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LETTER

Large enteroviral vaccination studies to prevent type 1 diabetes should be well founded and rely on scientific evidence. Reply to Skog O, Klingel K, Roivainen M et al [letter]

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Abbreviations
PKR Protein kinase R
VP1 Viral protein 1

To the Editor: We are pleased that Skog et al [1] have accepted the challenge to respond to our recent 'For Debate' article [2]. In this, we proposed that both vaccination and trials with antiviral therapies are warranted, based on decades of studies implicating an association between enterovirus infections and type 1 diabetes. It is pleasing to observe these conclusions were similarly supported by Skog et al [1].

However, while supporting our overall conclusions, the authors also challenged several pieces of evidence cited in our article, and we believe it important to respond to each challenge. First, Skog et al question the value of antiviral therapies at clinical onset of type 1 diabetes because they contend that the evidence for viral infection post diagnosis is weak [1]. In response, we would cite the growing evidence for: (1) chronic autoimmunity and persistent insulin secretion extending for many years after diagnosis [3–5]; (2) the existence of beta cell dysfunction at onset (reviewed in [6, 7]); (3) the negative impact of viral infections on beta cell function [8, 9]; and (4) the increasing evidence (some of which is unpublished) supporting pancreatic viral infections near diagnosis and several years thereafter [10]. Furthermore, enterovirus nucleic acids/proteins in human samples (including blood) have been associated with type 1 diabetes in several meta-

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analyses [11, 12]. We believe the best way to resolve this issue is to undertake a randomised controlled trial using antiviral agents at diagnosis; one such trial, led from Oslo, Norway, is already recruiting across Scandinavia. Of course, one factor that may influence the outcome is the fact that the efficacy of antiviral agents against persistent enteroviral infections is largely unknown and this must be borne in mind when evaluating the results. Nevertheless, even a modest effect would provide supportive evidence for the continued pursuit of antiviral strategies.

In our article [2], we did not discuss in detail how vaccination trials for type 1 diabetes prevention might be designed, but we are happy to clarify here that, in the first phase, relevant target groups might include those with increased genetic risk for type 1 diabetes, populations with very high disease incidence, and children acquiring multiple autoantibodies by 3 years of age as a surrogate for disease progression. We agree that enteroviral vaccines should target multiple strains, and we would not expect that all cases of type 1 diabetes would be prevented, given the possible role of other viruses or that some individuals may develop disease in the absence of viral infections.

Clearly, our key conclusion that the weight of evidence favours the hypothesis that enteroviruses are associated with type 1 diabetes [2] is not based solely on reports claiming to have isolated viruses from the pancreas. We accept that these are few and that in one study [13] the isolate may have resulted from laboratory contamination, as we have emphasised previously [14]. Nevertheless, in our view, Skog et al [1] have overstated the problem of contamination; especially in relation to the Diabetes Virus Detection Study (DiViD). In that work, contamination is extremely unlikely as an explanation for the source of enterovirus because: (1) the biopsy collections were performed under sterile conditions in the operating room; (2) enterovirus RNA was amplified from islet supernatants, but could not be amplified from the equivalent cultures of pancreatic exocrine cells; and (3) enterovirus sequences differed among the participants. We agree, however, that studies that compare the viral RNA sequences detected in tissue and supernatants are needed, and these too are ongoing.

Skog et al [1] have also proposed that 'any evidence for an association between enterovirus and type 1 diabetes based on IHC [immunohistochemistry] with the Dako VP1 [viral protein 1] antibody should be disregarded'. This is a singular claim given that the fidelity of this antibody has been tested rigorously under the stringent conditions we use [15]. In addition, and as they suggested, we have also tested additional antibodies and find that the majority are much less sensitive than the Dako anti-VP1 reagent [16]. However, as noted in one earlier study [17], some additional antisera do label equivalent islet cells to those stained by the Dako clone in pancreas sections from people with type 1 diabetes. Skog et al [1] also drew attention to the poor correlation between VP1 immunopositivity in pancreas sections and the ability to amplify enteroviral sequences by PCR. Unlike Skog and colleagues, we expect that a perfect correlation will be difficult to achieve if one considers that the two assays (detection of immunoreactive VP1 and PCR amplification of viral nucleic acid sequences) are performed on paraffin and frozen tissues, respectively, and that there can be significant variability in the detection of viral signals when adjacent tissue blocks are examined. Indeed, anecdotally, we have found such discrepancies in our own studies but these have been resolved when further regions of the tissue are analysed.

In terms of protein kinase R (PKR) staining, Skog and colleagues [1, 18] argue that PKR is expressed uniformly in all cells of the pancreas. We contend that this is incorrect. PKR is certainly present in the islet at low levels (this point has never been in dispute) but its level varies dramatically between those cells that stain positively for enterovirus VP1 compared with those that do not. This is true both in control individuals (where the number of such cells is very low) and in people with type 1 diabetes (where more islet cells stain positively) [10, 14]. These observations are firmly consistent with the activation of an antiviral response involving upregulation of PKR in a small subset of islet cells [14]. We agree that selectively detecting the phosphorylated form of PKR would also be desirable, but we have not been able to find suitable antisera to label this form reliably in fixed tissue samples. We assume from their recent paper that Skog and colleagues [1, 18] have also failed in this objective, although we note that they did not analyse virally infected samples in their study. In support of the proposal that PKR activation follows from viral infection in islet cells, we have demonstrated that the labile anti-apoptotic protein myeloid cell leukaemia sequence-1 (Mcl-1) is selectively lost from islet cells in which PKR expression is increased, a response that is entirely consistent with the activation of translational arrest induced by active PKR [10].

We acknowledge that our article [2] cited information from ongoing (currently unpublished) studies, in which novel approaches have been employed including, for example, the application of proteomics to pancreas extracts as a means to identify the sequence of the enterovirus VP1 peptide recognised by the Dako antibody (J. Nyalwidhe and J. Nadler, Eastern Virginia Medical School, Norfolk, VA, USA, personal communication). We felt it important to include such emerging information as representative of the current state-of-the-art.

In closing, we stand by our conclusion, with which Skog et al [1] seem to agree, that there is sufficient evidence deriving from multiple studies using a wide range of approaches to implicate enteroviruses in type 1 diabetes. At the same time, we agree that many questions remain and that scientists must work collegially to address these outstanding issues. However, we also suggest that a point has been reached where

vaccination trials represent the most effective means to resolve the debate once and for all. In support of this, we drew attention to the fact that one company has weighed the evidence independently and has reached the conclusion that investment in the development of a polyvalent enteroviral vaccine represents a sound scientific and commercial venture [19]. We expect that the outcome of such vaccination studies will establish or disprove a role for enteroviruses in type 1 diabetes. Supporting this notion, we are interested to note the very recent publication of an Australian study reporting a decreased incidence of type 1 diabetes following the introduction of a rotavirus vaccine [20]. The authors cite previous evidence associating rotavirus infections with type 1 diabetes [20, 21] and the possibility should not be overlooked that co-infection with rotavirus and other viruses (including different enterovirus types) might contribute to the development of type 1 diabetes.

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