SHORT COMMUNICATION

No Evidence for Presence of Mucosal-Associated Invariant T Cells in the Insulitic Lesions in Patients Recently Diagnosed with Type 1 Diabetes

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Mucosal-associated invariant T (MAIT) cells are innate T cells that recognize bacteria-infected cells and are thought to play a role in autoimmune diseases. Translocation of duodenal bacteria and viruses to the pancreas through the pancreatic duct has been hypothesized to initiate an innate inflammatory response that could contribute to the development of type 1 diabetes, a process that could involve MAIT cells. In this study, we used immunohistochemistry and quantitative PCR to search for evidence of MAIT cells in the insulitic lesions in the pancreas of human patients recently diagnosed with type 1 diabetes. Only a few scattered MAIT cells were found within the exocrine parenchyma in all pancreatic samples, but no MAIT cells were found in association to the islets. Also, only low gene expression levels of the MAIT T-cell receptor $V\alpha 7.2-J\alpha 33$ were found in the pancreas of patients with type 1 diabetes, in similar levels as that in nondiabetic organ donors used as control. The absence of MAIT cells shown in insulitic lesions in humans questions the direct cytotoxic role of these cells in β -cell destruction. (*Am J Pathol* 2018; 188: 1744–1748; <https://doi.org/10.1016/j.ajpath.2018.04.009>)

The roles of the innate and acquired immune response in the etiology of type 1 diabetes (T1D) remain unresolved. Insulitis in human T1D is discrete and affects only few islets compared with animal models of the disease, and it is predominantly located in the interface between the islet and the surrounding exocrine parenchyma.¹ It is known that CD8⁺ T cells are the most common cell type in the insulitic lesions when insulitis is present,² and a significant portion of these are of a tissue resident memory phenotype.³ An important contribution was recently presented by Rouxel et al⁴ showing an essential role for mucosal-associated invariant T (MAIT) cells in the disease process in nonobese diabetic (NOD) mice and possibly in T1D in humans. The authors highlighted the role of MAIT cells in the maintenance of gut integrity as well as cytotoxic MAIT cells directly causing loss of the insulin-producing β cells. MAIT cells are innate T cells that express a conserved T-cell receptor (TCR) that

recognizes bacterial metabolites from the synthesis of riboflavin presented by major histocompatibility complex class I–related protein MR1⁵ and are thought to play a role in autoimmune diseases such as multiple sclerosis^{6,7} and

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rheumatoid arthritis.⁸ It has been hypothesized that translocation of duodenal bacteria to the pancreas through the pancreatic duct can initiate an innate inflammatory response that contributes to the development of T1D,^{9,10} a process that could involve MAIT cells.

By using our unique access to high-quality pancreas biopsy specimens from recent-onset T1D patients [the Diabetes Virus Detection study (DiViD) cohort¹¹] and matched nondiabetic organ donors, we present data on the scarcity of MAIT cells in pancreatic tissue as well as in insulitic lesions.

Materials and Methods

Human Tissue Samples

The Government's Regional Ethics Committee in Norway and the Regional Ethics Committee in Uppsala approved this study. Pancreatic tissue from six living patients participating in the DiViD study were obtained 3 to 9 weeks after diagnosis of T1D.¹¹ Pancreatic tissue from six nondiabetic organ donors and duodenal tissue from four nondiabetic organ donors procured within the Nordic Network for Clinical Islet Transplantation were used as controls. These were age-matched to the patients participating in the DiViD study (range, 24 to 35 years). Tissue frozen in liquid nitrogen and stored at -80°C was used for immunohistochemistry and tissue immersed in RNeasy Lysis Buffer (Qiagen, Sollentuna, Sweden) and stored at -80°C was used for quantitative PCR.

Immunohistochemistry

Ten-micron-thick tissue sections were fixed in 4% paraformaldehyde (Sigma Aldrich, Darmstadt, Germany). Thereafter, sections were rinsed in Tris-buffered saline and stained with the EnVision G2 Double stain System (Dako, Glostrup, Denmark) according to the manufacturer's protocol. In brief, endogenous peroxidase and alkaline phosphatase were blocked, followed by incubation with mouse anti-human T cell receptor alpha chain segment V alpha 7.2 (TCRV α 7.2) (dilution 1:50; BioLegend, San Diego, CA) for 30 minutes at room temperature. Thereafter, sections were incubated with horseradish peroxidase-conjugated polymers carrying mouse and rabbit immunoglobulins. TCRV α 7.2 expression was visualized by diaminobenzidine chromogen. Thereafter, sections were incubated with rabbit anti-human CD3 (1:75; Dako) for 30 minutes at room temperature. Alkaline phosphatase-conjugated polymers were added and CD3 was visualized with Permanent Red Chromogen (Dako). Sections were counterstained with hematoxylin and mounted with Faramount aqueous mounting medium (Dako). To evaluate the staining, human duodenum served as positive control. Isotype control was used to assess the background.

