Insulinitis and characterisation of infiltrating T cells in surgical pancreatic tail resections from patients at onset of type 1 diabetes

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Abstract

Aims/hypothesis It is thought that T cells play a major role in the immune-mediated destruction of beta cells in type 1 diabetes, causing inflammation of the islets of Langerhans (insulinitis). The significance of insulinitis at the onset of type 1 diabetes is debated, and the role of the T cells poorly understood.

Methods In the Diabetes Virus Detection (DiViD) study, pancreatic tissue from six living patients with recent-onset type 1 diabetes was collected. The insulinitis was characterised quantitatively by counting CD3⁺ T cells, and qualitatively by transcriptome analysis targeting 84 T and B lymphocyte genes of laser-captured microdissected islets. The findings were compared with gene expression in T cells collected from kidney biopsies from allografts with ongoing cellular rejection. Cytokine and chemokine release from isolated islets was characterised and compared with that from islets from non-diabetic organ donors.

Results All six patients fulfilled the criteria for insulinitis (5–58% of the insulin-containing islets in the six patients had ≥ 15 T cells/islet). Of all the islets, 36% contained insulin, with several resembling completely normal islets. The majority (61–83%) of T cells were found as peri-insulitis rather than within the islet parenchyma. The expression pattern of T cell genes was found to be markedly different in islets compared with the rejected kidneys. The islet-infiltrating T cells showed only background levels of cytokine/chemokine release in vitro.

Conclusions/interpretation Insulinitis and a significant reserve reservoir for insulin production were present in all six cases of recent-onset type 1 diabetes. Furthermore, the expression patterns and levels of cytokines argue for a different role of the T cells in type 1 diabetes when compared with allograft rejection.

Keywords Gene expression · Inflammation · Insulin · Insulinitis · Pancreas · T cells · Type 1 diabetes

Lars Krogvold and Anna Wiberg contributed equally to the study and are listed in alphabetical order.

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Abbreviations

DiViD	Diabetes Virus Detection (study)
GRO	CXC chemokines growth-regulated oncogene
ICI	Insulin-containing islet
IDI	Insulin-deficient islet
IEQ	Standard islet equivalent
IP-10	Interferon γ inducible protein 10
LCM	Laser-capture microdissection
MCP-1	Monocyte chemotactic protein
RPKM	Reads per kilobase per million mapped reads
TCR	T cell receptor

Introduction

Type 1 diabetes is a disease characterised by a progressive loss of insulin-producing beta cells. A model for the natural history of type 1 diabetes, originally published by Eisenbarth in 1986 [1], suggests that the disease is triggered by one or several environmental factors in genetically susceptible individuals. The model has been modified and expanded [2]. The degree of beta cell destruction required to develop type 1 diabetes has been shown to depend on individual factors, such as age, BMI and physical activity [3]; in adults it has been predicted that as many as 40–50% of beta cells remain at the time of diagnosis [4].

Histologically, a discrete inflammatory infiltration of the islets of Langerhans is found in about 68% of children and 29% of young adults when examined within 1 month of diagnosis [5]. This lesion, first described in a diabetic child who died from ketoacidosis [6], was later named insulinitis [7]. Insulinitis, together with the presence of circulating autoantibodies directed against islet cell antigens, has been regarded as evidence for an immune-mediated aetiology of type 1 diabetes [1]. The infiltrating cells may be found in the islet–exocrine interface, when it is called peri-insulinitis, or be present within the islet parenchyma, in which case it is called intra-insulinitis [8]. The lesions mainly affect islets containing insulin-producing cells and, according to a recent consensus report, should be present in a minimum of three islets, with a threshold level of ≥ 15 CD45⁺ cells/islet to be classified as insulinitis [8]. Surprisingly, since 1902 only a total of 48 out of 247 examined cases of type 1 diabetic patients with insulinitis (according to the previous definition of insulinitis, demanding ≥ 5 T cells/islet) have been described in the literature [5].

