Type 1 diabetes (T1D) results from a complex interplay between genetic and environmental factors, leading to chronic immune-mediated destruction of pancreatic \( \beta \)-cells. The inflammatory process is initiated by one or more environmental triggers, such as a viral infection, stimulating release of autoantigens, inflammatory mediators including cytokines and chemokines, and death effectors, with resultant \( \beta \)-cell loss. While multiple enterovirus (EV) serotypes demonstrate \( \beta \)-cell tropism, most studies support a role for the coxsackievirus B (CVB) group in the pathogenesis of T1D. Experimental studies using animal models, insulin-producing cell lines and human islets indicate that the major mechanism of EV-induced \( \beta \)-cell destruction is apoptosis.

Human EV infection has long been implicated in the pathogenesis of T1D. Many epidemiological and cross-sectional studies have demonstrated higher rates of EV infection among individuals with T1D compared with non-diabetic controls. In our meta-analysis of 26 studies using molecular methods for virus detection, there were significant associations between EVs and initiation of autoimmunity (OR 3.7, 95% CI 2.1–6.8) as well as development of T1D (OR 9.8, 95% CI 5.5–17.4). The detection of EV particles in pancreatic tissue from people with recent onset and long-standing T1D, with prominent islet tropism, provides direct evidence for the involvement of EVs in T1D. Furthermore, EVs isolated at T1D onset effectively destroyed human islets in culture and the pancreas of experimental animals, supporting a potential causal role for specific EV serotypes in the pathogenesis of T1D. Recently, the EV capsid protein VP1 was detected by immunostaining in 44/72 (61%) of pancreatic autopsy specimens from patients with recent onset T1D. The capsid protein was detected in multiple islets and immunostaining was specific to \( \beta \)-cells, with no involvement of other pancreatic cell types. These studies provide some of the most compelling evidence in support of an aetiological role for EVs in at least some cases of T1D.

While pancreatic autopsy specimens provide \textit{in vivo} data, we and others have shown that a range of EV genotypes, including CVBs...
and several enteric cytopathic human orphan (ECHO) viruses infect, replicate, impair β-cell function and cause cell death in human islets and insulin producing cells in vitro. In vitro, CVB3 and CVB4 can cause persistent infection in human β-cells, with release of infectious particles up to 1 month after infection, without cell lysis. Interestingly, EVs isolated from individuals discordant for development of T1D differed in their capacity to infect β-cells in vivo. Four isolates, from a mother and her son diagnosed with T1D on the same day (both infected with CVB5) and from twins (both infected with ECHO 21), one of whom subsequently developed T1D, replicated in human islets and caused slowly progressive β-cell lysis. However, β-cell tropism varied across these isolates, with the least cytolysis manifested by the isolate from the non-diabetic twin, suggesting divergence of species between individuals. This suggests that recombination events might have resulted in changes in virulence and/or viral persistence, rather than the host immune response determining whether T1D ensues following an EV infection.

It is generally accepted that activation and infiltration of autoreactive cells within the islets is initiated largely by cytokines and chemokines produced by macrophages and other immune cells. Recently, several studies have demonstrated that pancreatic islets are actively involved in signalling immune cells to invade the site of EV infection. CVB4 infection upregulates expression of cytokines such as interleukins (IL) eg; IL-1β, IL-6 and IL-8 and the chemokines; C-C motif ligand-5 (CCL-5) and C-C motif ligand-2 (CCL-2) by human islets. Similarly, CVB5 induces β-cell expression of IL-15, CCL-5 and interferon (IFN)-γ induced protein 10 (IP-10), as we and others have shown. The constant activation of pro-inflammatory cytokines leads to the expression of high levels acute inflammatory mediators, such as IL-1β, CCL-2, tumour necrosis factor-α (TNF-α) and IL-8, with progression to T1D through activation of apoptotic signalling factors. In addition, acute or chronic viral infection activates the innate immune response via interaction of ssRNA with pattern recognition receptors such as toll-like receptor (TLR) 7 and TLR8, activation of signalling pathways and production of antiviral cytokines such as IFN-α.

There are two major apoptotic pathways: intrinsic and extrinsic. The intrinsic pathway is regulated by B-cell lymphoma 2 (Bcl-2), which is associated with the outer mitochondrial membrane, while the extrinsic pathway is induced by death receptors such as Fas and TNF receptor-1 (TNF-R1). Either pathway involves activation of MAPK kinase, NF-κB and JAK/STAT pathways triggering, downstream cysteine proteases (caspases) – the final step of apoptosis.

Most studies examining mechanisms of EV mediated cell death have utilised cell types other than human β-cells. There is some evidence that the MAPK kinase pathway is involved (Figure 2); for example CVB3 infection in HeLa cells and Jurkat T cells phosphorylated p38 MAPK, JNK and ERK1/2. Similarly, c-Jun and p44/42 MAPK were phosphorylated following EV71 infection in rat brain astrocytes.

There is also more limited evidence that EVs induce β-cell death via apoptosis. Following CVB5 and 4 infection of islets derived from human pancreatic progenitor cells, we observed increases in ERK1/2, JNK and p38 (data not shown), confirming activation of the MAPK pathway (Figure 2). Similarly, EV infection in pancreatic islets activated the intrinsic pathway via an increase in Bim and a
decrease in induced myeloid leukemia cell differentiation protein (Mcl-1), an anti-apoptotic factor, as well as the NF-κB promoter.

Collectively, these data indicate that apoptosis is the major mechanism of cell death following EV infection of β-cells. However, the involvement of other signalling pathways has not been investigated.

Although EVs are ubiquitous, their contribution to the burden of T1D remains poorly understood. Furthermore, the putative role of other viruses such as rotavirus, rubella and mumps, as initiators and/or accelerators of T1D, is even less studied. While it is essential to identify specific serotypes and molecular characteristics of EVs that infect and destroy β-cells, a better understanding of the mechanisms of β-cell death may provide insights into development of novel strategies for prevention and treatment of T1D. In particular, EV induced β-cell death may be prevented through intervening in the production and/or action of immune mediators and apoptotic pathways. Development of vaccines targeting ‘diabetogenic’ EVs is another promising approach that might pave the way to reducing the burden of this chronic life-long disease.

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Under the Microscope


Biographies

Sandhya Nair completed a year of honours with the Virology Research Laboratory at the Prince of Wales Hospital and continued as a PhD student, furthering her research into the mechanism behind type 1 diabetes and enterovirus. Sandhya’s current research is examining viral induction of signalling pathways in beta cells infected with different enterovirus subtypes, to further understand the pathogenesis of virus-induced diabetes.

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Associate Professor Maria E Craig is a NHMRC Practitioner Fellow and a Staff Specialist in Paediatric Endocrinology at The Children’s Hospital at Westmead. After training in paediatric endocrinology, she was awarded a NHMRC Postgraduate Medical Research Scholarship for her PhD studies at the Virology Research Laboratory, investigating the association between enterovirus infection and the onset of type 1 diabetes. She has since been undertaking further larger-scale cohort studies of at-risk children to investigate the link between viruses and diabetes, including several NHMRC-funded project grants.

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