Loss of IDO1 Expression From Human Pancreatic β-Cells Precedes Their Destruction During the Development of Type 1 Diabetes

Florence Anquetil,[1] Giada Mondanelli,[2] Nathaly Gonzalez,[1] Teresa Rodriguez Calvo,[1] Jose Zapardiel Gonzalo,[1] Lars Krogvold,[3,4] Knut Dahl-Jørgensen,[3] Benoit Van den Eynde,[5,6] Ciriana Orabona,[2] Ursula Grohmann,[2] and Matthias G. von Herrath[1,7]

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PATHOPHYSIOLOGY

Indoleamine 2,3 dioxygenase-1 (IDO1) is a powerful immunoregulatory enzyme that is deficient in patients with type 1 diabetes (T1D). In this study, we present the first systematic evaluation of IDO1 expression and localization in human pancreatic tissue. Although IDO1 was constitutively expressed in β-cells from donors without diabetes, less IDO1 was expressed in insulin-containing islets from double autoantibody-positive donors and patients with recent-onset T1D, although it was virtually absent in insulin-deficient islets from donors with T1D. Scatter plot analysis suggested that IDO1 decay occurred in individuals with multiple autoantibodies, prior to β-cell demise. IDO1 impairment might therefore contribute to β-cell demise and could potentially emerge as a promising therapeutic target.

Type 1 diabetes (T1D) results from a breakdown of immune tolerance that leads to the selective destruction of β-cells in the pancreas, but the circumstances driving this dysfunction remain unclear. Indoleamine 2,3-dioxygenase-1 (IDO1) is a metabolic enzyme that catalyzes the first rate-limiting step of tryptophan catabolism, ultimately leading to the production of immunoregulatory molecules known as kynurenines. Its catalytic and noncatalytic effects are involved in the regulation of immunity (1), including the induction of tolerogenic dendritic cells (2) and regulatory T cells (3). However, IDO appears to be involved in selective immune regulation mechanisms, as IDO knockout mice do not develop a fulminant autoimmune phenotype (4). Interestingly, the dysregulation of the tryptophan metabolic pathway was suggested to contribute to the development of T1D in NOD mice (5–7).

A recent report from Orabona et al. (8) reveals that the majority of children with T1D have a defect in IDO1 expression in peripheral blood mononuclear cells. This defect is characterized by very low or absent levels of the protein IDO1. The same study reports that tocilizumab, a humanized interleukin-6 (IL-6) receptor antibody that blocks the IL-6 receptor, reverses this phenotype and controls hyperglycemia in NOD mice with overt diabetes (8). Therefore, the restoration of IDO1 immunoregulatory mechanisms may also be clinically beneficial in patients with T1D.

In light of these promising results, we investigated IDO1 expression in pancreata of individuals with T1D. We obtained pancreatic tissue sections from donors without diabetes and with diabetes collected by the Network for Pancreatic Organ Donors with Diabetes (nPOD) and from live patients with recent-onset T1D included in the Diabetes Virus Detection study (DiViD) (9) and systematically analyzed IDO1 and insulin expression by immunofluorescence assay. Although IDO1 was constitutively expressed

[1]La Jolla Institute for Allergy and Immunology, La Jolla, CA
[2]University of Perugia, Perugia, Italy
[3]Division of Paediatric and Adolescent Medicine, Oslo University Hospital, Oslo, Norway
[4]Faculty of Dentistry, University of Oslo, Oslo, Norway
[5]de Duve Institute, Brussels, Belgium
[6]Ludwig Institute for Cancer Research, Brussels, Belgium
[7]Novo Nordisk Diabetes Research & Development Center, Seattle, WA

Corresponding author: Matthias G. von Herrath, matthias@lji.org.

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F.A. is currently affiliated with the Novo Nordisk Diabetes Research & Development Center, Seattle, WA.

T.R.C. is currently affiliated with the Institute of Diabetes Research, Helmholtz Zentrum München, Neuherberg, Germany.

in β-cells from donors without diabetes, it was nearly absent in insulin-deficient islets. Moreover, we observed that IDO1 was seldom to not expressed in certain insulin-containing islets from donors with multiple positive autoantibodies (AAb^+) or with T1D, suggesting an impairment of IDO1 in the early stages of islet dysfunction. These findings could have important implications for the development of drugs able to target IDO1 expression in β-cells.

RESEARCH DESIGN AND METHODS

Subjects

Pancreata were collected and processed by the nPOD and DiViD as previously described (9,10). Forty pancreatic sections from the tail region were analyzed: 8 donors without diabetes, 10 AAb^+ donors with prediabetes, 6 patients with recent-onset T1D, 11 donors with T1D of longer duration, and 5 donors with type 2 diabetes (T2D) (Table 1). Tonsil control tissues were provided by the laboratory of Shane Crotty at La Jolla Institute for Allergy and Immunology. The La Jolla Institute for Allergy and Immunology Institutional Review Board approved all experimental procedures (protocol #DI3-054-0315).