The objective of the Diabetes Virus Detection (DiViD) study was to collect pancreatic tissue from clinically well-characterised patients with type 1 diabetes soon after the diagnosis. The sampling procedures and clinical data, including autoantibody titres and HLA haplotypes, have been reported previously [9]. Here, we report on the degree of T cell inflammation in endocrine and exocrine tissue and characterise the islet-infiltrating cells using laser-capture microdissection (LCM) and transcriptome analysis targeting 84 T and B

lymphocyte genes. The findings were compared with T cells collected from biopsies of kidney allografts with ongoing cellular rejection (Banff 1A and 1B). Furthermore, cytokine and chemokine release from isolated islets is characterised and compared with that from islets of non-diabetic organ donors.

Methods

A total of six patients, aged 24–35 (median 28) years were recruited to the study. Pancreatic biopsies, consisting of approximately 3 cm of the tail of each pancreas, were laparoscopically taken 3–9 weeks after diagnosis of type 1 diabetes (median 5 weeks). The surgery was performed under general anaesthesia; it involved a standard spleen-preserving procedure with dissection of the pancreatic tail and the splenic vein before division of the tail from the pancreatic body with an endoscopic GIA-stapler (Medtronic, Minneapolis, MN, USA). The clinical details of the patients have been reported previously [9, 10]. Pancreatic tissue from three non-diabetic organ donors of normal weight, matched for age (20, 22 and 25 years), and kidney graft biopsies from two patients with ongoing cell-mediated alloreactivity were used as controls for the gene-expression analysis. The kidney transplant rejections were classified as Banff 1A (recipient HLA: A1,A2 B8,B60, DRB1*03,DRB1*11, DQB1*02,DQB1*03; donor HLA: A2,A2 B3,B60, DRB1*03,DRB1*11, DQB1*02, DQB1*03) and 1B (recipient HLA: A1,A2 B18,B39 DRB1*01,DRB1*08, DQB1*05,DQB1*04; donor HLA: A2, A32, B15,B44, DRB1*04,DRB1*15, DQB1*03,DQB1*06) respectively, and occurred after immunosuppression with ciclosporin A and prednisolone was switched to tacrolimus, mycophenolate mofetil and prednisolone (patient with Banff 1A), and tacrolimus and prednisolone (patient with Banff 1B). Islets isolated from six donors (mean age 58 years [range 54–61 years], BMI 26.6 kg/m² [range 24.6–31.1 kg/m²]) were used as controls for the analysis of cytokines and chemokines. In order to run the case and control islets in parallel, islets from unmatched donors that were available for research at the time were used. Collection of pancreatic tissue in the DiViD study was approved by the Norwegian Governments Regional Ethics Committee. Written informed consent was obtained from all individuals with type 1 diabetes after they had received oral and written information from the diabetologist and the surgeon separately. Ethics permission for using kidney biopsies for research was approved by the Regional Ethics Committee in Uppsala (Dnr 2011/349/2). Consent for using isolated islets and pancreatic tissue for research was obtained verbally from the deceased person's next of kin by the attending physician and was documented in the medical records of the deceased person in accordance with Swedish law and as approved by the Regional Ethics Committee (Dnr 2009/043 and 2009/371).

The pancreatic biopsies from type 1 diabetic patients were processed as described previously [10]. Sections, 4 μ m, from

two different paraffin blocks for each of the six patients were processed and labelled using a standard immunoperoxidase technique. Double immunostainings were performed on three consecutive sections from each block: anti-CD3 (rabbit antibody, dilution 1:150, Neomarkers, Fremont, CA, USA)/insulin (mouse antibody, clone 2D11/H5, dilution 1:100, Novocastra, Newcastle upon Tyne, UK), anti-CD3/glucagon (rabbit antibody, clone EP74, dilution 1:400, Epitomics, Burlingame, CA, USA) and insulin/glucagon. This gave a total of 36 sections (6 cases $\times$ 2 blocks $\times$ 3 stainings). Slides were counterstained with haematoxylin and analysed by light microscopy. The total number of islets was registered by counting glucagon-containing islets. Only islets containing at least five to ten endocrine cells were registered, while single glucagon- or insulin-positive cells scattered in the exocrine area were not. Insulin-containing islets (ICIs) were counted and their area estimated using an ocular grid consisting of 100 squares of 0.0016 mm² at a magnification of $\times$ 200. For each ICI, the numbers of CD3⁺ cells within the islet area (intra-insulitis) and in the endocrine–exocrine interface in close contact with the islet boundary (peri-insulitis) were counted and registered. Regarding the latter, cells in direct contact with an islet or in contact with another CD3⁺ cell in direct contact with an islet were registered. The new definition for insulitis was used, meaning that the islet should contain or be closely surrounded by $\geq$ 15 leucocytes (CD45⁺ cells) to be considered insulitic, but with the modification that only T cells (CD3⁺) were counted. By combining the three double immunostainings described, the percentage of four categories of islets [11], indicating the progress of disease, was calculated: A, ICIs without insulitis; B, ICIs with insulitis; C, insulin-deficient islets (IDIs) with insulitis; and D, IDIs without insulitis.

