Islet Cell Hyperexpression of HLA Class I Antigens: A Defining Feature in Type 1 Diabetes

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Aims/hypothesis: Human pancreatic beta-cells may be complicit in their own demise in type 1 diabetes but how this occurs remains unclear. One potentially contributing factor, hyperexpression of HLA class I antigens, was first described ~30 years ago but has never been fully characterised and was recently challenged as artefactual. Therefore, we investigated HLA class I expression at the protein and RNA levels in pancreata from three cohorts of patients with type 1 diabetes. The principal aims were to consider whether HLA class I hyperexpression is artefactual and to determine the factors driving it.

Methods: Pancreas samples from type 1 diabetes patients with residual insulin-containing islets (n=26) from the nPOD, DiViD and UK recent-onset type 1 diabetes collections were immunostained for HLA class I isoforms, STAT1, NLRC5 and islet hormones. RNA was extracted from islets isolated by laser capture microdissection (LCM) from nPOD and DiViD cases and analysed using Affymetrix gene expression arrays.

Results: Hyperexpression of HLA Class I was observed in the insulin containing islets (ICIs) of type 1 diabetes patients from all three tissue collections and was confirmed at both the RNA and protein level. Beta 2-microglobulin expression (β2M; a second component required for generation of functional HLA Class I complexes) was also elevated. Both "classical" HLA class I isoforms (HLA-A,B,C) as well as a "non-classical" HLA molecule, HLA-F, were hyperexpressed in ICIs. The hyperexpression did not correlate with detectable upregulation of the transcriptional regulator, NLRC5. However, it was strongly associated with increased STAT1 expression in all three cohorts. Islet hyperexpression of HLA class I occurred in the insulin-containing islets of recent onset type 1 diabetes patients and was also detectable in many patients with disease duration of up to 11 years, declining thereafter.

Conclusions: We conclude that islet cell HLA class I hyperexpression is not an artefact but is a hallmark in the immunopathogenesis of type 1 diabetes. The response is closely associated with elevated expression of STAT1 and, together, these occur uniquely in patients with type 1

diabetes, thereby contributing to their selective susceptibility to autoimmune-mediated destruction.

Introduction

The incidence of type 1 diabetes is increasing rapidly worldwide [1-3] probably due to changes in the environment that ultimately impact the development, functional activity and longevity of pancreatic beta-cells.

Against this background, the cellular and molecular events associated with initiation and progression of type 1 diabetes remain poorly understood; largely because the disease process cannot be studied non-invasively in the pancreata of living individuals. Hence, deductions regarding pathogenic processes is made from an analysis of tissue recovered either after death or by pancreas biopsy of living subjects [4-7]. Collectively, such approaches have been applied in few cases worldwide, reflecting the paucity of accessible samples in which the tissue architecture has been preserved and the destructive process is still present and amenable to study [8, 9].

Despite these limitations, a consensus model has emerged in which type 1 diabetes is envisaged to result from the selective destruction of beta-cells by immune cells infiltrating the islets of Langerhans [1-3, 8, 9]. In this scenario, CD8+ T-cells are considered the major effectors of beta-cell death and these are directed to the pancreatic islets to participate in the autoimmune assault against the beta-cells [10-13]. It is also likely, however, that the beta-cells are complicit in these events by processing and presenting cellular antigens aberrantly, thereby becoming visible to autoreactive CD8+ T cells [14]. This could be achieved in several ways, including via the up-regulated expression of the major histocompatibility complex (HLA class I molecules) [15-17].

Up-regulation of HLA class I (HLA-I) expression (often cited as “hyperexpression”) in pancreatic islets has been studied in relatively few type 1 diabetes patients and no previous attempts have been made to verify the phenomenon across multiple cohorts. Moreover, the concept has primarily been examined at the protein level using immunocytochemical

approaches and this has rarely been corroborated by gene expression data to verify that the two are concordant. Indeed, in one recent study, it was argued that such concordance may not exist [18].