Immunofluorescence

Pancreas sections were subjected to a double-indirect immunofluorescence staining for IDO1 (clone 4.16 H1 [11]) and insulin (clone ICBTACLS). A detailed protocol is provided in the Supplementary Data. Alternatively, pancreas sections were subjected to a double-indirect immunofluorescence staining for IDO1 and CD11c (clone 2F1C10; 1:100, 1 h; Proteintech) or MHC class I (MHCI; clone EMR8-5; 1:200; Abcam).

All sections were scanned with an Axio Scan.Z1 slide scanner (Carl Zeiss), and images were acquired with the ZEN 2 slidescan module (Carl Zeiss).

Quantitative Analysis

Blinded samples were evaluated by two investigators (F.A. and N.G.). Thirty islets were randomly selected to account for heterogeneity of the sections (12,13). IDO1-positive area, colocalization of IDO1, and insulin-positive area or the percentage of IDO1-positive areas within the insulin-positive area were quantified with Image-Pro Premier software 9.1 (Media Cybernetics, Inc.). Additional details can be found in the Supplementary Data.

Heat Maps

The ZEN 2 analysis module was used to determine IDO1- and insulin-positive areas in all of the islets of four case subjects (6073, 6267, 6247, and 6). Islet location and IDO1 percentage of positive area were then plotted as heat maps using MATLAB (MathWorks).

Statistical Analysis

Data are presented as mean ± SD and analyzed using a one-way ANOVA or a two-tailed unpaired Student *t* test. *P* values were adjusted for multiple comparisons using the Bonferroni correction. Analyses were performed using Prism version 7 (GraphPad Software). A value of $P < 0.05$ was considered significant.

RESULTS

Characteristics of the Cohorts

We selected a cohort that featured the different stages of the disease (i.e., prediabetes, recent-onset T1D, and T1D of longer duration). Mean age at time of tissue collection was not different between groups. As expected, the mean BMI of case subjects with T2D (35.4 ± 6.2) was higher than the mean BMI of those without diabetes, those who were AAb^+, or those with recent-onset and longer-duration T1D (26.6 ± 4.5 vs. 25.8 ± 5.8 vs. 26.2 ± 3.2 vs. 24.4 ± 3.1, respectively) (Table 1). Among 17 case subjects with T1D, age at onset was heterogeneous (14–35 years old), 7 out of 15 donors were C-peptide negative (2 C-peptide values were unknown), and 6 out of 17 had no remaining insulin-containing islets.

IDO1 Is Mainly Expressed in Insulin-Producing Cells and Nearly Absent From Insulin-Deficient Islets

IDO1 was detected in both exocrine and endocrine pancreas. We first investigated the localization of IDO1 in the endocrine pancreas. Insulin and IDO1 signals mostly overlapped, indicating that IDO1 was constitutively expressed by β-cells (Fig. 1*A*). IDO1 localization was confirmed with a second commercially available antibody (clone 10.1) (Supplementary Fig. 1). Furthermore, there were no statistical differences between groups in the localization of IDO1 (Fig. 1*B*), which confirmed that IDO1 was consistently expressed in β-cells, independently of the status of diabetes.

In the exocrine pancreas, IDO1 staining was notably dimmer than in the endocrine tissue. IDO1-positive cells were found at a very low density (≤1 cell/cm^2) (Fig. 1*C*) and identified as CD11c-positive cells (Fig. 1*D*), presumably dendritic cells.

Next, the percentage of IDO1-positive area present in the islets was assessed. We observed that IDO1 was significantly less expressed in insulin-containing islets from donors with T1D (6.6 ± 4.5%) regardless of disease duration than in islets from donors without diabetes (18.7 ± 2.3%) or donors with T2D (15.0 ± 3.2%). Moreover, in insulin-deficient islets from donors with T1D, IDO1 was mostly absent (1.4 ± 1.5%) (Fig. 1*E*).

Less IDO1 Expression in Some Islets of Donors With Prediabetes and Donors With Recent-Onset T1D

Next, we specifically assessed the expression of IDO1 in insulin-containing islets (percentage of IDO1 in the insulin-positive area) and discovered that IDO1 was heterogeneously expressed in insulin-containing islets (representative examples) (Fig. 2*A*). In order to visualize the distribution of IDO1 expression in the islets, heat maps showing insulin-deficient

Table 1—Clinical and histological features of individuals with diabetes and control subjects without diabetes

