



Immunoregulated insulinitis and slow-progressing type 1 diabetes after duodenopancreatectomy

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Abstract

Aims/hypothesis We report the case of a woman who underwent a partial pancreatectomy for a serous cystadenoma when aged 56 years. She had been diagnosed with diabetes 6 years before and had Hashimoto's thyroiditis. Despite positive anti-GAD autoantibodies (GADA) and previous surgery, she was transiently weaned off long-acting insulin. Blood glucose levels remained well controlled with low-dose long-acting insulin. Insulin needs eventually increased 8 years after surgery, in conjunction with anti-zinc transporter 8 (ZnT8) seroconversion and decreasing residual C-peptide. We hypothesised that the surgical pancreas specimens and blood autoimmune T cell responses may provide correlates of this indolent clinical course.

Methods Beta and alpha cell area and insulinitis were quantified on pancreas head tissue sections obtained at surgery. Blood T cell responses against beta cell antigens were analysed by enzyme-linked immunospot.

Results Pancreas sections displayed reduced beta cell and normal alpha cell area (0.27% and 0.85% of section area, respectively). High-grade insulinitis was observed, mostly in insulin-containing islets, with a peri-insulinitis pattern enriched in T cells positive for regulatory forkhead box protein 3 (FOXP3). In vitro challenge with beta cell antigens of circulating T cells collected 4 and 9 years after surgery revealed dominant and persistent IL-10 responses; IFN- γ responses increasing at 9 years, after anti-ZnT8 seroconversion, was observed.

Conclusions/interpretation Despite persistent GADA and the histopathological finding of insulinitis and decreased beta cell area 6 years after diabetes diagnosis, glycaemic control was maintained with low-dose insulin up to 8 years after surgery. Regulated T cell responses towards beta cell antigens and FOXP3-positive peri-insulinitis suggest spontaneous long-term regulation of islet autoimmunity after substantial beta cell loss, and eventual autoimmune progression upon anti-ZnT8 seroconversion.

Keywords Autoimmunity · Duodenopancreatectomy · Insulin secretion · Regulatory T cells · Type 1 diabetes

Abbreviations

aAb	Autoantibody	IA-2	Islet antigen 2
DiViD	Diabetes Virus Detection	ICI	Insulin-containing islet
FOXP3	Forkhead box protein 3	IDI	Insulin-deficient islet
GADA	GAD autoantibodies	PBMC	Peripheral blood mononuclear cell
		PHA	Phytohaemagglutinin

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Research in context

What is already known about this subject?

- In type 1 diabetes, the slope of beta cell decline is variable across individuals
- In particular, some people present with indolent, slowly evolving forms of type 1 diabetes and can preserve significant beta cell function for several years

What is the key question?

- What are the immune correlates of these slowly evolving forms of type 1 diabetes in the pancreas and blood?

What are the new findings?

- We present the case report of a woman positive for anti-GAD autoantibodies who maintained significant insulin secretion over 8 years
- The indolent clinical course correlated with reduced beta cell mass in the pancreas and with insulinitis lesions featuring both CD8-positive and forkhead box protein 3-positive T cells
- Beta cell loss eventually relapsed, concomitantly with seroconversion for anti-zinc transporter 8 autoantibodies and with increased proinflammatory vs regulatory responses against islet antigens by circulating T cells

How might this impact on clinical practice in the foreseeable future?

- This case report of autoimmune flare, long-term remission and eventual relapse suggests that boosting immune regulation may halt beta cell destruction

Teff	Effector T cell
Treg	Regulatory T cell
ZnT8	Zinc transporter 8

studied to search for histopathological and autoimmune T cell correlates of this indolent clinical course.

Introduction

Type 1 diabetes is the late consequence of an autoimmune process targeting insulin-producing pancreatic beta cells [1, 2]. The disease occurs on a susceptible genetic background following elusive environmental triggers [3] and is mediated by T cells, involving both CD4⁺ and CD8⁺ T cells that destroy beta cells. The presence of one or more autoantibodies (aAbs) against GAD, islet antigen 2 (IA-2) and zinc transporter 8 (ZnT8) in adults is the hallmark of an ongoing autoimmune process. An improper balance between regulatory T cells (Tregs), which express the lineage marker forkhead box protein 3 (FOXP3), and effector T cells (Teffs) is central to the progression of this autoimmune process towards beta cell destruction [4]. Up to the critical threshold whereupon it becomes insufficient to maintain euglycaemia, it is unknown whether the progression of beta-cell destruction is linear or follows a relapsing–remitting pattern, possibly reflecting cyclic disruption and restoration of the Treg/Teff balance [4].

We report the case of a patient positive for anti-GAD aAbs (GADA), whose diabetes remained well controlled with low-dose long-acting insulin for 8 years after partial pancreatectomy. Surgical pancreas specimens and blood samples were

Methods

Clinical case A 47-year-old woman was diagnosed with a multicystic-serous cystadenoma of the uncinate process, in 2002. The timeline of the clinical course is summarised in Fig. 1 and detailed in Electronic supplementary materials (ESM) Table 1. Diabetes was diagnosed 3 years later (2005); blood glucose levels were reported to be ‘moderately elevated’, at a weight of 96 kg and BMI of 37 kg/m². The woman had no family history of diabetes and no personal history of gestational diabetes during any of her three pregnancies. Metformin and gliclazide were started. She also had Hashimoto’s thyroiditis that required 112.5 µg/day L-thyroxine.