Therefore, in the present work, we have taken advantage of the unique collaborative strength achieved by combining three of the world's most significant collections of pancreas samples from individuals with type 1 diabetes; an archival collection of postmortem samples from the United Kingdom [4], the Network for Pancreatic Organ donors with Diabetes (nPOD) collection of organ donor pancreata (United States) [19, 20], and pancreatic biopsy material from living subjects participating in the Norwegian Diabetes Virus Detection study (DiViD) [7]. These tissues have been interrogated to provide definitive evidence as to whether islet cell hyperexpression of HLA-I antigens is an early and defining feature of human type 1 diabetes. Collectively, these new data allow us to report the most comprehensive examination of HLA-I expression ever undertaken involving the human pancreas in type 1 diabetes.

RESEARCH DESIGN AND METHODS

Tissue

Formalin-fixed paraffin embedded (FFPE) pancreas sections were available from three cohorts: the nPOD and DiViD collections and an archival collection from the United Kingdom (Supplementary Tables 1 & 2). Frozen tissue was also available from nPOD and DiViD. Analysis was performed on 17 controls and 26 type 1 diabetes cases for whom FFPE and frozen tissue were available. All samples were studied with appropriate ethical approval and, in the case of the DiViD study, participants provided written informed consent.

Immunohistochemistry & Immunofluorescence

Immunohistochemistry was performed using a standard immunoperoxidase approach as described previously [21]. To examine multiple antigens within the same FFPE section, samples were probed in a sequential manner with up to three different antibodies (Supplementary Tables 3 & 4). The mean fluorescence intensity (MFI) of stained antigens was measured using ImageJ. Some slides were processed with isotype control antisera to confirm the specificity of labelling (Supplementary Fig.1). Frozen sections were stained using a standard immunofluorescence approach [22].

Islet Microdissection and RNA Collection

OCT slides of pancreatic tissue were used for laser-capture microscopy. This was conducted on an Arcturus Pixcell II Laser-Capture Microdissection (LCM) system (Arcturus Bioscience, CA, USA). Islets were recognized by their natural auto-fluorescence [23]. All islets visible in 2-5 sections from each sample were pooled and RNA extracted using the Arcturus PicoPure RNA Isolation Kit (Applied Biosystems, Grand Island, NY, USA). RNA quantity and quality

was determined using a Bioanalyzer 2100 (Agilent Technologies, CA). RNA samples were subjected to gene expression analysis using Affymetrix expression arrays as described [24].

Affymetrix Array Analysis

CEL files using Affymetrix Human Gene 2.0ST array were generated from both control and type 1 diabetes donors, as described [24]. Raw intensity signal values from Affymetrix spike-in controls demonstrated that array hybridization was successful (i.e., bioB<bioC<bioD<Cre). Data quality was verified by measurement of the positive versus negative area under the curve. Raw signal intensity values from all arrays were Robust Multichip Average (RMA) background corrected, quantile normalized, median polish summarized, and log2transformed [25-27]. NetAffx-determined probeset annotations for HLA genes were re-mapped according to Refseq, release 73 (11/15/2015). For each HLA gene, where multiple mappings were possible (i.e., HLA-A, B, C, and F), probe sets were annotated according to 8 major haplotypes incorporated into the human genome assembly, as previously described [28]. Because probe sets shared mappings, it was not possible to identify HLA sub-types uniquely using this gene chip; rather, transcript clusters were used to examine changes in global gene expression. For each HLA gene, where multiple mappings were possible (i.e., HLA-A, C and F), probe sets were annotated according to 8 major haplotypes incorporated into the human genome assembly, as previously described Processing was carried out using Partek Genomics Suite, version 6.5, build 6.10.0810 copyright 2010 (Partek, St. Louis, MO). The resulting normalized expression data for specific genes of interest were then subjected to analysis as described below.