To normalise the number of CD3⁺ cells per islet to a standard islet equivalent (IEQ), the islet area was divided by the area of an average cross-section of an IEQ. In the field of islet isolation and in the consensus statement on insulitis [8] an IEQ is defined as an islet of 150 μ m diameter, giving an average standard cross-sectional area of 0.010936 mm² ($D_{\text{cross-section}} = [\pi/4] \times D_{\text{sphere}}$, where D=diameter). As the consensus definition of insulitis does not take into account the huge variation in islet size, leading to underestimation of inflammation in small islets and vice versa, we propose a normalisation to allow accurate comparisons between different studies. To estimate insulin area and CD3⁺ cell density in exocrine tissue, 15 grids constituting a total area of 2.4 mm² were randomly placed in each section and analysed by registering the content in each of the (121 $\times$ 15) 1,815 intersections.

Laser-capture microdissection Frozen pancreatic samples from the individuals with type 1 diabetes, the three non-diabetic organ donors and needle biopsies from rejected kidneys were prepared for LCM, essentially as described in Marselli et al and Skog et al [12, 13]. Sections mounted on

Superfrost Plus glass slides (Menzel-Gläser, Braunschweig, Germany) and stained for CD3 (rabbit anti-human CD3, Dako, Glostrup, Denmark), as described previously [14], were used to identify and localise infiltrating T cells in islets and in the rejected kidneys. Sections mounted on Arcturus PEN Membrane Glass Slides (Life Technologies, Carlsbad, CA, USA), and consecutive to the sections stained for CD3, were used for LCM. Only islets with insulitis ($\geq$ 15 CD3⁺ cells) were microdissected and pooled. Islets without insulitis from the three non-diabetic organ donors and renal cortex from rejected kidneys with infiltrating T-cells were used as control. An Arcturus^{XT} instrument with HS LCM Caps (Life Technologies) was used for LCM.

RNA isolation and expression analysis from microdissected tissue Total RNA was isolated from the microdissected tissue using RNeasy Micro Kit (Qiagen, Sollentuna, Sweden). Synthesis and pre-amplification of cDNA were performed with the RT2 PreAMP cDNA Synthesis Kit (Qiagen) using a pathway-specific primer mix (Human T-Cell & B-Cell Activation, PBH-053Z, Qiagen) according to the manufacturer's instructions. Expression analysis was performed using the RT2 Profiler PCR array for human T cell and B cell activation (PAHS-053Z) on a StepOne Plus instrument (Life Technologies). Beta actin (*ACTB*) and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) were chosen for normalisation as they are commonly used reference genes in human islets and display little variation between samples. To compare expression levels in immune cells from infiltrated islets and infiltrated kidney, *PTPRC* (*CD45*) was used for normalisation. All samples passed the quality control of PCR array reproducibility, reverse transcription control and genomic DNA contamination provided by the PCR array analysis software available at www.SABiosciences.com/pcrarraydataanalysis.php. The mRNA expression for each gene was normalised to the expression the reference genes using the ΔC_t method and the data are presented as relative expression representing $2^{-\Delta C_t}$.

Islet isolation Islets from the individuals with type 1 diabetes and non-diabetic controls were isolated as described previously [13, 15]. Islets from the controls were isolated from the entire pancreas with the aim of clinical transplantation and were available for research only because the islet yield was too low.

Cytokine/chemokine analysis Islets (100 handpicked) from the individuals with type 1 diabetes and six non-diabetic controls were analysed 1 day after isolation for cytokine/chemokine content by Luminex xMAP (Millipore, Billerica, MA, USA). Each sample was lysed in 1 μ l cell lysis factor QG and 89 μ l lysis buffer (Bio-Plex Pro, BioRad, Hercules, CA, USA), containing $\times$ 1 protease inhibitor (SIGMAFAST, Sigma-Aldrich, St Louis, MO, USA) and sonicated for 20 s.