RNA Extraction and Quantitative PCR

Approximately 10 mg of each tissue sample stored in RNeasy Lysis Buffer (Qiagen) was used for extraction of RNA. The tissue was

homogenized in buffer RLT plus (Qiagen) containing 1% β -mercaptoethanol using the Precellys soft-tissue homogenizing kit in a Minilys homogenizer (Bertin Technologies, Montigny-le Bretonneux, France). This was followed by passage through a QIAshredder spin column (Qiagen), after which the RNA was extracted with the RNeasy Plus MiniKit (Qiagen) according to the manufacturer's instructions. Two microgram of total RNA was reverse-transcribed to cDNA using the Superscript VILO kit (Fisher Scientific, Gothenburg, Sweden). After dilution in nuclease-free water, cDNA transcribed from 20 ng total RNA was used for quantitative PCR with the QuantiFast SYBR Green PCR kit (Qiagen). Primers specific for TCR V α 7.2-J α 33,⁶ CD3D, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Quantitect Primer Assay; Qiagen) were used for amplification. Melt curve analyses were used to verify the specificity of the amplified targets. Expression levels of TCR V α 7.2-J α 33 were normalized to the expression of CD3D or GAPDH with the comparative cycle quantification method ($2^{-\Delta\text{cycle quantification}}$).

Results

No MAIT Cells in Insulitic Lesions in Patients Recently Diagnosed with Type 1 Diabetes

All subjects with recent-onset T1D, but none of the nondiabetic controls, fulfilled the criteria for insulitis, and most of the T cells in subjects with T1D resided in the endocrine-exocrine interface. In the examined tissue, the median number of CD3⁺ T cells per islet was 7, and 36% of all islets fulfilled the criteria for insulitis (≥ 15 CD3⁺ T cells).¹² Antibodies directed to the V α 7.2 TCR chain^{4,13} and CD3 were used to identify MAIT cells and, as previously shown, MAIT cells were found in high numbers in the human intestine (Figure 1, A and B). In pancreatic samples from subjects with recent-onset T1D, as well as in nondiabetic controls, no TCRV α 7.2-positive cells could be detected in any islets or in the islet vicinity. Only occasional MAIT cells were found residing in the exocrine parenchyma (Figure 1, C and D).

Low Expression of the MAIT TCR in Human Pancreatic Samples

Quantitative PCR on frozen pancreatic and duodenal tissue with primers specific for TCR V α 7.2-J α 33,¹⁴ CD3D, and GAPDH confirmed the immunohistochemical data; TCR V α 7.2-J α 33 was detected at low levels in all pancreatic samples and no difference in the expression level was found between recently diagnosed T1D patients and controls (Figure 2). No difference was seen when the expression was compared with the reference gene GAPDH (Figure 2A) or with expression of the T-cell marker CD3D (Figure 2B). As expected, higher expression levels of the MAIT TCR were found in duodenal samples (cycle quantification values indicating at least 32-fold).

Figure 1 Mucosal-associated invariant T (MAIT) cells in human pancreas and duodenum. **A–D:** Expression of T-cell receptor (TCR) $V\alpha 7.2$ (diaminobenzidine, brown) and CD3 (red) in human intestine (**A** and **B**) and pancreas (**C** and **D**) of patients with recent-onset type 1 diabetes. **Arrows** indicate MAIT cells (TCRV $\alpha 7.2+$ and CD3+ cells). The **boxed areas** in **A** and **C** are shown in higher magnification in **B** and **D**, respectively. Scale bars: 100 μ m (**A** and **C**); 50 μ m (**B** and **D**).

Discussion

These results from subjects with recent-onset T1D are in sharp contrast to that in the NOD mouse model⁴ and cast doubt on the role of MAIT cells as cytotoxic cells directly causing β -cell destruction in the development of T1D in humans. In contrast, previous studies have shown an accumulation of activated MAIT cells in multiple sclerosis lesions.⁷ This discrepancy between the lesions of T1D and multiple sclerosis suggests different etiologies of the two diseases involving disparate immunologic responses, or that after T1D diagnosis the tentative process involving MAIT cells already has ended.

Access to pancreatic tissue from human subjects recently diagnosed with T1D is rare and pancreas from only six such subjects were examined in this study. Nevertheless, these six subjects, included in the DiViD cohort, represent one of the largest collections of well-preserved pancreatic tissue from recent-onset T1D subjects available worldwide. Importantly, all six subjects fulfilled the definitions of insulinitis,^{12,15} as reported previously,¹ enabling the search for MAIT cells in ongoing lesions.