Statistical analysis

Individual comparisons of MFI, RNA, or protein were performed using either a Satterhwaite corrected two-sample or paired/unpaired Student's t-test and considered significant if $P<0.05$.

In the case of multiple comparisons, statistical significance was indicated at a Bonferroni-corrected nominal α level of 0.025. Correlations were evaluated and considered strong if $P<0.05$ and a Spearman's rank correlation coefficient (r) is >0.80. All reported *P*-values are 2-tailed and unadjusted. Statistical analysis was performed using SAS software version 9.4 (SAS Institute, Cary, NC).

RESULTS

Lobular Hyperexpression of HLA-ABC in Type 1 Diabetes

In accord with earlier reports [9, 14, 21, 22] hyperexpression of HLA-I A, B & C (HLA-ABC) was observed consistently in the islets of patients with type 1 diabetes among all cohorts examined (Fig.1) but not in controls. The pattern was lobular and mainly restricted to ICI (Fig.1*a,b*) while IDIs displayed normal expression. Islet hyperexpression of HLA-ABC was not confined solely to beta-cells but occurred in all islet endocrine cells (Fig.1, Supplementary Fig.2).

Classification of Donors Based on HLA-ABC Expression

Since islet hyperexpression of HLA-ABC has been claimed to be artefactual [18] we monitored the levels of HLA-ABC in a subset of nPOD donors in two independent laboratories using pancreas preserved by different methods (frozen vs formalin-fixed). Staining for HLA-ABC was performed using either an immunoperoxidase method coupled with a mouse primary antiserum in FFPE tissue (Supplementary Fig.2*a*) or via immunofluorescence in OCT sections (Supplementary Fig.2*b*) from the same donor, using a different primary antiserum. A blinded analysis was conducted with donors classified into three categories: normal (Supplementary Fig.2;left panel); elevated (Supplementary Fig.2, middle panel); hyperexpression (at least one islet with extremely high expression of HLA-ABC affecting all endocrine cells (Supplementary Fig.2;right panel)). Un-blinding of the analysis revealed a 100% concordance rate between laboratories (Supplementary Fig.2c).

Further confirmation of the staining specificity in FFPE tissue was obtained by staining of serial islet sections with two different HLA-ABC antibodies. In all cases where hyperexpression of HLA-ABC was detected with one antiserum, this was confirmed in the

same islet on the serial section with the second antiserum (Supplementary Fig.3; Supplementary Tables 3 & 4).

Examination of patients with increasing disease duration revealed that HLA-ABC hyperexpression was not restricted only to recent-onset cases of type 1 diabetes but could still be observed in subjects with longer-term disease (i.e. up to 11 years) when ICIs were retained (Supplementary Fig.4). However, the proportion of ICIs hyperexpressing HLA-ABC decreased as type 1 diabetes duration increased (r=-0.883, P<0.0001; Supplementary Fig.4). HLA-I hyperexpression was not found in patients lacking residual ICIs. It was also absent from the ICIs of patients with still longer disease duration (>11 years), even among those who retained insulin immunopositivity (3 nPOD cases with a total of 110 ICIs) after this time (Supplementary Fig.4).

β2 Microglobulin is Elevated in Islets in Type 1 Diabetes

Functional HLA-I complexes are heterodimers comprised of an isoform of HLA-I plus β2-microglobulin (β2M). Therefore, the levels of β2M were also assessed and found to be expressed differentially in type 1 diabetes and controls. β2M was present in the islets of individuals without type 1 diabetes (Fig.2*a*) but its expression was increased in the ICIs of patients with type 1 diabetes, which also hyper-expressed HLA-ABC. IDI from the same individuals expressed levels of β2M and HLA-ABC comparable to those seen in non-diabetic controls (Fig.2*b*,*c*).

