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RESEARCH ARTICLE

Type 1 diabetes could begin with alterations in innate anti-viral immunity, which are already at this stage associated with HLA risk haplotypes

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Abstract

Aims: To investigate if HLA risk haplotypes and HbA1c levels are associated with the expression levels of innate anti-viral immune pathway genes in type 1 diabetes.

Materials and Methods: We investigated RNA expression levels of innate anti-viral immune pathway genes in laser-dissected islets from two to five tissue sections per donor from the Diabetes Virus Detection study and the network of Pancreatic Organ Donors in relation to HLA risk haplotypes (non-predisposed and predisposed) and HbA1c levels (normal, elevated, and high).

Results: The expression of innate anti-viral immune genes (TLR7, OAS1, OAS3 etc.) was significantly increased in individuals with predisposing vs non-predisposing HLA haplotypes. Also, the expression of several of the innate anti-viral immune genes from the HLA risk haplotype analysis was significantly increased in the group with high vs normal HbA1c. Furthermore, the gene expression of OAS2 was significantly increased in the group with high HbA1c vs elevated HbA1c.

Conclusions: Expression of innate anti-viral immune pathway genes was increased in individuals with predisposing HLA risk haplotypes and those with high HbA1c. This indicates that type 1 diabetes might well begin with alterations in innate anti-viral immunity, and already at this stage be associated with HLA risk haplotypes.

KEYWORDS
enterovirus, HbA1c, HLA distribution, innate antiviral immunity, TLR7, type 1 diabetes

We were excited to read the recent commentary by Bruzzaniti et al. in Diabetes Metabolism Research and Reviews that is highlighting an important and until now rather overlooked observation of dysregulation in innate anti-viral immune pathways,[1] which might be central in the aetiology of type 1 diabetes. The authors cite our recent publication that observed type 1 diabetes-associated single-nucleotide polymorphisms and marked transcriptional dysregulation of several innate anti-viral immune pathways in laser-dissected islets from individuals with various stages of type 1 diabetes.[2] However, a few central points need to be emphasised, which is the purpose of this article. First, we discovered that the innate anti-viral immune pathways Toll-like

receptor 7 (TLR7) and Interferon Regulatory Factor 7 were downregulated in auto-antibody positive individuals compared to healthy controls from the nPOD programme, while TLR7 and other innate anti-viral immune pathways, such as 2–5′ oligoadenylate synthetase (OAS)1–3, were upregulated in new-onset type 1 diabetes patients from the DiViD study.[2] The latter has also been shown for multiple interferon-stimulated genes in insulitic islets.[3] This is an important observation for two reasons, namely that these pathways are involved in sensing viral single-stranded RNA that includes enteroviruses, which the DiViD study identified as the only virus present in pancreata from new-onset type 1 diabetes patients,[4] and for the reason that a downregulation of these pathways might lead to persistent latent enterovirus infections in genetically predisposed individuals, resulting in beta-cell autoimmunity and type 1 diabetes. Second, it indicates that either the islets as such or single islet cells (beta cells, macrophages, NK cells) are behind the observed dysregulations of innate anti-viral immune pathways, although our study did not answer that question, as it was done on laser-dissected whole islets and not on single cells. Nevertheless, it was previously shown that mimicking a viral infection with interferon alpha (IFN-α) and/or polyinosinic:polycytidylic acid (poly [I:C]) results in a markedly higher OAS response in beta cells compared to alpha cells,[5] which points to the possibility that specifically beta cells are involved in this problem.

Our new unpublished observation on the HLA risk genotype and HbA1c being associated with the expression of innate anti-viral immune pathway genes seems appropriate to put forward here (Figure 1).

This sub-study, using nPOD and DiViD data, indicates that the expression of innate anti-viral immune genes (*TLR7*, *OAS1*, *OAS3* etc.) is significantly increased in individuals (patients and healthy controls pooled) with predisposing HLA haplotypes (as defined for patients and healthy controls in electronic supplemental material, table 1 in Ref. 2) versus non-predisposing HLA haplotypes (neutral or protective haplotypes) (Figure 1A). Furthermore, we divided individuals (patients and healthy controls pooled) into 3 sub-groups according to normal (<5.7%), elevated (5.7%–6.4%), and high (6.4% >) HbA1c levels. Analysis showed that the expression of several of the innate anti-viral immune genes evaluated in the HLA risk

