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LETTER

Large enteroviral vaccination studies to prevent type 1 diabetes should be well founded and rely on scientific evidence

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Abbreviations
IHC Immunohistochemistry
PKR Protein kinase R
VP1 Viral protein 1

To the Editor: We read with great interest the For Debate article by Dunne et al [1] and fully agree with the conclusion that intervention studies using a vaccine or antiviral drugs represent the way forward to finally establish whether enterovirus plays a role in type 1 diabetes. While we welcome and support such initiatives, we consider it crucial that funders investing in the huge vaccination trials required to prove or disprove the link are provided with solid scientific evidence. Also, in the risk–benefit analysis and ethical considerations, it is essential that the available evidence is viewed critically. Therefore, we feel compelled to point out some issues regarding the evidence cited in the article by Dunne et al.

First, the sequence data of the enterovirus isolated by Dotta et al from an individual with recent-onset type 1 diabetes [2] shared 99% identity with the coxsackievirus B4 prototype strain JVB Benschoten, originally isolated in 1951, clearly indicating that it was in fact a laboratory contaminant. This is well known and non-controversial among virologists and others within the field [3–5] and we find it problematic that this paper is uncritically cited as supporting evidence for the presence of pancreatic enterovirus in type 1 diabetes.

Second, in the Diabetes Virus Detection (DiViD) study, we detected enterovirus RNA with RT-PCR in pancreatic tissue samples from only one of the six patients with recent-onset type 1 diabetes [6]. This patient was also RT-PCR-positive for enterovirus RNA in peripheral blood mononuclear cells, suggesting that the positive signal could have come from infected circulating blood cells present in the pancreas, particularly given that this biopsy was collected as a surgical specimen and not perfused with preservation solution to remove blood, as is usually done during organ procurement for transplantation. The reported, and frequently cited, enterovirus RNA-positive samples from four out of six DiViD patients were from the culture medium of isolated islets. These islets were isolated from the tip of the pancreatic tail under non-sterile conditions in a research laboratory for the purpose of functional islet studies [7]. The enterovirus-negative islets from six non-diabetic organ donors [6], referred to by Dunne et al, were isolated in our Good Manufacturing Process (GMP) facility for the purpose of clinical islet transplantation and, therefore, these results cannot be used to rule out the possibility of viral contamination in the culture medium of islets isolated from the type 1 diabetic individuals. Also, the discrepancy in PCR positivity between tissue samples and the culture medium of isolated islets is difficult to explain by a difference in sensitivity [8], and the possibility that the positive signal was derived from contaminating viral sequences introduced during islet isolation or culture cannot be excluded. Other studies (not cited in the article by Dunne et al) have failed to find evidence of enterovirus RNA in pancreatic tissue from individuals with type 1 diabetes [8–11] or reported no difference between type 1 diabetes and non-diabetic control individuals [12].

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Third, the combined virus culture–PCR approach, stated in the article by Dunne et al as detecting enterovirus sequences in samples from all six type 1 diabetes pancreases included in the DiViD study, has only been published as an abstract [13], and so the validity of the results cannot be evaluated. It will be essential to compare the nucleotide sequences of the amplified enterovirus with the amplicons (located in the 5′ non-coding region of the enterovirus genome) previously reported in the DiViD islet supernatants [6]. In addition, sequencing of coding regions of the detected viruses would allow genotyping and comparison with previous isolates and laboratory strains. It is likely that enough viral genome would be obtained for amplification and sequencing if, as according to the article by Dunne et al, the virus could be isolated and propagated between cell cultures. While this approach sounds promising, it is of low value as evidence for enterovirus infection of islets at type 1 diabetes onset until these data are published and available for review by the scientific community.

Fourth, we have previously questioned the validity of immunohistochemistry (IHC) staining for the enterovirus viral protein 1 (VP1) in the human pancreas and have shown that the antibody used (clone 5D8/1, Dako) can cross-react with mitochondrial antigens [14]. In addition, this antibody was found to have strong cross-reactivity with cellular proteins in the heart of humans and mice [15]. It has been argued that, under specific conditions, this antibody can still be used to detect enteroviruses in the pancreas [16]. However, the presence of enterovirus protein specifically in pancreatic tissue immunopositive for VP1 by IHC with the Dako antibody has not been confirmed by other methods, and recently it was reported that, in the Network for Pancreatic Organ Donors with Diabetes (nPOD) cohort, there was no correlation between immunopositivity for VP1 and the presence of enterovirus genome as demonstrated by PCR [12]. These findings suggest that any evidence for an association between enterovirus and type 1 diabetes based on IHC with the Dako VP1 antibody should be disregarded.

The ‘unbiased proteomic analysis’ claimed in the article by Dunne et al to verify the presence of viral proteins in type 1 diabetic pancreases sounds interesting but is based on unpublished data only, and it is not clear whether the presence of VP1 correlates with IHC positivity or type 1 diabetes. A number of novel antibodies with demonstrated high specificity for structural [15, 17] and non-structural [18] enterovirus proteins have been developed but, to date, none of these have been used to confirm the presence of enterovirus protein in the pancreas of individuals with type 1 diabetes.

Fifth, Dunne et al refer to studies claiming that staining for protein kinase R (PKR) overlaps more or less completely with staining for enterovirus VP1 [19] and argue that this is consistent with an innate immune response initiated by a chronic enterovirus infection. However, we recently demonstrated that PKR is expressed in the majority of cells in the pancreas, regardless of enterovirus infection [20]. In order to study a potential antiviral response inducing translational arrest in infected beta cells, methods that specifically detect active phosphorylated PKR need to be used. To our knowledge, this has not been performed to date in human pancreatic samples.

Sixth, evidence of persistent enterovirus infection in thyroid disease is weak and based on immunopositivity for VP1 using the same antibody clone as demonstrated to cross-react with cellular proteins in pancreatic [14] and heart [15] tissue. This must be considered when estimating the commercial viability of developing an enterovirus vaccine.

In summary, the evidence for the presence of enterovirus in islets predominantly from individuals with type 1 diabetes compared with non-diabetic control individuals is at best weak. However, we know that enteroviruses can infect beta cells in vitro and in vivo [21] and it is possible that such infections play a role in the induction of type 1 diabetes but are resolved at the time of clinical diagnosis. A viral aetiology of type 1 diabetes is still plausible as several observations are in line with this hypothesis, and we agree that intervention trials in type 1 diabetes directed against enterovirus could finally prove a causal relationship. However, type 1 diabetes is not likely to be caused by a single enterovirus strain or serotype and vaccination should therefore be as broad as possible. Therapy with antiviral drugs initiated after diagnosis of type 1 diabetes is less likely to have any effect, as the evidence of enterovirus infection in islets post diagnosis is weak. Overinterpretation and uncritical review of current evidence risks obscuring a real link between enterovirus and type 1 diabetes and damaging our common goal of understanding the aetiology of type 1 diabetes in order to prevent the disease. That said, we are looking forward to seeing the results of broad vaccination trials, which will hopefully prevent at least a fraction of type 1 diabetes cases.

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Contribution statement OS conceptualised and drafted the article. All authors were responsible for revising the article critically for important intellectual content. All authors approved the version to be published.

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