Expression of RNA Transcripts Encoding HLA or β2M in Laser-Captured, Microdissected Islets

Next, the expression of HLA-isoforms and β2M was examined at the RNA level in LCM islets. RNA was extracted from pooled islets harvested in a manner that did not differentiate between

islets with hyperexpression or normal expression of HLA-I or between ICIs and IDIs (Fig.3). Initially, RNA expression profiles were analysed in islets from the DiViD cohort since these represent patients with recent-onset disease who retain ICIs [29, 30]. Age matched control subjects were selected from the nPOD collection. When displayed in a "heat map" format to indicate relative RNA levels using multiple probe-sets (Fig.3*a*) each of the HLA isoforms (A,B,C) and β2M was shown to be markedly elevated. Quantification yielded mean ± SEM increases of 1.9 ± 0.14-fold, 2.15±0.16-fold, 2.02±0.09-fold for HLA-A,B&C respectively and 2.07-fold for β2M. Data from the nPOD cohort revealed similar trends (Supplementary Fig.5) although the effects were less marked. When analysis of the nPOD cases was refined by exclusion of individuals in whom no ICIs could be found in sections adjacent to the pancreatic blocks used for islet RNA isolation, the trend for increased expression of HLA-ABC and β2M was more pronounced (Fig.3*b*).

HLA-F Expression is Also Elevated in the Insulin-Containing Islets of Individuals with Recent-Onset Type 1 Diabetes

During analysis of RNA expression in islets from the DiViD patients it was observed that a non-classical HLA, HLA-F, was also upregulated (by 1.71±0.04-fold) when analysed across all probe-sets (Fig.4*a*). Therefore expression at the protein level was assessed in FFPE tissue. This revealed that HLA-F is expressed at low levels in the islets of non-diabetic controls but was upregulated in the ICIs of patients with recent-onset type 1 diabetes (Fig.4*b;* Supplementary Fig.6a). The elevated expression was not restricted to beta-cells but could also be observed in α-cells (Fig.4*c*). Similar findings were made in pancreas tissue from the nPOD, DiViD and UK cohorts (Supplementary Fig.6b). Surface localisation of HLA-ABC and HLA-F were observed, but HLA-ABC was also seen in the cytosol of ICIs (Supplementary Fig.6*c*).

NLRC5 Expression does not Correlate with HLA-ABC Hyperexpression

In order to understand the factors that might drive islet HLA-I hyperexpression in type 1 diabetes, we studied NLRC5, a known transcriptional regulator of HLA-ABC and β2M [31]. NLRC5 was readily detected at the protein level in the cytoplasm of beta-cells in healthy control pancreas (Fig.5*a* – upper panel). Expression of NLRC5 was similarly detected in islets of patients with type 1 diabetes but it was not elevated, even in those islets having demonstrably elevated HLA-ABC expression (Fig.5*a* – lower panel; Fig 5*b*). This was confirmed at the RNA level in LCM captured islets (Fig. 5*c*; P=0.4504).

STAT1 Expression Correlates Positively with HLA-ABC Hyperexpression in Type 1 Diabetes

Given that NLRC5 expression does not change in parallel with HLA-ABC or β2M in the islets of patients with type 1 diabetes, a second transcriptional regulator, STAT1, was investigated. This protein was present at low levels in the islets of non-diabetic controls (Fig.6*a* – upper panel) and the pattern of staining was similar in each of the three tissue cohorts examined (UK, nPOD, DiViD). STAT1 expression was also low in the insulin deficient islets of type 1 diabetes donors (Fig.6*a* – middle panel). However, STAT1 levels were markedly elevated in ICIs which hyperexpressed HLA-ABC (Fig.6*a* – lower panel). STAT1 expression was highest in beta-cells and appeared to be localised within both the cytoplasm and the nucleus (Fig.6*a*). The fluorescence intensity for immunolabelling of STAT1 and HLA-ABC was measured across a minimum of 7 ICIs in 7 different cases. This revealed a striking positive correlation between STAT1 and HLA-ABC expression (Fig.6*b*, overall Spearman's r = 0.5454, P<0.0001).