The lysate was centrifuged at 15,000g for 10 min at 4°C, the supernatant fractions filtered through Pierce Detergent-Removal spin columns (Thermo Scientific, Rockford, IL, USA) and the protein concentrations were determined by Bradford assay (Thermo Scientific). From each sample, 5 µg total protein was used. Cytokines and chemokines were analysed with the HCYTMAG-60K-PX41 Milliplex kit (Millipore). The procedure described in the manual (HCYTOMAG-60K Rev.07-JUN-2012) was followed except for the volume of beads, detection antibodies and streptavidin–phycoerythrin (12.5 µl instead of 25 µl as described). The analysis was performed on a Magpix platform (Luminex, Austin, TX, USA).

Whole transcriptome sequencing Samples of RNA were extracted from isolated islets from individuals with type 1 diabetes and three non-diabetic controls and whole transcriptome sequencing was performed and analysed as described previously [13]. Normalised reads per kilobase per million mapped reads (RPKM) for the genes encoding the cytokines and chemokines included in the Luminex analysis are presented in this study and the full data set (reads) has been openly available in the BILS DOI repository (<http://doi.bils.se/>) under doi: 10.17044/BILS/g000002.

Statistical analysis Differences between group pairs were analysed by Wilcoxon matched-pairs signed rank test, or between groups by the Mann–Whitney test, using GraphPad Prism v6 (La Jolla, CA, USA). Findings were considered statistically significant at $p < 0.05$.

Results

Insulin content Figure 1 shows the distribution of islets in the different categories in the 12 sections examined. On average, 36% of all islets investigated ($n=1,596$) contained insulin, either with or without signs of insulinitis. Of these ICIs, 74.2% did not seem to be affected by the disease, resembling completely normal islets. In some areas of the pancreatic biopsies the insulin-positive area was completely normal. Large variations were observed in the percentage of ICI, both between individuals (18–66%) and between sections from different formalin-fixed blocks from the same individual (0–94% in case 6). The insulin area comprised, on average, 0.58% of the pancreatic tissue (0.44–1.2%).

Insulinitis and infiltrating T cells All six patients fulfilled the criteria for insulinitis [8]. The variation between the cases was large. Only 6 of 130 (5%) ICIs were affected in case 6 (≥ 15 CD3⁺ cells) vs 42 of 73 (58%) in case 1 (Table 1). There was also large variation in the mean total number of CD3⁺ cells per ICI (peri- and intra-insulinitis), ranging from 2.2 in case 6 to

Fig. 1 Histological analysis of all islets in two pancreatic sections (a and b) from each of the six individuals with recent-onset type 1 diabetes. The islets were categorised as ICI or IDI with or without insulinitis. Insulinitis was defined as ≥ 15 CD3⁺ cells in or in close association with the islet. Black, ICI without insulinitis; light grey, ICI with insulinitis; dark grey, IDI with insulinitis; white, IDI without insulinitis

31.6 in case 1 (Table 1). Although normalisation of the number of CD3⁺ cells to the size of an IEQ (150 µm diameter) changed the number of CD3⁺ cells in individual islets by altering the denominator in the fraction, the mean number of CD3⁺ cells per islet and the fraction of insulitic islets per case remained basically the same (Table 1).

In total, 11% of all islets were infiltrated with ≥ 15 CD3⁺ cells. Most of the inflamed islets, 82%, were ICIs. Again, large inter-individual (4–26%) and intra-individual (2–20% in case 5) variations were observed (Fig. 1). Of the ICIs, in total 25.8% showed insulinitis. In contrast, insulinitis affected only 2.9% of IDIs.

A significantly higher density of CD3⁺ cells was found in ICIs (328 ± 232 per mm²) than in exocrine tissue (51 ± 27 per mm²) ($p < 0.05$, Fig. 2). They were found more frequently in the periphery of the islet (peri-insulinitis) than within the islet area (intra-insulinitis) (Table 1 and Figs 3 and 4). This difference was significant ($p < 0.001$) in all cases, but the fraction of total islet-associated CD3⁺ cells located inside the islets varied from only 17% in case 3 to 39% in case 4 (Table 1). As the islets of Langerhans constitute only 1–2% of the pancreas, in absolute numbers most of the CD3⁺ cells were located in the exocrine tissue.

