surface, which allows the virus entry into the cell. Ylipaasto et al. showed co-localization of enterovirus receptors PVR and integrin αβ3 with virus particles in human islet cells [30]. After entrance into the cells, viruses organized their replication and effect on islet morphology and insulin release. In fact, several studies [26–32] in tissue cultures of isolated human
pancreatic islets infected with different strains of Coxsackievirus showed that viruses are not only able to infect human pancreatic islets cells in vitro, but also to cause morphological and functional damage or trigger beta cell death characterized by nuclear pyknosis. Specifically, authors showed that Coxsackievirus B3, few strains of Coxsackievirus B4 (e.g. CBV-4-E2 and CBV-89-4557) and Coxsackievirus B5 induced beta cell cytolysis resulting in a reduced glucose-stimulated insulin response of islets infected with viruses within 1 week post-infection, even if the susceptibility of islets to infection with different viruses was variable. In contrast, other studies showed that one strain of Coxsackievirus B4, strain VD2921, was able to infect human islet cells and to replicate, without causing any cytopathic effect or affecting the ability to respond to high glucose in terms of insulin release during the first few days post-infection, compared to non-infected islets. The authors concluded that infection with Coxsackievirus B4 VD2921 or related strains may, in vivo, predispose to progressive autoimmune islet cell damage and/or may affect the ability to establish a persistent infection in human beta cells. Ylipaasto et al., with the purpose of elucidating the molecular mechanisms involved in virus-induced beta cell destruction, infected three independent human islet preparations with Coxsackievirus B5 or exposed them to IL-1β plus IFN-γ, and then analysed the global pattern of gene expression by microarray analysis [33]. It was found that 484 genes were modified similarly by cytokines and by viral infection providing new evidence for potentially common molecular mechanisms involved in viral- and cytokine-induced human beta cell dysfunction and damage. More specifically, it was observed that both cytokines and viral infection increased the expression of several chemokines significantly, such as CXCL1 (MIP-2α), CXCL2 (MIP-2β) and CXCL10 (IP-10) and of cytokine IL-15. A separate study reported the specific expression of a number of inflammatory genes, including IL-1β, IL-6, IL-8 and CCL2 (MCP-1) after infection of isolated human islets with a Coxsackievirus B4 strain causing lytic infection (V89-4557) and a Coxsackievirus B4 strain establishing persistent infection (VD2921) [34,35]. Both studies provide strong evidence about the link between viral infections and the induction of factors having a key role in triggering insulitis and development of type 1 diabetes in man. Our group demonstrated [24] the specific expression of CCL2 in virus-infected pancreatic beta cells of type 1 diabetic patients (Fig. 3).

Viruses may cause beta cell destruction either by cytopathic effects on the target cells or indirectly by triggering or potentiating the autoimmune response. In this regard, it is of interest that recent studies showed IFN-γ-induced protein 10
(IP-10) expression, a chemokine that promotes the migration of activated T cells, in the islets of recent-onset type 1 diabetic patients [36] and in the sera of newly diagnosed children with type 1 diabetes [37].

The established role of inflammation in the different stages of the insulitic process [38] and increasing evidence in support of the contribution of viral infections to a proinflammatory islet scenario are strongly suggestive that viruses may indeed contribute to beta cell damage both directly (e.g. causing beta cell damage and/or functional impairment) and indirectly (e.g. by inducing the expression of proinflammatory cytokines and chemokines).

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Disclosure

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References

Viruses and beta cell function