Case identification number	Age (years)	Sex	Race	Treatment	BMI (kg/m^2)	Antibody status	C-peptide (ng/mL)	Duration of disease (years)	ICI	Histology (external evaluation)
No diabetes (nPOD)										
6029	24.0	F	Hispanic/Latino	NA	22.6	Negative	Unknown	NA	Y	Mild fatty infiltrate, endothelium in islets fairly prominent
6034	32.0	F	Caucasian	NA	25.2	Negative	3.14	NA	Y	Normal islets, no significant infiltrates
6073	19.2	M	Caucasian	NA	36.0	Negative	0.69	NA	Y	Mild, multifocal parenchymal mixed infiltrate
6098	17.8	M	Caucasian	NA	22.8	Negative	1.41	NA	Y	Normal islets, few with vascular stasis
6165	45.8	F	Caucasian	NA	25.0	Negative	4.45	NA	Y	Numerous islets, no infiltrates
6251	33.0	F	Caucasian	NA	29.5	Negative	1.92	NA	Y	Normal islets, no significant lesions
6290	58.0	M	Caucasian	NA	22.5	Negative	7.46	NA	Y	Mild focal chronic pancreatitis
6295	47.0	F	African American	NA	30.4	Negative	12.47	NA	Y	Hypertrophic islets, mild fatty replacement, and atrophy in the exocrine regions
AAb$^+$ (nPOD)										
6080	69.2	F	Caucasian	NA	21.3	GADA, mIAA	1.84	NA	Y	No islet infiltrates, chronic pancreatitis, mild, multifocal
6123	23.2	F	Caucasian	NA	17.6	GADA	2.01	NA	Y	Various size islets, no infiltrates
6147	23.8	F	Caucasian	NA	32.9	GADA	3.19	NA	Y	Normal islets, no infiltrates
6151	30	M	Caucasian	NA	24.2	GADA	5.49	NA	Y	Normal islets, no infiltrates
6158	40.3	M	Caucasian	NA	29.7	GADA, mIAA	0.51	NA	Y	Exocrine atrophy, mild ductal dysplasia, focal mild chronic pancreatitis
6167	37	M	Caucasian	NA	26.3	IA-2A, ZnT8A	5.43	NA	Y	Normal islets, no infiltrates, mild acinar fat
6184	47.6	F	Hispanic/Latino	NA	27	GADA	3.42	NA	Y	Normal islet numbers and morphology
6197	22.0	M	African American	NA	28.2	GADA, IA-2A	17.48	NA	Y	Rare insulitis, mild, multifocal chronic pancreatitis
6267	23.0	F	Caucasian	NA	23.5	GADA, IA-2A	16.59	NA	Y	Focal islet hyperplasia, insulitis, mild CD3$^+$ infiltrates, and exocrine atrophy
6301	26.0	M	African American	NA	32.1	GADA	3.92	NA	Y	Numerous islets, mild acinar atrophy
T1D of longer duration (nPOD)										
6038	37.2	F	Caucasian	Humulin, insulin	30.9	Negative	0.2	20	Y	Amyloid islets, no infiltrates
6039	28.7	F	Caucasian	Yes, UTH	23.4	GADA, IA-2A ZnT8A, mIAA	<0.05	12	Y	Islet atrophy, mild peri- and intraislet CD3$^+$ infiltrates
6040	50.0	F	Caucasian	Humulin, insulin	31.6	mIAA	<0.05	20	N	Acinar atrophy, vascular occlusion, mild CD3$^+$ infiltrates
6076	25.8	M	Caucasian	Yes, UTH	18.8	GADA, mIAA	10.6	15	N	Rare insulitis, diffuse chronic pancreatitis, mild atrophy, and fibrosis
6081	31.4	M	Hispanic/Latino	Yes, noncompliant	28.0	Negative	0.24	15	Y	Moderate chronic pancreatitis, atherosclerosis mild, focal
6084	14.2	M	Caucasian	Insulin	26.3	mIAA	<0.05	4	N	Lobular adipose infiltration, mild exocrine, periductal CD3$^+$ infiltrates
6173	44.1	M	Caucasian	Lantus (Sanofi), Humalog (Eli Lilly and Company)	23.9	Negative	<0.05	15	N	Reduced islet density, acinar atrophy, chronic pancreatitis, CD3$^+$ infiltrates