Analysis of the expression of STAT1 at the mRNA level in laser-captured islets confirmed the data obtained at the protein level. Moreover, in common with HLA-ABC (Supplementary Fig.*4*) the extent of this increase declined with disease duration (Fig.6*c*).

Consistent with this, the most pronounced rise was seen in islets harvested by laser capture microdissection from the six DiViD cases (Fig. 6*d*, $P = 0.0263$ and Supplementary Fig.7) who were studied very close to disease onset.

DISCUSSION

An elevation in the expression of HLA-I antigens in the islet cells of patients with recent onset type 1 diabetes was first reported ~30 years ago [15, 17]. However, neither the significance of this response for disease etiology nor the mechanism by which it is achieved has been revealed. Moreover, the concept of islet cell HLA-I hyperexpression in type 1 diabetes has been challenged recently; largely on the basis that immunostaining protocols are subject to artefact [18]. Here, we counter this by providing firm evidence that the phenomenon occurs in each of the various cohorts of patients examined, using multiple different HLA-ABC antisera and testing both paraffin and frozen sections. In considering the differing conclusions reached by ourselves and Skog et al [18] we note that the respective IHC data are similar but that those authors did not find an elevation at the RNA level in isolated islets of T1D patients vs controls. They acknowledge, however, that this is not definitive since down-regulation of HLA class I might occur as a consequence of islet isolation. The studies also differ in that Skog et al. [18] studied RNA expression in laser captured islets from only two patients with T1D whereas we examined islets from many more patients. Therefore, we conclude that hyperexpression of HLA-I is a characteristic feature of the islets in human type 1 diabetes, usually linked to remaining insulin, and that it is not an artefact unique to any particular geographical region, mode of tissue preservation or mechanism of pancreas retrieval. These conclusions are supported by evidence of concurrent induction of β2M production with HLA-I and were confirmed at both the protein level (for all three cohorts) and in RNA studies for the nPOD and DiViD collections, where frozen material was available. The increase at the RNA level was most marked in the group of DiViD patients who were all studied soon after disease onset.

We also found that the increased transcription of HLA-I isoforms was not restricted solely to the commonly studied HLA-ABC isoforms but discovered that another, atypical, isoform (HLA-F) was also enhanced. This was especially pronounced in the DiViD cohort and

was confirmed at the protein level. Of interest, expression of HLA-F was localised to the cell surface rather than intracellularly [32]. It has been demonstrated that HLA-F can interact with open conformation HLA-I heavy chains (without bound peptide), facilitating migration of the complex to the cell surface [33, 34].

Extending these novel data, we have also discovered that islet cell hyperexpression of HLA-I can persist, at the protein level, beyond the initial phases of the disease since it was seen in patients who had been diagnosed with type 1 diabetes for 11 years prior to death (although it was lost beyond this time). Thus, while there was a tendency towards normalized HLA-I expression over time in the disease course; residual beta cells with elevated HLA-I expression could still be found in some patients long after disease onset. It follows, therefore, that the surviving beta cells in these patients had apparently evaded elimination despite displaying an altered HLA-I phenotype; possibly over many years. The reasons for this are unclear and will require further study. It is also possible that the elevated expression of HLA-I occurs at different times in the disease course and that the islets we examined were at various stages of this progression. However, earlier studies do not support this since the proportion of ICIs displaying HLA-I hyperexpression in children with recent-onset disease was extremely high, suggesting that essentially all such islets hyperexpress HLA-I at diagnosis [15]. We also conclude that beta-cells are necessary to initiate and sustain the response, since HLA-I hyperexpression was not present in islets devoid of beta-cells in the plane of the section. This may reflect the egress of immune cells from insulin-deficient islets [11] but it is also possible that elaboration of a diffusible molecule, such as one of the interferons, by beta-cells may drive islet HLA-I hyperexpression in surrounding cells [35, 36] since HLA class I hyperexpression does not correlate directly with insulitis.