MAIT cells are known to be activated in the presence of active bacterial infection. Duodenal bacteria entering the pancreas through the pancreatic duct have been proposed as a possible mechanism by which the inflammatory process in T1D could be initiated.⁹ It is possible that this translocation of bacteria to the pancreas could lead to migration of MAIT cells to the pancreas and their activation. The absence of MAIT cells shown in the insulinitic lesions, 3 to 9 weeks after the diagnosis of T1D, suggests that if bacterial translocation occurs in T1D, the infection may already have been cleared within a few weeks of diagnosis. Alternatively, exposure of bacteria to the pancreatic enzymes present in the duct kills

the bacteria, precluding their synthesis of riboflavin and presentation on MR1. In the latter case, no activation of MAIT cells would occur but inflammation could be initiated by Toll-like receptor–mediated detection of the remaining endotoxins of dying bacteria.¹⁰

In a recent study,⁴ it was shown that in children with recent-onset T1D the proportion and number of circulating MAIT cells were significantly lower than in controls, whereas no such difference was found between children with Rouxel et al⁴ established T1D and children without T1D. The authors suggested that the decreased frequency of MAIT cells in blood might have reflected their migration to inflamed tissues and/or their death by apoptosis subsequent to their activation.⁴ This notion is supported by the increased presence of MAIT cells in pancreatic islets of

Figure 2 Low expression of the mucosal-associated invariant T (MAIT) T-cell receptor (TCR) cells in human pancreatic samples. **A** and **B:** The expression of TCR $V\alpha 7.2$ - $J\alpha 33$ in human pancreatic samples from six patients recently diagnosed with type 1 diabetes (T1D) and six nondiabetic organ donors was evaluated by quantitative PCR and is shown relative to the expression of *GAPDH* (**A**) or *CD3D* (**B**) ($2^{-\Delta\Delta\text{cycle}}$ quantification).

NOD mice as well as their production of Granzyme B and interferon- γ , which are thought to participate in the destruction of the insulin-producing cells. However, contradictory results were also presented in the same article as illustrated by the exacerbation of diabetes development in mice lacking MAIT cells. Moreover, it is not surprising that Rouxel et al⁴ showed that MAIT cells from nondiabetic subjects are able to kill a human β -cell line exposed *in vitro* to 5-amino-6-D-ribitylaminouracil, an early intermediate in bacterial riboflavin synthesis, because this would be expected for any cell expressing MR1.¹⁶

Our data from subjects with recent-onset T1D provide no support for a direct role of MAIT cells in β -cell destruction, at least in adults. Notably, in obesity and type 2 diabetes (two nonautoimmune conditions but with impaired glucose metabolism), a reduced circulating number of MAIT cells but an increased production of inflammatory cytokines (interleukin-2, interleukin-17 α , interferon- γ , and tumor necrosis factor- α) also has been observed.¹⁷ These changes in absolute numbers and functional activation of MAIT cells were normalized rapidly after bypass surgery and stabilization of glucose metabolism.¹⁷ The notion that MAIT cell numbers and phenotype are influenced to a large extent by impairment of glucose metabolism is further supported by the correlation between MAIT cell numbers and their phenotype to the hemoglobin A1c levels in children with T1D.¹⁸ Thus, it is possible that the increased MAIT cell activation and reduced circulating numbers observed in children with T1D may be directly linked to hyperglycemia rather than being part of the β -cell destructive process.

In this study, using well-preserved tissue from humans recently diagnosed with T1D and with ongoing insulitis, we showed a lack of accumulation of MAIT cells in the islets of Langerhans. Although an accumulation of MAIT cells earlier in the disease process cannot be excluded, if MAIT cells were directly involved in the destruction of β cells in T1D, we would expect to show their presence in the pancreatic biopsy specimens of the DiViD cohort with a large number of remaining insulin-producing cells in islets with ongoing insulitis (ie, examined at a similar stage of disease progression as the NOD mice in the report by Rouxel et al⁴). NOD mice are frequently used as a small animal model of T1D; however, the immunopathologic findings show limited similarities with human disease and the model has low predictive value in screening for intervention therapies.¹⁹ Based on our data presented here, a direct role for MAIT cells in β -cell destruction in T1D is unlikely.

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E.K. and O.S. performed the experiments; E.K., O.S., and O.K. analyzed the data; and all authors wrote the manuscript. K.D.-J. was the Principal Investigator of the DiViD study.

Supplemental Data

Supplemental material for this article can be found at <https://doi.org/10.1016/j.ajpath.2018.04.009>.

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