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Table 1—Continued

Case identification number	Age (years)	Sex	Race	Treatment	BMI (kg/m^2)	Antibody status	C-peptide (ng/mL)	Duration of disease (years)	ICI	Histology (external evaluation)
6195	19.2	M	Caucasian	Insulin	23.7	GADA, IA-2A ZnT8A, mIAA	<0.05	5	N	Insulitis in a few islets, moderate acinar atrophy with chronic multifocal, mild pancreatitis
6198	22.0	F	Hispanic/Latino	Insulin	23.1	GADA, IA-2A ZnT8A, mIAA	<0.05	3	N	Diffuse mild insulitis, mild diffuse chronic pancreatitis
6212	20.0	M	Caucasian	Insulin, Humalog	29.1	mIAA	<0.05	5	Y	Mild insulitis in a few islets, focal ductal epithelial proliferation
6247	24.0	M	Caucasian	Lantus, Humalog	24.3	mIAA	0.47	0.6	Y	Mild insulitis in a few islets, mild exocrine atrophy
T2D (nPOD)										
6028	33.2	M	African American	Levemir (Novo Nordisk), FlexPen (Novo Nordisk)	30.2	Negative	22.4	17	Y	Very mild, diffuse CD3$^+$ acinar infiltrates
6109	48.8	F	Hispanic/Latino	None	32.5	mIAA	<0.05	New Dg	Y	Reduced density of islets, no fatty infiltrate, no CD3$^+$ infiltrates
6110	20.7	F	African American	Yes, UTH	40.0	Negative	0.58	New Dg	Y	Some atrophied islets, no fatty infiltrates, no CD3$^+$ infiltrates
6139	37.2	F	Hispanic/Latino	UTH	45.4	Negative	0.6	1.5	Y	Minimal fibrosis, no pancreatitis
6149	39.3	F	African American	NovoLog (Novo Nordisk), insulin	29.1	GADA	11.55	16	Y	Some hypertrophied islets, islet amyloidosis, moderate acinar atrophy and atherosclerosis, periductal and acinar infiltrates
Recent-onset T1D (DiViD)										
Case 1	25	F	Caucasian	Insulin 0.5 units/kg/day	21.0	IA-2A, ZnT8A, GADA, mIAA	0.46	4 weeks	Y	Intra- and peri-islet infiltration of CD3$^+$ T cells, 15% of islets with insulitis
Case 2	24	M	Caucasian	Insulin 0.35 units/kg/day	20.9	IA-2A, ZnT8A, GADA	0.350	3 weeks	Y	Intra- and peri-islet infiltration of CD3$^+$ T cells, 5–10% of islets with insulitis
Case 3	34	F	Caucasian	Insulin 0.17 units/kg/day	23.7	IA-2A, ZnT8A, GADA	0.74	9 weeks	Y	Intra- and peri-islet infiltration of CD3$^+$ T cells, 25% of islets with insulitis
Case 4	31	M	Caucasian	Insulin 0.4 units/kg/day	25.6	IA-2A, GADA, mIAA	Unknown	5 weeks	Y	Intra- and peri-islet infiltration of CD3$^+$ T cells, 4–7% of islets with insulitis
Case 5	24	F	Caucasian	Insulin 0.36 units/kg/day	28.6	IA-2A, GADA, mIAA	Unknown	5 weeks	Y	Intra- and peri-islet infiltration of CD3$^+$ T cells, 2–18% of islets with insulitis
Case 6	35	M	Caucasian	Insulin 0.52 units/kg/day	26.7	GADA	0.24	5 weeks	Y	Intra- and peri-islet infiltration of CD3$^+$ T cells, 0–5% of islets with insulitis

F, female; GADA, GAD autoantibody; IA-2A, insulinoma-2–associated autoantibody; mIAA, microinsulin autoantibody; ICI, insulin-containing islet; M, male; N, no; NA, not applicable; New Dg, diagnosed at time of death; UTH, unknown treatment history; Y, yes; ZnT8A, zinc transporter 8 autoantibodies.