An uneventful duodenopancreatectomy (Whipple procedure with removal of the head of the pancreas) was performed 9 years later (February 2011) because of increasing tumour size (70 mm diameter) accompanied by abdominal pain, signs of vena cava compression with limb oedema and deteriorating diabetes (HbA_{1c} 83 mmol/mol [9.7%]). Add-on insulin glargine was started before surgery. Postoperative treatment included metformin, gliclazide, insulin glargine and pancreatic enzyme replacement therapy (lipase [Creon], 150,000 U/day). Faecal elastase-1 concentration was 18 µg/g (normal

values >200 µg/g), confirming severe pancreatic exocrine failure and the need for replacement therapy.

This woman was referred to our Diabetology unit in July 2012, 17 months after surgery. She had lost 16 kg since surgery, and gliclazide was stopped due to hypoglycaemia. HLA typing was *DQB1*03:01/DRB1*04:01* (neutral for type 1 diabetes susceptibility) and *DQB1*05:03/DRB1*14:01* (a rare haplotype with unknown association with disease susceptibility or protection). Anti-thyroid peroxidase aAbs were positive (118 U/ml; positive threshold >34 U/ml), GADA were also positive at high titre, and IA-2 and ZnT8 aAbs were negative.

Insulin glargine was stopped 3 months later (October 2012) and was resumed after 2 months because of positivity for GADA and intolerance to metformin doses higher than 1500 mg/day. With this treatment, blood glucose levels remained at target, with HbA_{1c} values of 43–55 mmol/mol (6.1–7.2%) for more than 6 years. In 2015 (i.e. 3 years after surgery), fasting C-peptide concentration was 0.27 nmol/l (normal range 0.27–1.27 nmol/l) and increased to 0.35 nmol/l 6 min after i.v. challenge with 1 mg glucagon. Peripheral blood mononuclear cells (PBMCs) were collected for T cell assays.

Another aAb measurement performed 6 years after the initial one (2019) documented increasing GADA titres and

the appearance of high-titre anti-ZnT8, both persisting 1 year later. Anti-smooth muscle cell aAbs were positive on two occasions 1 year apart (1:2000, then 1:50; positive threshold >1:20). One year later, fasting C-peptide values were lower than before (0.13 nmol/l), but still increased to 0.21 nmol/l 2 h after stimulation by a mixed meal test, documenting some residual insulin secretion. At the last follow-up in November 2020, fasting C-peptide was 0.10 nmol/l, and 0.15 nmol/l after a mixed meal test. A second PBMC sample was collected.

The patient gave written informed consent and the study was approved by the Cochin Hospital Ethics Committee.

Immunohistochemistry Beta cell area, alpha cell area and CD8⁺, CD4⁺ and FOXP3⁺ insulinitis were assessed on 4 µm sections taken from four different distal regions of peri-tumoral pancreatic tissue (excluding the uncinate process). Immunohistochemical staining employed an avidin–biotin–peroxidase system. Briefly, sections were placed in a Bond III Automated Immunohistochemistry Vision Biosystem (Leica, Nanterre, France). Tissues were deparaffinised and pre-treated with EDTA buffer (pH 8.8) at 98°C for 20 min. After washing, peroxidase activity was blocked for 10 min using the Bond Polymer Refine Detection Kit DC9800 (Leica). Tissues were