Expression analysis of infiltrating cells Of the 84 T and B cell genes studied, all except *TLR1*, *CXCR3*, *TNFSF14*, *CD5* and *TLR9* were expressed ($C_t < 35$) in the infiltrated islets from at least two of the six patients. Islets from non-diabetic control donors had no or low expression of all genes analysed, except *CD47*, *DPP4*, *CD81*, *CD276*, *TGFB1*, *MAP3K7* and *NCK1*. Since these genes were expressed in control islets that did not contain lymphocytes, they were excluded from further analysis (electronic supplementary material [ESM] Fig. 1). When compared with the expression pattern in islets from those with cell-mediated kidney allograft rejection, the immune

Table 1 Peri- and intra-islet T cells in ICIs from the six individuals with recent-onset type 1 diabetes

Individual	ICI (n)	Area of ICIs, mean±SD, mm ² (median)	Number of intra-islet CD3 ⁺ , mean±SD (median)		Number of peri-islet CD3 ⁺ , mean±SD (median)		Intra CD3 ⁺ , % total CD3 ⁺ (intra+peri)	ICI with insulinitis, n (%)	
			Per islet	Per IEQ ^a	Per islet	Per IEQ ^a		≥15 CD3 ⁺ per islet	≥15 CD3 ⁺ per IEQ ^a
1	73	0.016±0.017 (0.0080)	8.16±15.48 (2)	3.75±4.34 (2.3)	23.42±27.78 (16)	29.63±115 (11.8)	26	42 (58)	40 (55)
2	78	0.014±0.012 (0.010)	1.37±2.08 (0)	1.16±2.10 (0)	4.92±5.86 (3)	7.70±17.1 (2.9)	22	9 (12)	12 (15)
3	206	0.012±0.011 (0.0096)	3.92±8.33 (1)	3.24±5.14 (1.17)	18.70±21.55 (12)	22.20±26.07 (14.3)	17	107 (52)	114 (55)
4	55	0.016±0.014 (0.011)	7.36±11.53 (3)	5.05±6.08 (2.9)	11.35±14.11 (7)	11.24±15.87 (6.08)	39	18 (33)	21 (38)
5	73	0.016±0.015 (0.011)	3.71±5.65 (2)	2.71±2.98 (1.95)	6.56±6.04 (5)	8.45±13.35 (4.88)	36	17 (23)	18 (25)
6	130	0.0093±0.010 (0.0064)	0.56±2.11 (0)	0.46±1.94 (0)	1.65±3.92 (0)	2.30±5.76 (0)	25	6 (5)	6 (5)

^a Each islet is normalised to the area of an IEQ, defined as an islet with a diameter of 150 µm or an average cross-sectional area of 0.010936 mm² CD3⁺, CD3⁺ cells; intra, intra-islet; peri, peri-islet

Fig. 2 CD3⁺ cells per ICI (intra-islet) and exocrine area (excluding peri-islet CD3) in sections from individuals with recent-onset type 1 diabetes. Black circles, case 1; white circles, case 2; black squares, case 3; white squares, case 4; black triangles, case 5; and white triangles, case 6. * $p < 0.05$ calculated from the mean of two sections per patient

infiltrates in type 1 diabetic islets revealed a markedly different expression pattern (Fig. 5).

Cytokines and chemokines in isolated islets Luminex analysis of 41 cytokines and chemokines in isolated handpicked islets from the six type 1 diabetes patients showed signs of moderate inflammation, i.e. moderate levels of monocyte chemoattractant protein 1 (MCP-1), IL-8, interferon γ inducible protein 10 (IP-10) and CXC chemokines growth-regulated oncogene (GRO), that did not differ from isolated islets from brain-dead organ donors (Fig. 6a, b). Of the 41 analytes, 25 were above the detection limit in islets from at least one patient.