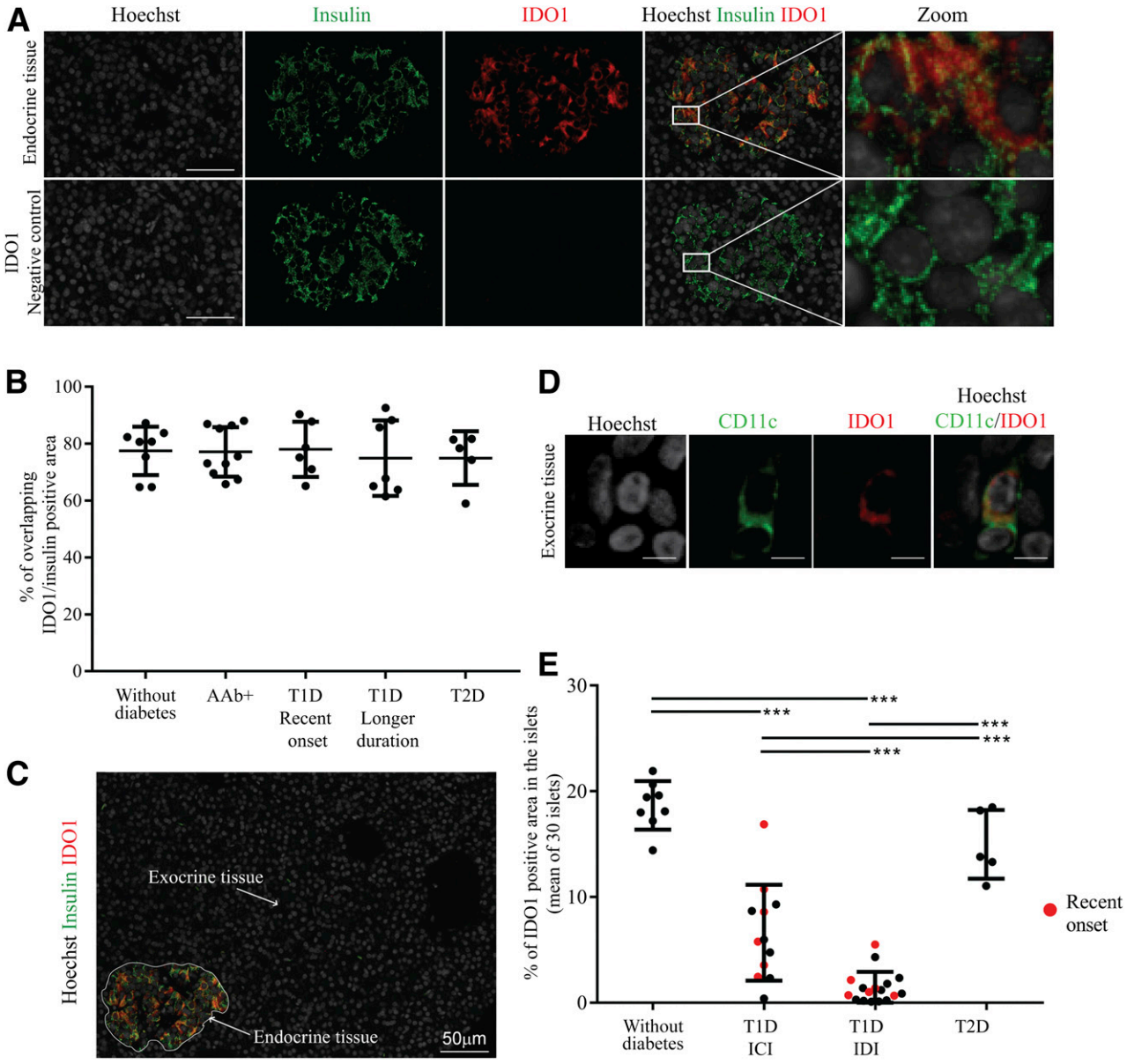

Figure 1—IDO1 is mainly expressed in β-cells independently of status of diabetes. *A*: Representative images of IDO1 expression in an islet from nPOD case 6029 without diabetes. The section was stained for Hoechst (white), insulin (green), and IDO1 (red); the merged image of the three channels is displayed in the fourth column from left. The second row shows the IDO1-negative control (secondary antibody alone) from the same islet in a consecutive section. *B*: Localization of IDO1 in endocrine pancreas represented as the percentage of overlapping insulin- and IDO1-positive signal. Each dot represents a case subject (mean of 30 islets). *C*: Representative image of IDO1 expression in the exocrine pancreas; the islet from *A* is in the bottom left corner of the image. *D*: Representative image of an IDO1/CD11c-positive cell found in the exocrine pancreas. *E*: Percentage of IDO1-positive area in islets was quantified and presented as a mean of 30 islets (each dot represents a case subject). In the groups with T1D, red dots represent case subjects with recent-onset (DiViD), and black dots represent case subjects with T1D of longer duration (nPOD). Bars represent SD, and significance was determined using unpaired Student *t* tests corrected post hoc with Bonferroni. Images were acquired with a ×20 objective. Scale bars, 50 μm (*A* and *C*) or 10 μm (*D*). ***$P < 0.001$. ICI, insulin-containing islets; IDI, insulin-deficient islets.

islets (purple dots) and the percentage of IDO1 in insulin-containing islets (gradient green to red) were created. In donors without diabetes and single AAb$^+$ donors, IDO1 expression was high (>50%), whereas it was markedly reduced in individuals with T1D of longer duration (<20%). Interestingly, double AAb$^+$ donors and patients with recent-onset T1D presented higher heterogeneity in IDO1 distribution. Both of these groups showed lobe-specific impairment of IDO1 expression (Fig. 2*B*), similar to the lobular pattern of β-cell loss in T1D.

Loss of IDO1 Expression Precedes β-Cell Decay

Finally, in order to clarify at which stage of T1D IDO1 expression was impaired, the percentage of insulin-positive area and percentage of IDO1 in β-cells from all