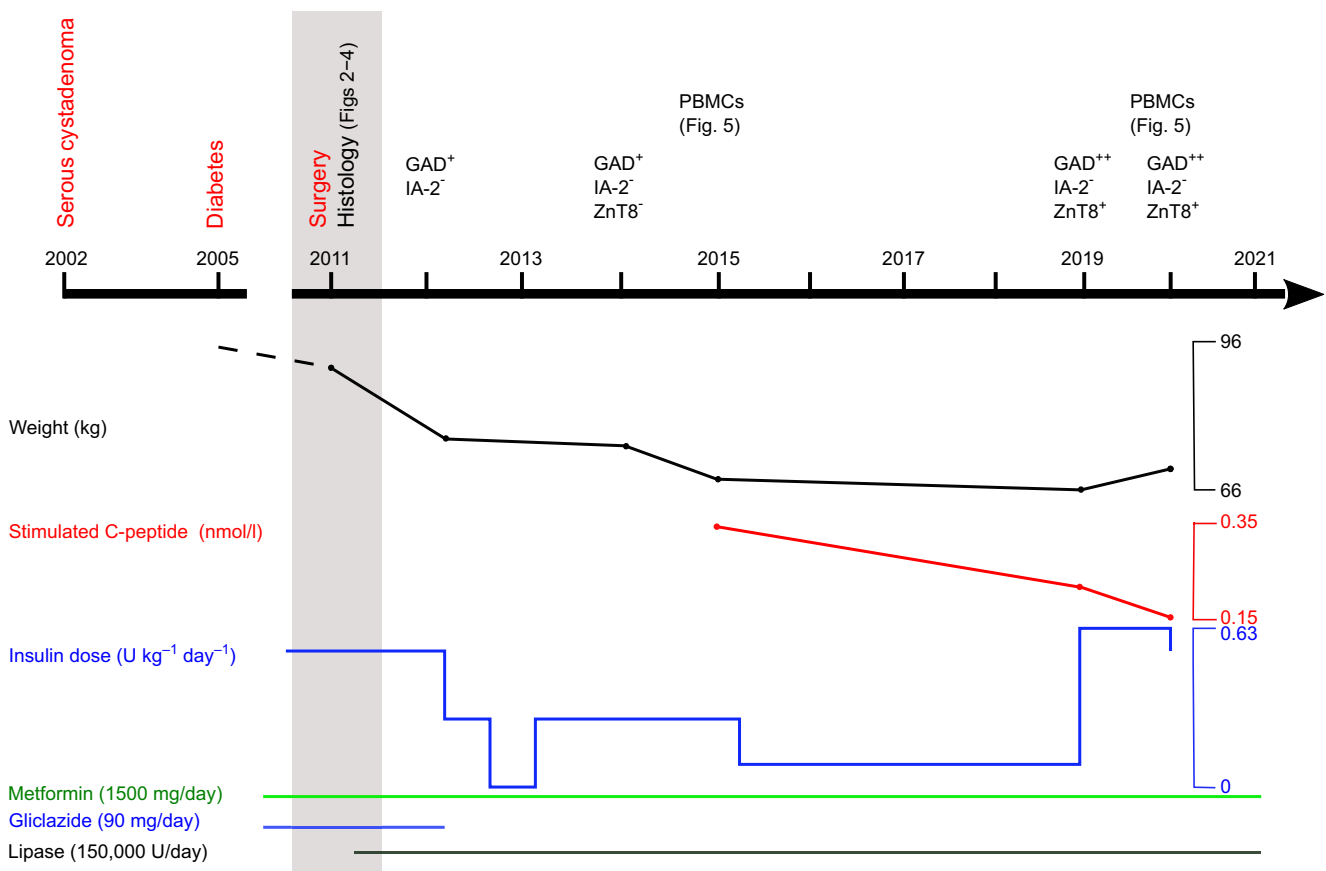


Fig. 1 Schematic timeline of the evolution of aAbs, clinical variables, treatments and related pancreas histopathology and blood T cell analyses. The patient’s clinical history before and after surgery (2011) is depicted.

The time of sampling for pancreas tissue (2011) and PBMCs (2015, 2020) is indicated

washed again and incubated with primary antibodies for 20 min: anti-insulin (RRID:AB_10013624 [Dako/Agilent, Les Ulis, France], 1:500 vol./vol.); anti-glucagon (RRID:AB_10013726 [Dako], 1:1000 vol./vol.); anti-CD8 (clone C8/144B, RRID:AB_2075537 [Dako]; 1:100 vol./vol.); anti-CD4 (clone 4B12, RRID:AB_10554438 [Dako]; 1:200 vol./vol.), anti-FOXP3 (clone 236A/E7, RRID:AB_445284 (Abcam, Cambridge, UK); 1:80 vol./vol.), as previously described [5]. Tissues were subsequently coated with polymer for 10 min and developed with 3,3'-diaminobenzidine for 8 min. Slices were washed with distilled water and counterstained with Acid Hemalum (Mayer). Quantifications were performed with a Hamamatsu Nanozoomer (Hamamatsu, Massy, France) and the NDP.view software. Pancreatic and islet areas on insulin- or glucagon-stained slides were manually defined based on morphology, irrespective of islet size (possibly excluding clusters of few [<10] cells lacking an identifiable islet architecture). Insulin and glucagon area is expressed as the percentage of pancreatic area. Insulinitis was scored as positive according to a threshold level of ≥ 15 CD8⁺ cells/islet, a more restrictive definition than the consensus criterion of ≥ 15 CD45⁺ cells/islet [6]. CD4⁺ insulinitis was assessed using the same criteria.

Immunofluorescence To validate FOXP3 staining, CD25^{hi}CD127^{lo} Tregs and CD25⁻CD127⁺ Teffs were sorted and expanded as described [7], fixed and permeabilised for flow cytometry staining with anti-FOXP3 antibody (clone 259D/C7, RRID:AB_11153143 (BD Biosciences, Le Pont de Claix, France), or paraffin-embedded for immunofluorescence staining. Two consecutive pancreatic sections were analysed by triple immunofluorescence to simultaneously evaluate the expression of CD8 or FOXP3 along with insulin and glucagon. After stepwise deparaffinisation and rehydration (xylene-I and xylene II, 20 min/each; ethanol 100%, 95%, 80%, 75%, all vol./vol., 5 min/each), sections were subjected to heat-induced antigen retrieval using Tris-EDTA buffer (10 mmol/l Tris, 1 mmol/l EDTA, 0.05% vol./vol. Tween-20, pH 9.0) for 20 min at 100°C. After cooling and incubation in 5% vol./vol. goat serum (Sigma, Saint Quentin Fallavier, France) to reduce non-specific reactions, sections were stained in 5% goat serum overnight at 4°C with antibodies against CD8 (RRID:AB_2075537 [Dako]; 1:50 vol./vol., final concentration 3.2 µg/ml) or FOXP3 (clone 236A/E7, RRID:AB_467556 [eBioscience, Le Pont de Claix, France]; 1:40 vol./vol., 12.5 µg/ml). After three washes in PBS, sections were incubated with pre-diluted guinea pig anti-insulin (RRID:AB_2800361 [Dako]) for 20 min, followed by rabbit anti-glucagon (RRID:AB_10013726 [Dako]; 1:100 vol./vol., final concentration 352 µg/ml) in 5% goat serum for 1 h. Secondary antibodies were subsequently added at 1:500 vol./vol. in PBS for 1 h: Alexa-Fluor594-labelled goat anti-guinea pig IgG (RRID:AB_2535856 [Dako]); Alexa-Fluor647-labelled goat anti-rabbit IgG (RRID:AB_2535813

[Dako]); and Alexa-Fluor488-labelled goat anti-mouse IgG (RRID:AB_2534088 [Dako]). Sections were counterstained with DAPI (Sigma; 1:3000) and mounted with Vectashield antifade (RRID:AB_2336789, Eurobio, Les Ulis, France) or fluorescence mounting medium (Dako) and analysed immediately or stored at 4°C prior to acquisition on a Leica TCS SP5 confocal laser scanning microscope with LAS software and manual counting of CD8⁺ and FOXP3⁺ cells. CD8⁺ insulinitis was defined based on ≥ 15 CD8⁺ cells/islet. FOXP3⁺ insulinitis was defined based on ≥ 1 FOXP3⁺ cells/islet.