Elevated expression of HLA-I has been observed in mouse models of type 1 diabetes, where IFNgamma release from infiltrating immune cells has been shown to be the driver [37,

38]. As noted above, however, we emphasise that, contrary to the situation in mice, hyperexpression of HLA-I can occur in human islets without evidence of insulitis [15, 22, 39, 40]. Of course, this does not exclude the likelihood that, in inflamed islets, the response might be enhanced or sustained by cytokines produced by immune cells.

An important additional new finding in the present work is the striking correlation between the levels of STAT1 measured in the beta-cells of patients with type 1 diabetes and the hyperexpression of HLA class I. This was confirmed in multiple individual islets across a range of different cases and was demonstrated at the protein and RNA levels. STAT1 is a critical protein involved in mediating antiviral responses to interferons and its early upregulation in the progression of type 1 diabetes would be expected to place the beta-cells in a heightened state of responsiveness to these cytokines [41-43].

Although STAT1 was elevated in beta-cells soon after disease diagnosis, its expression declined with disease duration, thereby correlating with a similar decline in HLA class I, as discussed above. On this basis, it seems possible that the two may be regulated coordinately or that increased production of HLA-I in beta-cells occurs as a consequence of enhanced STAT1 expression. In support of this (and in accord with others [18]) we could find no significant increase in levels of the putative HLA-I transcriptional regulator, NLRC5, in islets hyperexpressing HLA class I. Thus, an alternative transcriptional regulator must exist in beta cells and this could be STAT1. Importantly, however, cytosolic STAT1 expression was not increased in the non-β islet endocrine cells in type 1 diabetes, despite these having elevated HLA class I. This implies that a separate mechanism may control HLA expression in these cells, although it is also possible that a modest level of STAT1 activation might occur in the absence of a dramatically altered cytosolic protein level and, in support of this, we did detect nuclear STAT1 in some non-beta cells (Figure 6a).

Taken together, our observations provide solid evidence that islet cell HLA-I hyperexpression is a genuine pathological feature in type 1 diabetes and this raises important questions about the role of this phenomenon in disease progression in humans. One hypothesis, which is consistent with our data, conceives that enhanced expression of HLA-I antigens is critical for early disease progression, promoting the effective engagement of influent CD8+ cytotoxic T-cells specific to defined islet antigens. Finally, our findings also emphasize the complicity of beta cells in their own demise in type 1 diabetes.

AUTHOR CONTRIBUTIONS. S.J.R and N.G.M. designed the study, performed data analysis and interpretation, drafted, revised and approved the manuscript.T.R-C., I.G., M.Z, M.A.R., P.L. performed data collection, analysis, and interpretation, revised and approved the manuscript. L.K, and K.D-J. collected patient material, revised and approved the manuscript; I.G. and M.C. designed the Affymetrix array component of the study, revised and approved the manuscript. J.K. provided statistical expertise and revised and approved the manuscript. M.V.H, A.P., M.A. provided critical analysis of the results, revised and approved the manuscript.

S.J.R and N.G.M are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Duality of Interest. The authors declare that there is no duality of interest associated with this manuscript.

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FIGURE LEGENDS

Figure 1 - Immunocytochemical analysis of the expression HLA-ABC in pancreas tissue. (*a*) Pancreas sections from two recent-onset DiViD type 1 diabetes cases showing insulin (upper panels) and HLA-ABC immunostaining (lower panels) on serial sections. ICIs are indicated with red asterisks. (*b*) Immunofluorescence analysis of HLA-ABC expression in frozen pancreas from an nPOD recent-onset type 1 diabetes case. Hyperexpression of HLA-ABC (red) predominantly occurs only in ICIs (green; inset).