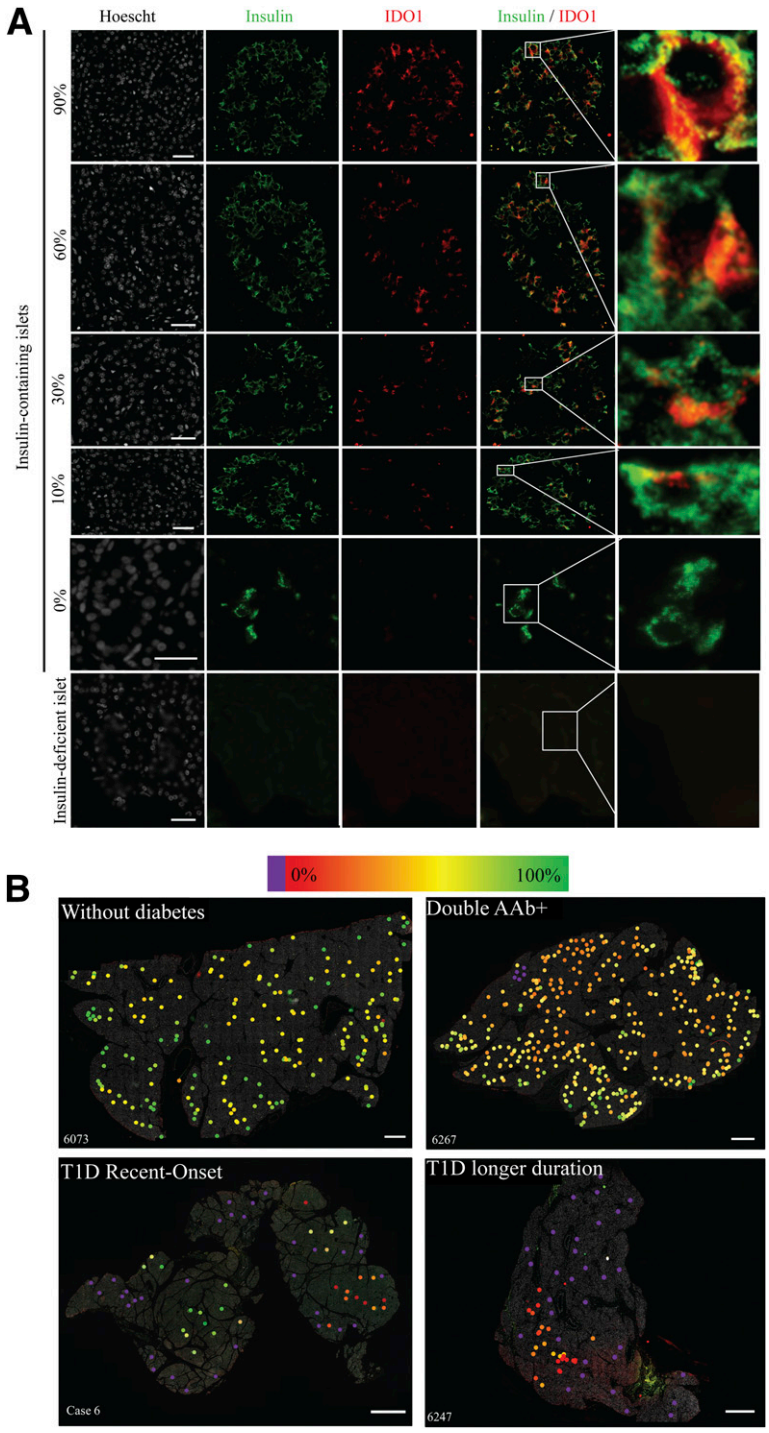

Figure 2—Some islets express less IDO1 in β-cells prior to β-cell loss. *A*: Representative image of the percentage of IDO1-positive area in insulin-positive area found in a donor (nPOD case 6267: 90, 60, 30, and 10%; DiViD case 6: 0%, insulin-deficient islet). Sections were stained for Hoechst (white), insulin (green), and IDO1 (red). The merged image of insulin/IDO1 is displayed in the fourth column from left. *B*: Heat maps of IDO1 islet expression and heterogeneity presented as the percentage of IDO1 in insulin-positive area in whole pancreatic tissue sections from donors without diabetes, double AAb$^+$ donors with prediabetes, and donors with recent-onset T1D and T1D of longer duration. Gradient indicates range from 0% (red dots) to 100% (dark green dots) in insulin-containing islets, and purple dots represent insulin-deficient islets. Images were acquired with a ×20 objective. Scale bars, 50 μm (*A*) or 300 μm (*B*).

of the cases were quantified, and the results were displayed as scatter plots (Fig. 3*A*). The heterogeneity of IDO1 expression in double AAb$^+$ donors and patients with recent-onset T1D (8–89 and 0–88% percentage of positive insulin area, respectively) was found to be substantially higher than in donors without diabetes, single AAb$^+$ donors, donors with T1D of longer duration, or donors with T2D (48–98, 43–90, 1–58, and 40–88%, respectively),