T cell assays Frozen-thawed PBMCs were processed as described [8, 9], plated at 10⁶ cells/well in 96-well flat-bottom plates and stimulated for 48 h, as described [8], in the presence of 10 µg/ml of the following antigens: proinsulin (kindly provided by Lilly, Neuilly sur Seine, France); insulin (Actrapid; Novo Nordisk, Puteaux, France); C-peptide (SynPeptide, Shanghai, China); preproinsulin signal peptide (GL Biochem, Shanghai, China); GAD65 (Diamed, Stockholm, Sweden); intracellular IA-2 (amino acids 214–591; kindly provided by J.F. Elliott, University of Alberta, Canada); adenoviral lysate (produced in-house); and phytohaemagglutinin (PHA, 1 µg/ml; Sigma). All antigens were $>95\%$ pure, with an endotoxin concentration of <5 U/mg. At the end of the 48 h culture, non-adherent cells were washed and plated at 2×10^5 cells/well in triplicate wells of 96-well PVDF enzyme-linked immunospot (ELISpot) plates coated with either an anti-IFN- γ or anti-IL-10 antibody (UCyTech, Utrecht, the Netherlands). After 6 h of incubation, plates were developed as described [8], counted on a Bio-Sys 5000 Pro-SF Bioreader (Bio-Sys, Karben, Germany) and results expressed as mean spot-forming cells/10⁶ PBMCs normalised across samples. T cell responses were scored as positive for counts at >2 SD above spontaneous background responses in the absence of antigen, as determined by receiver operator characteristic analysis.

Results

Decreased beta cell area and mixed CD8/FOXP3⁺ insulinitis at the time of surgery Quantitative immunochemistry was performed on four blocks from the head of the pancreas, distant from the tumour and from areas of chronic pancreatitis (Fig. 2a). On slides stained for insulin (section area 56–250 mm², total 513 mm²), a total of 119 islets were identified. On slides stained for glucagon (section area 44–219 mm², total 416 mm²) a total of 320 islets were identified. Representative images are shown in Fig. 2b,c. The total fractional islet areas were 0.54% and 1.31% of the pancreas area on slides stained for insulin and glucagon, respectively, ranging from 0.19% to 3.6% in different sections (Fig. 2d). Pancreas sections displayed reduced beta cell and normal