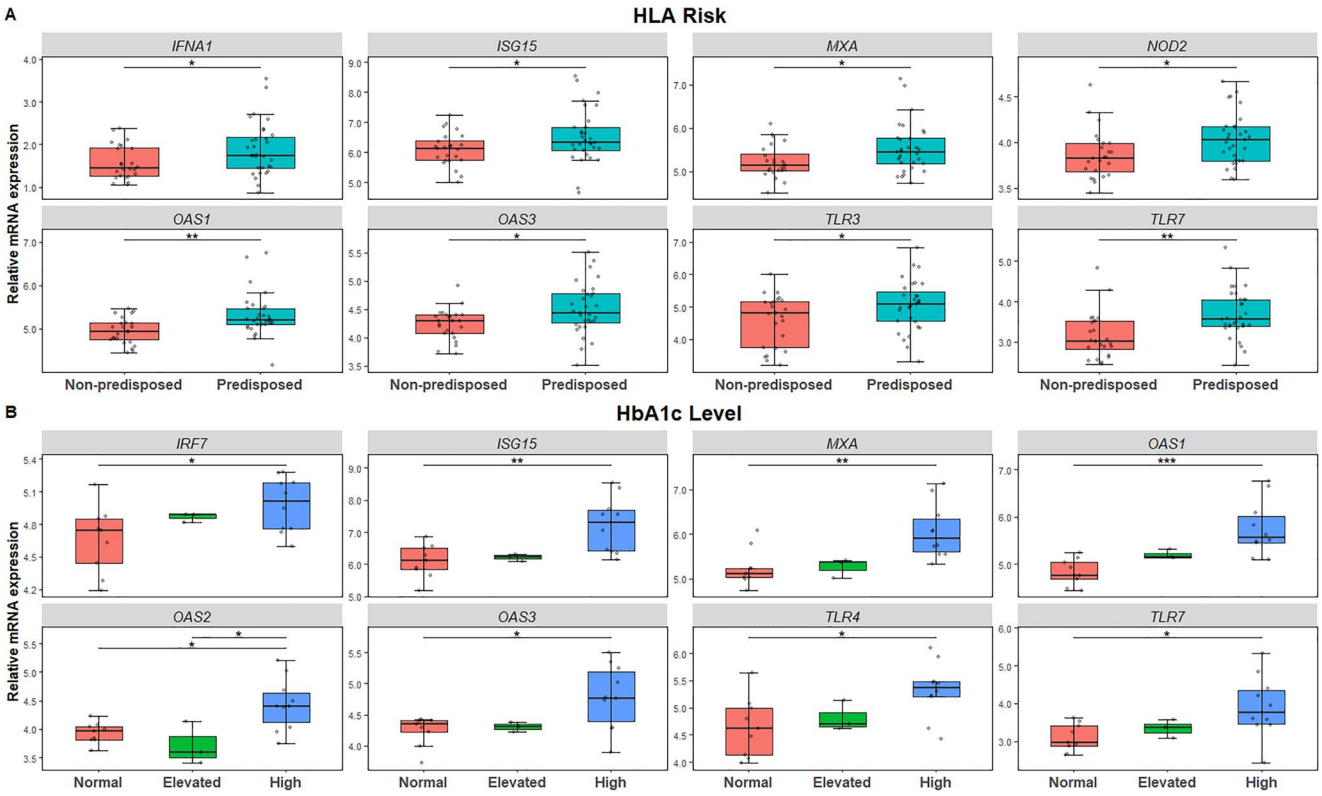

FIGURE 1 Relative mRNA expression of innate anti-viral immune genes. (A) HLA risk correlates with the mRNA expression of innate anti-viral immune genes in laser-dissected islets from non-predisposed individuals with neutral or protective haplotypes and predisposed individuals with predisposing haplotypes for T1D. The data included healthy controls, Ab+ individuals, new-onset type 1 diabetes, and long-standing type 1 diabetes patients (ESM table 1 in Ref. 2). (B) HbA1c level correlated with mRNA expression of innate anti-viral immune genes from laser-dissected islets. The range of normal, elevated, and high HbA1c is based on percentage and the guidelines from the American Diabetes Association. The data include healthy controls, Ab+ individuals, new-onset type 1 diabetes, and long-standing type 1 diabetes patients (ESM table 1 in Ref. 2). Rstudio 2022.07.1 build.554 was used for graphical representation and statistical analysis using one-way ANOVA with Tukey HSD correcting for multiple comparisons. ANOVA, analysis of variance; ESM, electronic supplementary material; HLA, human leukocyte antigen; HSD, honestly significant difference. *p value < 0.05; **p value < 0.01; ***p value < 0.001.