Figure 2 - Correlation between HLA-ABC, β2-microglobulin (β2M) and insulin expression in controls and those with type 1 diabetes. (*a*) Analysis of HLA-ABC (green) β2M (red) and insulin (light blue) in an ICI from a control individual (upper panels), a type 1 diabetes patient (middle panels) and an insulin-deficient (IDI) from the same patient (lower panels). Scale bar 25μm. (*b*) The mean fluorescent intensity (MFI) of islet HLA-ABC expression was measured in 5-14 islets among non-diabetic control cases (n=4) and in the ICI & IDIs of 8 type 1 diabetes cases (5 DiViD; 3 UK; 1 nPOD). * $P<0.001$. (*c*) The MFI of islet B2M expression was measured in 15 ICIs from each of 3 type 1 diabetes patients and 15 IDIs from the same cases (2 UK; 1 nPOD). This was compared with the expression in 20 islets from each of 4 non-diabetic control subjects (ND; 3UK; 1 nPOD). *$P<0.001$.

Figure 3 - Heat map illustrating the relative expression of HLA-A, B, C and β2M genes in control individuals and those with type 1 diabetes. Expression of each probe-set is displayed separately in islets of (a) 7 nPOD non-diabetic controls age-matched to 5 DiViD patients or (b) 8 nPOD non-diabetic controls and 14 nPOD type 1 diabetes donors. Expression values are shown in arbitrary units and the heat map illustrates relative expression ranging from low (green) to high (red). In (b), a comparison with the level of expression scored after

immunohistochemical analysis of islets present in nearby pancreatic blocks from the same patients is provided (hyperexpression – black; elevated expression – blue; normal expression - grey) together with an indication of the extent of insulin immunopositivity.

Figure 4 - Expression of HLA-F in control and type 1 diabetes cases. (*a*) Heat map illustrating the relative expression of the HLA-F probe sets in 7 nPOD non-diabetic controls age-matched to 5 DiViD patients. (*b*) Representative immunostaining of islets from an individual without diabetes (left panel) and a patient with type 1 diabetes (right panel) with anti-HLA-F. (*c*) Immunofluorescence staining of HLA-F (green) insulin (light blue) and glucagon (red) in an ICI (white arrow) and an IDI (orange arrow) of a DiViD type 1 diabetes patient (lower panel) and an islet from an nPOD control donor (upper panel). Scale bar 25μm.

Figure 5 - Expression of NLRC5 in the islets of control individuals and those with type 1 diabetes. (*a*) Representative islets from a subject without diabetes (upper panel) and an individual with type 1 diabetes (lower panel) are shown (NLRC5 (green), HLA-ABC (red), insulin (light blue) and DAPI (dark blue)). Scale bar 25μm. (*b*) MFI values for NLRC5 expression were quantified after immunostaining in 5 islets/section from 4 control and 4 type 1 diabetes (3 from the UK; 1 from nPOD) samples (P=0.0704). (*c*) Expression of NLRC5 was compared in RNA isolated from islets of subjects without diabetes and those with type 1 diabetes (P=0.4504).

Figure 6 - Expression of STAT1 and HLA-ABC in islets from control individuals and those with type 1 diabetes. (*a*) Representative islets from a control individual (upper panel) and from a type 1 diabetes patient (middle and lower panels) were immunostained for STAT1 (green), HLA-ABC (red), insulin (light blue) and DAPI (dark blue). The localisation of STAT1 is

shown in beta cells (white arrows) and non-beta cells (orange). Scale bar 25μm. (*b***)** MFI values for STAT1 and HLA-ABC expression were quantified and correlated from a minimum of 7 ICIs in 7 patients with type 1 diabetes among the DiViD and UK (E560) cohorts (Spearman's rank coefficient = 0.5454, $P<0.0001$). (*c*) Correlation between the expression of STAT1 mRNA and disease duration in nPOD type 1 diabetes patients with residual ICIs ($P<0.05$). (*d*) Analysis of STAT1 expression in RNA isolated from islets from DiViD cases and age matched control donors (nPOD) ($P=0.0263$).

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