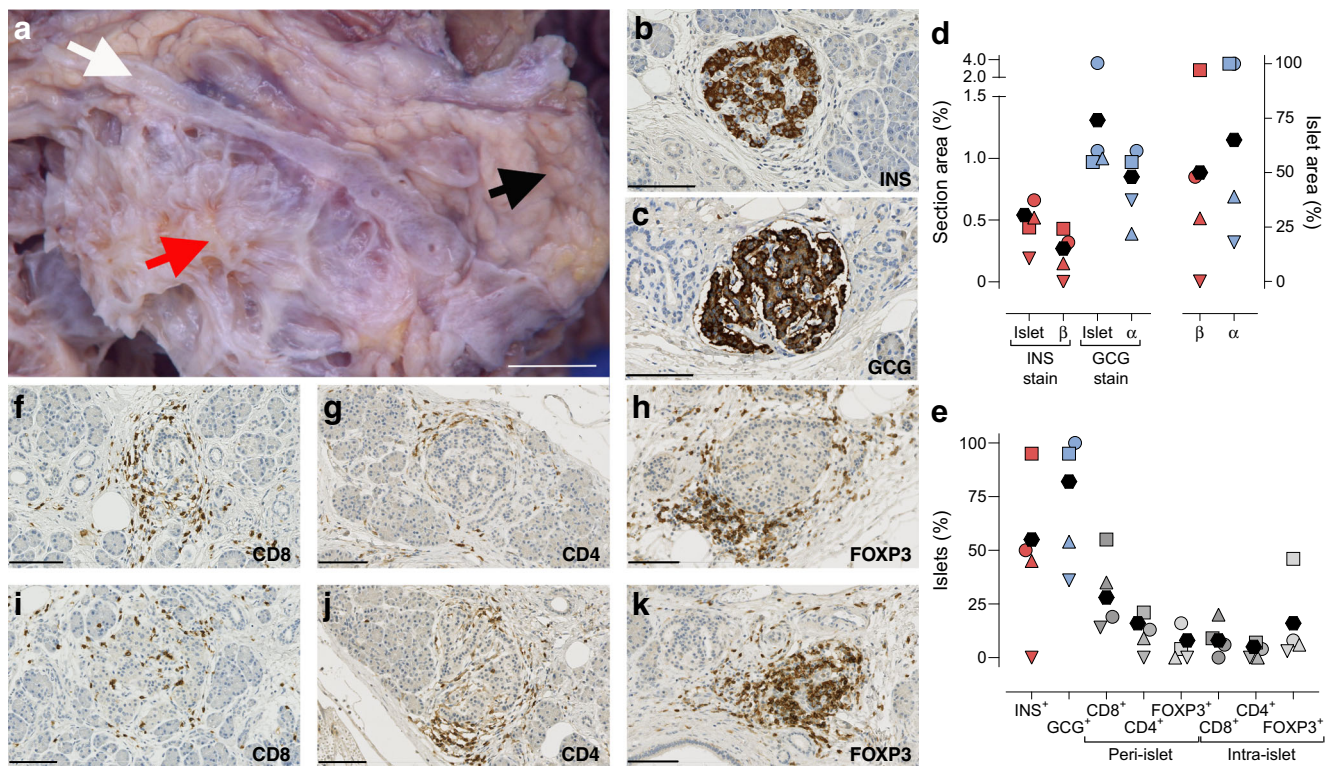


Fig. 2 Tissue macroscopy, immunohistochemistry and quantification of endocrine areas and insulinitis at surgery. **(a)** Macroscopy of the tumour and surrounding pancreas. The tumour (red arrow), the duct of Wirsung (white arrow) and the head of the pancreas where tissue sections were taken (black arrow) are indicated. Scale bar, 10 mm. **(b, c)** Representative images of tissue sections stained for insulin (INS, **b**) and glucagon (GCG, **c**). **(d)** Per cent tissue section area for islets, beta (β) cells and alpha (α) cells (first four columns) and per cent islet tissue area for beta and alpha

cells (fifth and sixth column). **(e)** Per cent positive islets for the indicated markers. Each symbol represents one of the four tissue blocks analysed; black symbols display values for the four blocks considered altogether. **(f–h)** Representative images showing peri-insulinitis stained for CD8 (**f**), CD4 (**g**) and FOXP3 (**h**). Scale bar, 100 μ m. **(i–k)** Representative images showing invasive insulinitis (intra-islet) stained for CD8 (**i**), CD4 (**j**) and FOXP3 (**k**). Scale bar, 100 μ m

alpha cell area (0.27% and 0.85% of section area, respectively). When considering the four blocks altogether, the beta cell/islet area and alpha cell/islet area was 50% (range 0–97%) and 65% (range 18–100%), respectively (Fig. 2d). Sixty-five islets (55%) were insulin-positive (referred to as insulin-containing islets [ICIs] from hereon), with wide heterogeneity across blocks (range 0–95%); 263 islets (82%) were glucagon-positive (range 36–100%) (Fig. 2e).

Figure 2f–h show representative images of islets that display peri-insulinitis and Fig. 2i–k show representative images of islets that display invasive (intra-islet) insulinitis. Overall, CD8⁺, CD4⁺ and regulatory (FOXP3⁺) peri-insulinitis was observed in 28%, 16% and 8% of the islets, respectively. CD8⁺, CD4⁺ and FOXP3⁺ intra-islet insulinitis was observed in 8%, 5% and 16% of the islets, respectively (Fig. 2e).

In type 1 diabetes, insulinitis is preferentially localised in ICIs as compared with insulin-deficient islets (IDIs; i.e. insulin-negative and glucagon-positive) [10]. We therefore analysed adjacent sections by triple immunofluorescence to verify whether this was the case in our patient and to quantify the number of CD8⁺ vs FOXP3⁺ cells. First, the specificity of

FOXP3 staining was confirmed on tonsil tissue sections and on sorted, in vitro expanded Tregs and Teffs (ESM Fig. 1). Representative images of CD8/insulin/glucagon and FOXP3/insulin/glucagon staining are shown in Fig. 3. Results were remarkably similar when comparing sections stained for CD8 and FOXP3 (Fig. 4a,b). ICIs accounted for 58–63% of islets. CD8⁺ and FOXP3⁺ insulinitis (defined as ≥ 15 CD8⁺ cells/islet and ≥ 1 FOXP3⁺ cells/islet, respectively) was present in 90% and 85% of ICIs vs 32% and 35% of IDIs, respectively ($p \leq 0.001$). Insulinitis displayed a predominant peri-islet pattern for both CD8⁺ and FOXP3⁺ cells (85% and 73% in ICIs vs 100% and 100% in IDIs, respectively). In addition, when analysing the number of CD8⁺ cells and FOXP3⁺ cells per islet (Fig. 4c,d), both were more abundant in ICIs than in IDIs. Overall, the number of CD8⁺ cells was approximately tenfold higher than for FOXP3⁺ cells in peri-islet infiltrates, while numbers were similar in the less common intra-islet infiltrates. As reported [11], some infiltration was also noted in the exocrine tissue for CD8⁺ cells and, to a lesser extent, for FOXP3⁺ cells.

Collectively, these findings document a heterogeneous pattern of beta cell loss and ICI-polarised insulinitis, as commonly observed in individuals with type 1 diabetes [12].