Fig. 3 Number of peri-islet (grey) and intra-islet (white) CD3⁺ cells per ICI in two pancreatic sections from each of the recent-onset type 1 diabetes cases. The total number of ICIs, the percentage ICI of all islets and the number and percentage of ICIs with insulinitis are shown. Boxes, interquartile range; horizontal line, median; +, mean; and whiskers, maximum/minimum

Fig. 4 Pancreatic tissue from individuals with type 1 diabetes stained for insulin (brown) and CD3 (red). (a) An islet with peri-insulinitis in which the majority of CD3⁺ T cells are found in the islet-exocrine interface. (b) An apparently healthy islet (bottom) located next to an islet with intense peri-insulinitis (top). (c–e) Examples of the three different double stainings of the same area are shown: (c) CD3 red, glucagon brown; (d) CD3 red, insulin brown; and (e) glucagon red, insulin brown. Scale bar, 100 μ m

Transcriptome data from the six type 1 diabetes patients and three non-diabetic controls confirmed that the expression of these 41 cytokines and chemokines was similar in the two groups (Fig. 6c, d).

Discussion

All six patients showed substantial intra- and peri-islet infiltration of CD3⁺ T cells. A majority of these islets contained insulin, whereas insulinitis was seldom observed in IDIs. The number of CD3⁺ cells per islet varied greatly between individuals with type 1 diabetes despite the patients in this study being clinically homogeneous [9]. The cause of this variation

Fig. 5 Expression of T and B cell genes in: (a) infiltrated pancreatic islets from six individuals with recent-onset type 1 diabetes; and (b) kidney biopsies from two cases with ongoing cell-mediated alloreactivity. (c)

Expression ratio for islets/kidney (on a log₂ scale). The expression of each gene is normalised to the expression of *PTPRC* (*CD45*)

is likely to be the previously described lobular pattern of disease progression with large variation between different pancreatic regions [16].

Notably, many islets contained insulin and seemed unaffected by the disease. Also, in some areas of the pancreatic biopsies the insulin-positive area was completely normal. This corresponds well with previous findings of focal and temporal distribution of the injurious inflammatory processes in type 1 diabetes [11]. Recent publications have shown that most patients with a long duration of type 1 diabetes continue to secrete low levels of endogenous insulin [17, 18], and our findings demonstrate that large numbers of insulin-positive, albeit perhaps dysfunctional, cells remain in individuals at the onset of type 1 diabetes. If disease progression could be halted, this beta cell volume would be of significant clinical importance.

The biopsies were collected shortly after diagnosis and when near-normoglycaemia had been re-established with exogenous insulin administration, precluding a potential

influence of superimposed metabolic phenomena such as ongoing ketoacidosis or postmortem alterations on local conditions in the pancreas. The size of the resected specimen from the pancreatic tail allowed us to investigate several formalin-fixed blocks from each patient, which is important as there is great variation in the presence of insulinitis and other disease-related processes across the pancreas [16]. One major limitation of our study is the small number of cases examined compared with the larger number of autopsy cases that have been previously published [19]. Nevertheless, this study is, so far, the largest on well-preserved pancreatic tissue from individuals examined at onset of type 1 diabetes, allowing molecular characterisation that cannot be performed elsewhere. Also, our data may supplement the relatively limited cohort of recent-onset type 1 diabetes cases described previously [5, 16, 19].

The prevalence of insulinitis in the age group 15–39 years with duration of disease less than 1 month has been reported to be only 29% (5 of 17 cases) [19]. The higher prevalence of

Fig. 6 Expression of cytokines and chemokines in isolated islets; proteins analysed by Luminex (**a**, **b**) and mRNA analysed by RNAseq (**c**, **d**). Data were collected from six cases with recent-onset type 1 diabetes (**a**, **c**) and from non-diabetic controls (**b**, **d**). See ESM Table 1 for full names of proteins

insulinitis in children previously reported has led to speculation on whether the aetiology of type 1 diabetes is more heterogeneous in adults [20]. Our results indicate that the difference between children and adults may be overestimated, and that insulinitis might be as frequent in at least young adults as in children [21]. This corresponds with the results in a thoroughly conducted study recently published that found insulinitis in 11 out of 11 organ donors with type 1 diabetes, of whom five had disease duration of between 0.25 and 1.5 years [22].