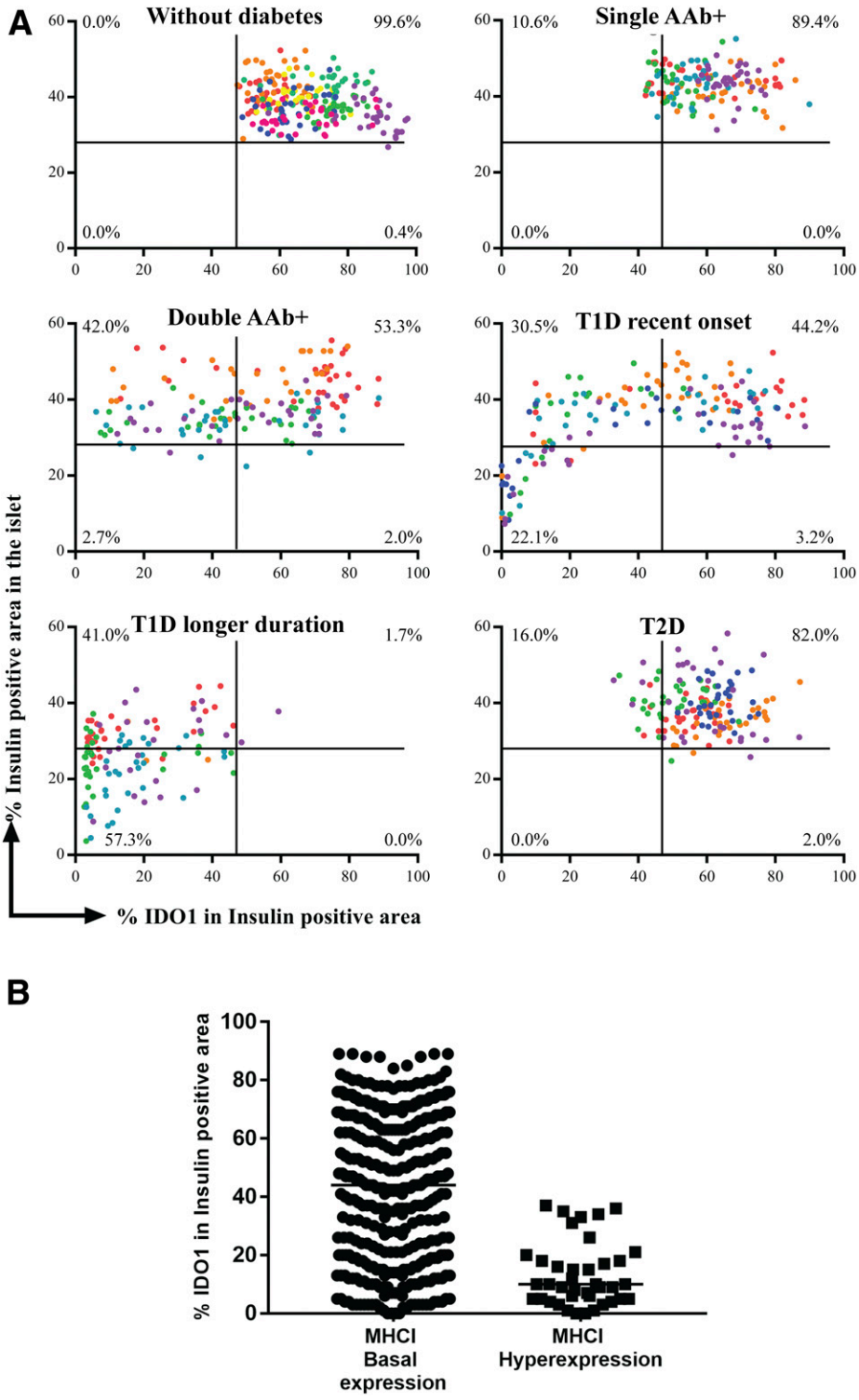

Figure 3—Early loss of IDO1 expression in insulin containing-islets during the course of T1D is not systematically associated to MHC hyperexpression. *A*: Scatter plots representing the percentage of insulin-positive area in islets (*y*-axis) and the percentage of IDO1 positive area in insulin-positive area (*x*-axis) from case subjects without diabetes (top left), case subjects who are single AAb$^+$ (top right) or double AAb$^+$ (middle left), and case subjects with recent-onset T1D (middle right), T1D of longer duration (bottom left), and T2D (bottom right). Thirty islets were assessed per case subject (each dot represents one insulin-containing islet; each color represents a case subject). Numbers represent the percentage of islets in each quadrant. *B*: Comparison of the percentage of IDO1 expression in β-cells with MHCI hyperexpression.

confirming observations from the heat maps. Moreover, we observed major differences in IDO1 expression depending on the antibody status and stage of disease. In islets from double AAb^+ donors and patients with recent-onset T1D, a higher percentage (30.5 and 42%, respectively) of $IDO1^{low}$ islets was observed when compared with donors without diabetes, single AAb^+ donors, or donors with T2D (0, 10.6, and 16%, respectively). Interestingly, the scatter plots suggested that the loss of IDO1 occurred before T1D onset (Fig. 3*A*, middle left panel), whereas notably less insulin was expressed around the time of diagnosis (Fig. 3*A*, middle right panel). In donors with T1D who still had remaining insulin-containing islets, $IDO1^{neg}Insulin^{pos}$ islets were found, whereas $IDO1^{pos}Insulin^{neg}$ islets were not, which supported the idea of an early IDO1 loss. Finally, we compared MHCI hyperexpression in islets with IDO1 expression. We observed that although $IDO1^{low}$ islets are more likely to hyperexpress MHCI, not all $IDO1^{low}$ islets displayed MHCI hyperexpression (Fig. 3*B*).