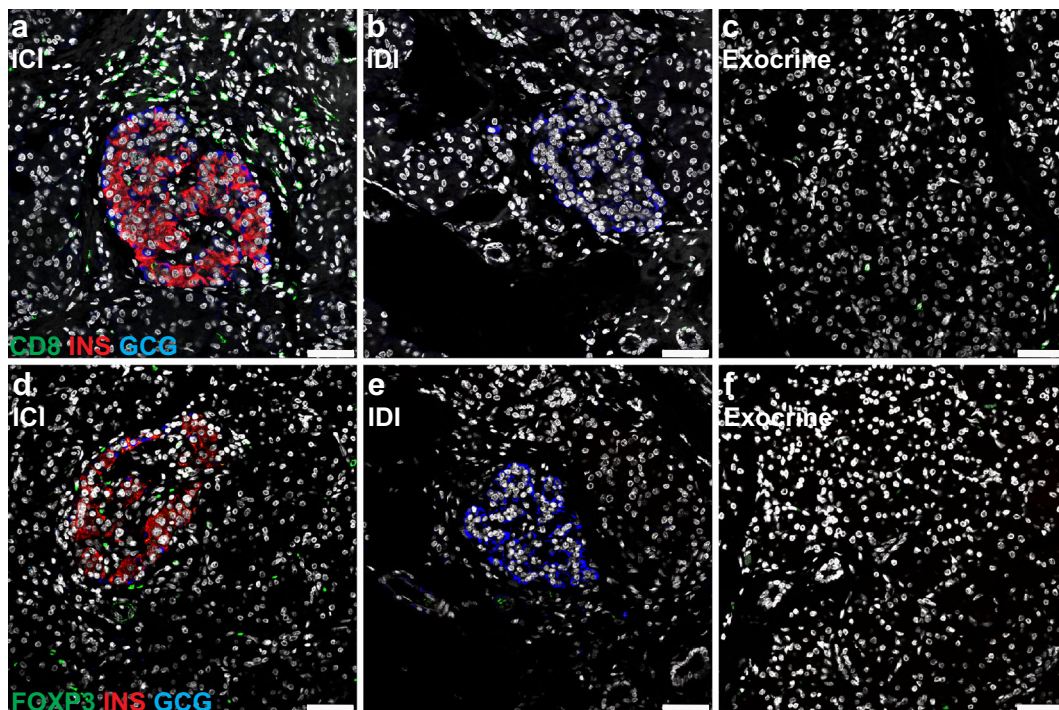


Fig. 3 Immunofluorescence images of pancreas sections at surgery. (a–c) Representative staining for CD8 (green), insulin (INS, red) and glucagon (GCG, blue) of one ICI (a), one IDI (b) and of exocrine tissue (c). (d–f)

Representative staining for FOXP3 (green), INS (red) and GCG (blue) of one ICI (d), one IDI (e) and of exocrine tissue (f). Scale bars, 50 μ m

This insulinitis displayed a preferential peri-islet pattern and an unusual FOXP3⁺ infiltrate suggestive of an ongoing regulatory process.

Regulatory polarisation of circulating beta cell-reactive T cell responses wanes over time Given the hallmarks of heterogeneous beta cell loss and preservation along with the regulatory features of insulinitis observed in pancreatic sections, we analysed the responses against beta cell antigens of circulating T cells obtained 4 and 9 years after surgery, in separate experiments (Fig. 5). We employed an assay that stimulates unfractionated PBMCs with protein/polypeptide antigens to detect CD4⁺ and, to a lesser extent, CD8⁺ T cell responses, without biasing towards IFN- γ or IL-10 responses [8]. Weak IFN- γ responses were initially present, mainly against proinsulin and IA-2, accompanied by a dominant IL-10 response against proinsulin and, to a lesser extent, C-peptide, GAD and insulin. In the follow-up sample taken 5 years later (i.e. after anti-ZnT8 seroconversion and the rise in insulin needs), IL-10 responses remained dominant but did not increase significantly. Conversely, IFN- γ responses were more robust against all the beta cell antigens tested, and significantly increased overall ($p = 0.03$). Both IFN- γ and IL-10 responses remained stable against the adenoviral lysate and PHA positive controls. The dominant IFN- γ responses detected in two age-matched individuals with type 1 diabetes are shown in Fig. 5 for comparison.

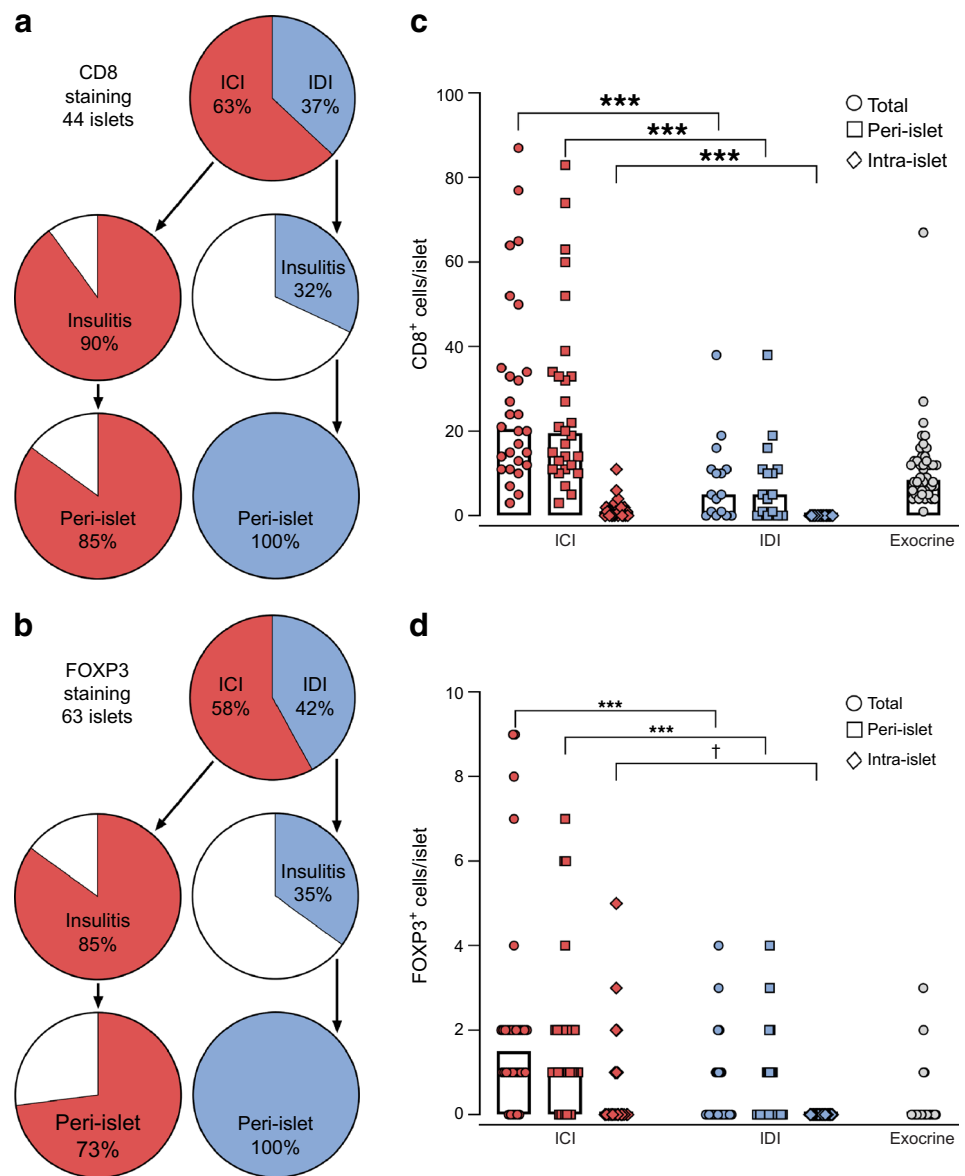
Collectively, these results suggest a pattern of T cell autoimmunity with an initial regulatory polarisation that waned over time.