A striking finding in this study is that the majority of islet-associated T cells were located in the exocrine–endocrine interface, i.e. outside the actual islet parenchyma. The pathogenic role of these T cells is unclear, but as they are not in direct contact with the endocrine cells they are unlikely to bind to islet auto-antigens presented on HLA molecules on the beta cells. Previous studies using tetramer staining to detect T cells with reactivity to distinct beta cell auto-antigens show the presence of only occasional T cells, also located in the islet periphery [23]. Notably, the frequency of beta cell-specific CD8⁺ T cells in peripheral blood is similar in those with newly diagnosed type 1 diabetes and HLA-matched healthy controls [24]. The incapacity of the CD8⁺ T cells to invade the islet and mediate efficient beta cell lysis could potentially be explained by the extraordinarily weak affinity between the T cell

receptor (TCR) and the HLA class I presenting the postulated type 1 diabetes auto-antigen [25, 26]. The TCR is abundant at such low levels of affinity and it was recently reported that they recognise more than a million different peptides presented on the appropriate HLA class I allele [27]. Drugs effective in preventing allograft rejection have been used in many intervention trials in type 1 diabetes with the assumption that the T cell-mediated destruction of beta cells resembles that of allograft rejection. Therefore, we chose to include a comparison of the T cells infiltrating the islets in type 1 diabetes with the T cells mediating kidney rejection in transplanted patients. The findings reveal both qualitative and quantitative differences in T and B cell activation in the insulinitic lesions in type 1 diabetes when compared with samples from individuals with ongoing allograft rejection (Fig. 5). The baseline cytokine/chemokine expression in islets isolated from individuals with type 1 diabetes compared with islets from non-diabetic organ donors (Fig. 6) are in agreement with previous observations [23–27], and although the number of cases is small, suggests a different role of T cells in type 1 diabetes compared with T cells observed in allograft rejection. The role and function of T cells infiltrating the islets in type 1 diabetes warrants further investigation, and also comparison with T cells in other, more well-defined, conditions.

It has recently been shown that, compared with non-diabetic controls, individuals with type 1 diabetes have an increased number of T cells in the peri-islet area [22] and the exocrine pancreas [28]. Their role in type 1 diabetes remains to be clarified: are they merely passive bystanders located in the inflamed peri-islet area? The role of these T cells needs to be urgently elucidated to obtain in-depth knowledge of the immunopathology of type 1 diabetes so that more efficient intervention therapies can be developed. Intervention trials in type 1 diabetes focusing on blocking T effector cells with drugs highly efficient at protecting an allograft have shown suboptimal protection of beta cell function: in the best situations, C-peptide production could be preserved temporarily and insulin requirements reduced, but no durable effects could be achieved [29–32].

Islets isolated from the type 1 diabetes patients expressed a number of chemokines (i.e. MCP-1, IL-8, IP-10 and GRO) involved in leucocyte recruitment, but the levels were not different from those expressed in islets isolated from brain-dead non-diabetic control donors, and were significantly lower, and with a much narrower repertoire, than demonstrated by Eizirik et al [33] in islets stimulated with IL-1 β and IFN γ (ESM Fig. 2). Whether CD3⁺ cells are present in these handpicked islets is unknown, and the isolation procedure itself can induce expression of some of these proteins. However, the low or absent expression of most of these chemokines and cytokines in islets from individuals with recent-onset type 1 diabetes described here fits well with the relatively low frequency of intra-islet T cells and lack of signatures in the transcriptome analysis of activation (Fig. 6).

In conclusion, all the individuals with newly diagnosed type 1 diabetes enrolled in the DiViD study fulfilled the criteria for insulinitis. Although it varied greatly, many islets contained insulin and several resembled completely normal islets, showing a significant reserve reservoir for insulin production. The islet-infiltrating T cells showed only background levels of cytokine/chemokine release and displayed marked differences at the messenger level when compared with allogeneic T cells.

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Contribution statement LK drafted the manuscript and was responsible for clinical coordination and the recruitment of patients and data collection, analysis and interpretation. AW was responsible for data analysis and interpretation of the results and participated in writing the manuscript. BE and TB made substantial contributions to the conception and design of the study, performed the surgery and participated in writing the manuscript. FLJ, EL, KFH and OK contributed to the study design, data analysis and interpretation and writing the manuscript. OS drafted the manuscript and participated in study design, data collection and analysis and interpretation of the results. KD-J was the principal investigator of the study, had the initial idea for the DiViD study, and participated in the study design, funding, regulatory issues, international collaboration, data collection, analysis and interpretation, and writing the manuscript. LK and KD-J 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. All authors had final approval of the version to be published.

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