DISCUSSION

IDO1, which leads the catabolism of tryptophan, is known to play multiple roles in the regulation of immunity through its antimicrobial effects and its activation of regulatory immune responses promoting immune tolerance (14). The enzyme therefore plays a role in controlling autoimmunity (15) and appears to be involved in several pathophysiological conditions, including autoimmune diseases (16). Interestingly, Orabona et al. (8) described a defect of IDO1 at the peripheral level in children with T1D. In light of these findings, we systematically investigated the pancreatic expression of IDO1 in patients with T1D.

IDO1 is expressed in various human tissues and cells, including antigen-presenting cells and regulatory T cells (11). In isolated rat islets, *IDO1* mRNA was not constitutively expressed, and its transcription was only activated by interferon-γ and IL-1β in β-cells (17). In isolated human islets, PDX1-positive cells (presumably β-cells) and other endocrine cells showed a strong immunoreactivity to IDO1, which was enhanced when the islets were treated with interferon-γ (18). In this study, we report for the first time, using two antibodies specific for IDO1, that human endocrine tissue expresses IDO1 primarily in β-cells. Moreover, we described the presence of scarce IDO1-positive cells in the exocrine pancreas that are likely to be tolerogenic dendritic cells (19). Previous studies have described low plasma levels of tryptophan catabolites in NOD mice (5) and patients with T1D (20,21). In this study, for the first time, we show that the peripheral deficiency of IDO1 in human T1D is concomitant with low expression of IDO1 in insulin-containing islets and its quasi-absence in insulin-deficient islets in the pancreas.

These major findings call into question whether the absence of IDO1 is a cause or a result of β-cell dysfunction. We therefore investigated IDO1 expression in β-cells only and discovered major differences depending on the stage of disease. Indeed, in islets from donors without diabetes, single AAb^+ donors, and donors with T2D, IDO1 expression was consistently high, whereas in islets from double AAb^+ donors and case subjects with recent-onset T1D, heterogeneity was notably higher, indicating a shift in IDO1 expression around the time of T1D diagnosis. Our observations imply that IDO1 decay may occur in the preclinical phases of T1D and might precede the time of β-cell destruction. Thus, reverting IDO1 loss might prevent or delay T1D outcome, as reported by Zhang et al. (22) in a NOD mice model in which fibroblasts overexpressing IDO1 protected β-cells from destruction and reversed hyperglycemia. Moreover, Mondanelli et al. (23) have reported a protective and therapeutic effect of bortezomib, a proteasomal inhibitor that attenuates IDO1 proteasomal degradation, in NOD mice.

By nature, any human histopathological investigation using tissues from deceased organ donors will be cross-sectional. However, because T1D is pathologically a highly heterogeneous disease that gradually affects selected lobes of the pancreas, all stages of T1D can essentially be observed in a single organ section (heat maps in Fig. 2*B*). This allowed us to conclude that IDO1 was lost before the decline of insulin secretion.

Our observations raise important questions for the role of IDO1 in β-cells. Previous studies have shown that the enzyme can be involved in either immune or nonimmune events (24,25). In the pancreatic islets, it may be that the loss of IDO1, and thus tryptophan metabolites, weaken the immunomodulatory microenvironment and make the β-cells more prone to immune attacks by activating resident or infiltrating immune cells. Alternatively, the fact that IDO1 is constitutively expressed in β-cells could suggest that the enzyme has a prominent role in their physiology. These theories will need to be developed in further studies using pancreatic islet models.

Clinical trials to reverse T1D or prevent loss of residual β-cell function have had limited success so far. One reason could be that β-cell dysfunction contributes more to the disease (especially early on) than autoimmune attacks. A striking finding of the current study is the early impairment (prior to insulin decline) of intraislet expression of IDO1 within the pancreata of donors with prediabetes and donors with T1D. Considering the potential role of IDO1 in immune and nonimmune events, its impairment might be involved in the cascade, which leads to β-cell dysfunction. Future studies should use isolated human islets to better understand the role of IDO1, which will also aide the development of future targeted therapies.

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Duality of Interest. B.V.d.E. is co-founder of and consultant for iTeos Therapeutics, a company involved in the development of IDO and tryptophan-2,3-dioxygenase inhibitors. M.G.v.H. is an employee of Novo Nordisk. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. F.A. designed, performed experiments, interpreted data, and wrote the manuscript. G.M. performed experiments and revised the manuscript. N.G. performed experiments and helped with the analyses. T.R.C. interpreted data and revised the manuscript. J.Z.G. assisted with the statistical analysis. L.K. and K.D.-J. collected patient material and revised the manuscript. K.D.-J. is principal investigator of the DiViD study. B.V.d.E. characterized and provided the IDO1 antibody. C.O. and U.G. revised the manuscript. M.G.v.H. designed experiments, interpreted data, and wrote the manuscript. M.G.v.H. is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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