Discussion

Several lines of evidence support the diagnosis of type 1 diabetes in this woman: positive and increasing GADA titres; the late appearance of anti-ZnT8 aAbs; the associated thyroid autoimmunity and positivity for anti-smooth muscle cell aAbs; and the histopathological finding of insulinitis and decreased beta cell area 6 years after diabetes diagnosis. However, this individual maintained some endogenous insulin secretion for up to 8 years post-surgery, suggesting a slowly evolving form of type 1 diabetes. A plausible histopathological correlate of this slow clinical progression was an insulinitis pattern enriched in FOXP3⁺ T cells. Additionally, in peripheral blood, T cell responses against islet antigens were skewed towards secretion of the regulatory cytokine IL-10, while IFN- γ is usually found in individuals with type 1 diabetes [13].

Given this person's age and the higher degree of beta cell loss and insulinitis observed in children [14–17], the most informative comparison is with published case series of adult recent-onset type 1 diabetes and at-risk single-aAb⁺ cases. Moreover, quantification of insulinitis in the pancreas of living

Fig. 4 Immunofluorescence quantification. **(a, b)** Fraction of ICIs and IDIs, insulinitis-positive ICIs and IDIs and peri-islet insulinitis in insulinitis-positive ICIs and IDIs in CD8-stained sections **(a)** and FOXP3-stained sections **(b)**. CD8⁺ and FOXP3⁺ insulinitis was scored as positive in islets with ≥ 15 CD8⁺ cells and ≥ 1 FOXP3⁺ cells, respectively. $p \leq 0.001$ for the comparison between insulinitis-positive ICIs and IDIs by Fisher's exact test (for both CD8 and FOXP3 staining). **(c, d)** Number of CD8⁺ cells **(c)** and FOXP3⁺ cells **(d)** in ICIs (red symbols), IDIs (blue symbols) and exocrine tissue (grey symbols). For ICIs and IDIs, cell numbers are displayed for total islets (circles) and for those displaying a peri-islet infiltrate (squares) and an intra-islet infiltrate (diamonds). *** $p \leq 0.001$, † $p = 0.02$ by Mann–Whitney *U* test



individuals with type 1 diabetes is seldom available [18, 19], and the best-matched case series may be the one reported by the Diabetes Virus Detection (DiViD) study on six living individuals aged 24–35 years with recent-onset type 1 diabetes [19].

First, in the present case, 55% of the islets were insulin-positive, which is in the 18–66% range observed in the DiViD study. The proportion of ICIs and insulinitis-positive islets was very variable across DiViD participants and, like in our case, from one section to another [19]. It should however be noted that the sections analysed herein were probably more distant between each other than the ones in the DiViD study. The beta cell area in our patient was 0.27% of the total pancreas surface, which is even lower than the 0.44–1.2% observed in DiViD participants [19], and much lower than the average 0.8% observed in single-aAb⁺ non-diabetic adult donors [20].

Moreover, the beta cell/islet area ratio was lower than for alpha cells, whereas this ratio, although highly variable, is higher for beta cells in non-diabetic individuals [21, 22]. Thus, several lines of evidence suggest that significant beta cell loss took place before surgery. Moreover, this beta cell loss may have been underestimated because we only had access to tissue from the head of the pancreas, which is the less affected region in individuals with type 1 diabetes [23].

Second, CD8⁺ lymphocytic infiltrates were detected in 36% of islets (28% peri-islet plus 8% intra-islet) as compared with 5–58% of CD3⁺ ICIs in the DiViD study (i.e. 5%, 12%, 23%, 33%, 52% and 58% for the six DiViD participants) [19]. Also in other type 1 diabetes case series, the fraction of infiltrated islets is typically low (<10%) [10, 14, 15]. At earlier stages of the disease, little or no insulinitis is observed (1–9% of islets) in single- and multiple-aAb⁺ donors, even in those with