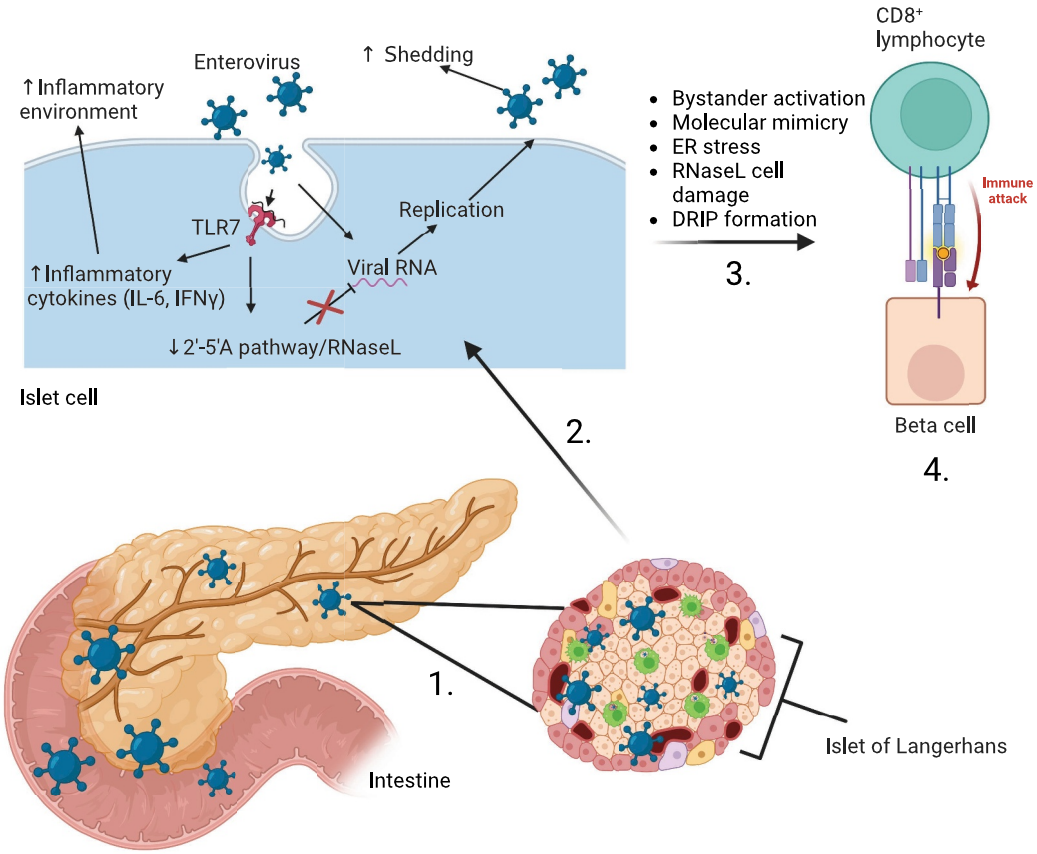

FIGURE 2 The hypothesis of the innate anti-viral immune response involvement in triggering type 1 diabetes. **1.** Potentiated by increased intestinal permeability, enterovirus infection spreads from the intestine to the islets of Langerhans in genetically type 1 diabetes-predisposed individuals. **2.** Early stage downregulation of innate anti-viral immune pathways may cause an increase in the inflammatory environment and shedding of enteroviruses. **3.** The resulting inadequate innate anti-viral immune response causes cell damage and stress in the islet cells, bystander activation, molecular mimicry, and/or defective ribosomal product formation. **4.** Activation of autoreactive T cells and destruction of beta cells, thereby also causing a significant upregulation of innate anti-viral immune pathways as seen in new-onset type 1 diabetes patients in Pedersen et al.[2] Illustration created using BioRender.com.

haplotype analysis significantly increased in the group with high HbA1c compared to the group with normal HbA1c, and for *OAS2*, there was a significantly increased gene expression in the group with high HbA1c versus elevated HbA1c (Figure 1B). Thus, our sub-study clearly shows that innate anti-viral immune gene expression is associated with HLA risk haplotypes and HbA1c. Most important is the former observation of HLA association, as it demonstrated that HLA risk is likely to have an effect before lymphocytes are extensively engaged in the pathogenesis of type 1 diabetes. Moreover, it could mean that the possession of HLA risk haplotypes is associated with the observed alterations in innate anti-viral immune pathways, which upon enterovirus infection may lead to persistent and latent viraemia and beta-cell autoimmunity with possible mediating mechanisms being bystander activation, molecular mimicry, endoplasmic reticulum (ER) stress, RNaseL cell damage, and defective ribosomal product (DRIP) formation. However, this causal relationship remains unproven.

Lastly, we would like to draw attention to the observation of increased intestinal permeability in pre- and manifest type 1 diabetes,[6] which very well could potentiate the pathogenetic process by spreading an enterovirus infection to the pancreas as well as other organs. Interestingly, stimulation of TLR7 with the receptor agonist imiquimod was recently shown to modulate the intestinal permeability in rats.[7] This observation indicates that local dysregulation of innate anti-viral immune pathways might influence the intestinal permeability, favouring the spread of enterovirus to the body, although confirmation of this hypothesis remains (Figure 2).

In conclusion, type 1 diabetes might well begin with alterations in innate anti-viral immunity, which seems to be HLA associated.

AUTHOR CONTRIBUTIONS

Karsten Buschard and Martin Haupt-Jorgensen wrote the manuscript. Mathias Høj Jensen, Lars Krogvold, Ivan C. Gerling, Knut Dahl-Jørgensen, and Kristina Pedersen critically reviewed the manuscript. Kristina Pedersen created the figures and illustrations. All authors have read and approved the final version of the manuscript.

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Illustrations were created using BioRender.com.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ETHICS STATEMENT

The DiViD study was approved by the Norwegian Government's Health Region South East Regional Ethics Committee (reference 2009/1907).

DATA AVAILABILITY STATEMENT

The datasets generated during the current study are available from the corresponding author upon reasonable request.

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PEER REVIEW

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