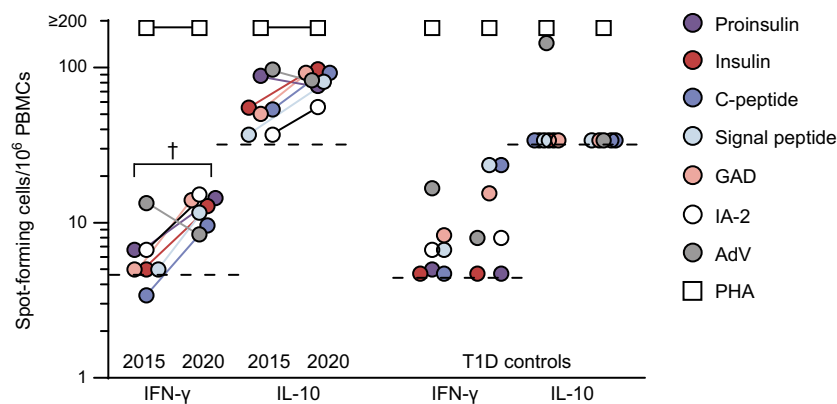


Fig. 5 IFN- γ and IL-10 T cell responses against beta cell autoantigens 4 and 9 years after surgery (2015, 2020). Frozen–thawed PBMCs were analysed by enzyme-linked immunospot (ELISpot) following a 48 h culture in the presence of the indicated beta cell antigens, an adenoviral (AdV) lysate and PHA positive controls or no antigen. Each symbol represents the mean number of spot-forming cells/ 10^6 PBMCs from

triplicate wells. The dashed line indicates the positive cut-off, corresponding to 2 SD above mean values from wells stimulated in the absence of antigen. The responses detected in two age-matched individuals with type 1 diabetes (T1D) are shown for comparison. $^\dagger p = 0.03$ by Wilcoxon signed rank test

predisposing HLA haplotypes [10, 15, 24, 25]. Thus, substantial insulinitis lesions were present in this patient; more extensive than those usually observed in type 1 diabetes.

Third, the striking histopathological hallmark of the present case was the abundance of infiltrating FOXP3⁺ cells, which are virtually absent in the pancreas of donors with type 1 diabetes, and even in non-diabetic individuals [10]. In a series of 16 individuals with recent-onset type 1 diabetes, scattered FOXP3⁺ cells were detected in two islets of one single individual and not in non-diabetic control individuals, using the same antibody employed herein [26]. Similarly, *FOXP3* gene expression in the DiViD series was modest [19]. Moreover, the insulinitis of individuals with type 1 diabetes mostly displays a peri-islet localisation, with very little islet invasion [10, 25]. This was also the case of the woman in our study, as all T cells were predominantly observed around the islets. However, FOXP3⁺ cells were also found inside the islets.

This FOXP3-enriched insulinitis was mirrored in the blood by the presence of islet-reactive T cells that were skewed towards a regulatory, IL-10-secreting phenotype, as reported in individuals with slow-progressing type 1 diabetes and healthy adults [13, 27], and in children and adolescents with ‘partially regulated’, IL-10⁺, pauci-aAb⁺, pauci-immune type 1 diabetes [16].

Despite significant beta cell loss and islet infiltration, insulin requirements remained modest over time, suggesting a slowly evolving islet destruction. Although the weight loss after surgery likely contributed to these reduced insulin needs, this lack of progression is striking when considering the additional beta cell deprivation imposed by duodenopancreatectomy. Collectively, these histopathological, immunological and clinical findings suggest that, after having destroyed a significant beta cell fraction, the autoimmune reaction remained efficiently regulated over several years. We may speculate that the autoimmune process in the islets may have been hindered by exposure to

the tumour microenvironment, which is often enriched in suppressive cytokines such as TGF- β that drive Treg differentiation [28]. Indeed, *TGFBI* mRNA was reportedly expressed in two pancreatic serous cystadenomas [29]. The strong CD8⁺ T cell infiltration may have been another driver for FOXP3⁺ T cells, as suggested in the NOD mouse [30].

Finally, the late increase in GADA titres, anti-ZnT8 seroconversion and increase in circulating IFN- γ T cell responses against islet antigens concomitant with increased insulin needs suggest that immune regulation was eventually lost. This progression may be in favour of a relapsing–remitting model of type 1 diabetes [4]. Several autoimmune diseases (e.g. vitiligo, multiple sclerosis and rheumatoid arthritis) follow a course marked by flares alternating with remission periods. In the present case, the early histopathological findings and late clinical and autoimmune progression are consistent with an initial autoimmune flare followed by several years of remission before eventual relapse. Nonetheless, some endogenous insulin secretion was maintained. In addition, in other autoimmune conditions, end-stage organ failure is not invariably attained even after significant tissue loss. For instance, subclinical hypothyroidism (i.e. positivity for anti-thyroperoxidase aAbs and increased thyroid-stimulating hormone levels with normal thyroxine levels) is reported to progress to overt hypothyroidism at a rate of only 4% per year [31]. Indeed, the prevalence of subclinical aAb⁺ conditions is largely higher than that of overt functional failure for most organ-specific autoimmune diseases [32, 33]. In the case of type 1 diabetes, most slow-onset forms are associated with isolated GADA, as initially observed in this person. While most individuals eventually require insulin treatment, one-third remains insulin-free [34]. However, in the context of pancreas transplantation, anti-ZnT8 seroconversion has been shown to predict loss of graft function [35]. A similar phenomenon may have occurred in our patient.

In conclusion, we here describe the case of a woman with type 1 diabetes who, despite positivity for GADA and other aAbs, significant insulinitis and beta cell loss, and partial pancreatectomy, remained well controlled with low-dose insulin until anti-ZnT8 seroconversion 14 years after diabetes onset. Both islet infiltrates and circulating islet-reactive T cells were skewed towards a regulatory phenotype. These observations suggest an islet autoimmune process that remained spontaneously regulated for several years after substantial beta cell destruction. Defining the mechanisms behind these indolent forms of more or less ‘benign’ autoimmunity may lead to better understanding of type 1 diabetes physiopathology and to the definition of novel therapeutic targets [36, 37].

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Data availability Data are available from the authors on request.

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