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Resident memory CD8(+) T cells dominate lymphoid immune cell population in human pancreatic islets in health and type 2 diabetes

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ABSTRACT

Introduction In type 2 diabetes (T2D), beta cell failure is often associated with islet inflammation driven by the innate immune response, with macrophages playing a significant role. However, the composition and phenotype of lymphoid immune cells in the islets of individuals with T2D have not been extensively studied. This study aims to characterize and compare the presence, phenotype, and frequency of islet-associated lymphocytes—specifically T, B, and natural killer (NK) cells—in patients with T2D and non-diabetic organ donors.

Research design and methods Multicolor flow cytometry was employed to detect NK, B, and T cells in dissociated pancreatic islets from 13 T2D and 44 non-diabetic donors. The frequencies and phenotypes of T cell subsets were determined using markers for memory differentiation status and tissue-resident T cells. The frequencies of alpha and beta cells were assessed by flow cytometry, and the insulin secretion level was measured by ELISA.

Results In both T2D and non-diabetic islets, CD3(+) T cells were the predominant lymphocytes, mainly central and effector memory phenotypes, with a bias toward CD8(+) T cells expressing canonical residency markers (CD69 and CD103). The frequencies of CD19(+) B cells and CD3(–) CD16(+) CD56(+) NK cells were low in both groups. The proportions of these immune and beta cells were similar between T2D and non-diabetic donors. However, T2D donors had a higher proportion of glucagon-producing alpha cells and significantly reduced glucose-stimulated insulin secretion compared with non-diabetic individuals.

Conclusions In T2D islets, resident CD8(+) T cells with a central memory phenotype dominate the lymphoid immune cell population, similar to non-diabetic donors. These findings provide the first insights into the memory T cell composition in human pancreatic islets in T2D, suggesting that the diabetic condition does not significantly alter the lymphoid landscape of pancreatic islets.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The progression of type 2 diabetes (T2D) involves a complex inflammatory process characterized by cytokines, chemokines, and immune cells, with macrophages being the primary drivers. Despite the well-documented influence of these innate immune processes, the role of lymphoid cells, particularly T lymphocytes, in T2D remains unclear.

WHAT THIS STUDY ADDS

⇒ The study identifies resident CD8(+) T cells, particularly those with a central memory profile, as the predominant lymphoid cell type in T2D pancreatic tissue.

⇒ The study reveals that the lymphoid cell composition and T cell memory status are similar between patients with T2D and non-diabetic donors, suggesting that diabetes does not significantly alter the immune landscape within the pancreatic islets.

⇒ The study highlights a higher proportion of glucagon-producing alpha cells in T2D donors, while insulin-producing beta cells remain stable in proportion despite decreased functionality.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The findings provide novel insights into the immune and endocrine cell composition of pancreatic islets in T2D, suggesting that alterations in immune cell function, rather than their quantity, may be crucial to beta-cell dysfunction. These insights could significantly impact future research and guide the development of more targeted and effective T2D treatments.

INTRODUCTION

Type 2 diabetes (T2D) is a chronic metabolic disorder characterized by low-grade inflammation, driven by immune dysregulation, metabolic syndrome, and obesity.[1] Innate immune mechanisms are involved in the inflammatory processes observed in T2D, with early and advanced stages marked by immune cell infiltration in the pancreatic islets, particularly by monocytes and macrophages, and elevated levels of proinflammatory cytokines.[2–4] Clinical trials targeting macrophages and the IL-1 signaling pathway have demonstrated potential in reducing inflammation and improving insulin secretion and glycemic control in T2D, further supporting the notion

that inflammation significantly contributes to T2D pathogenesis.[5 6]

Emerging evidence suggests that adaptive immune cells also contribute to islet dysfunction in T2D.[7–10] Early studies identified an increase in CD45(+) cells, notably CD20(+) B lymphocytes, in the pancreas of patients with T2D.[11] While some research indicated elevated CD8(+) T cells in the exocrine pancreas of individuals with T2D, these cells were not found in the islets.[12] Recent single-cell and multispectral imaging mass cytometry studies, however, have revealed an increased density of human leucocyte antigen (HLA)-DR-positive CD8(+) T cells within the islets of the human pancreas in T2D, suggesting that activated cytotoxic T cells may contribute to islet inflammation in T2D.[13] Despite these findings, the precise role of resident CD8(+) T lymphocytes in islet biology and the progression of islet dysfunction in T2D remains unclear.

Tissue-resident memory T (TRM) cells are a distinct subset of memory T cells that reside permanently in non-lymphoid organs without recirculating through the bloodstream or lymph nodes.[14–16] Under normal conditions, TRM cells provide immediate local immune protection against pathogens. Defective accumulation of TRM cells can impair immune responses, increase viral loads, and predispose individuals to recurrent infections.[17] TRM cells also play a crucial role in recruiting immune cells from the bloodstream to maintain ongoing immune activity.[18]

Building on our previous identification of TRM cells as the predominant T cell population in human pancreatic islets from non-diabetic donors, predominantly exhibiting a central memory phenotype,[19] this study addresses a significant gap in understanding the lymphoid immune cell composition and phenotype in the islets of individuals with T2D. We aim to characterize and compare the lymphocyte populations—specifically T, B, and NK cells—in isolated and dispersed islets from patients with T2D and non-diabetic organ donors using fluorescence-activated cell sorting (FACS) analysis.

RESEARCH DESIGN AND METHODS

Human pancreatic islets

The human pancreatic islets used in this study were obtained from 57 brain-dead organ donors, including 44 non-diabetic donors without pancreatic disease and 13 donors with T2D. These islets were provided by the Nordic Network for Clinical Islet Transplantation Program (www.nordicislets.org) through the Human Tissue Laboratory at Lund University Diabetes Center in Malmö, Sweden, which is funded by the Excellence of Diabetes Research in Sweden (EXODIAB) network (www.exodiab.se/home). The islets were isolated and prepared at Uppsala University following a methodology approved by the local ethics committee, as previously described.[20] Quality tests were performed on homogenized isolated islets using a Gyrolab workstation (Gyros, Uppsala, Sweden), and purity was assessed by dithizone staining of individual islet preparations. The sex, donor age, body mass index (BMI), hemoglobin A1c values (expressed as both percentage and mmol/mol), and the stimulatory index (a measure of glucose-stimulated insulin secretion from human islets cultured in vitro) are presented in online supplemental tables S1–S3. Consent for organ donation (both for clinical transplantation and for use in research) was obtained from the relatives of the deceased donors by the donor's physicians and documented in the medical records. All procedures were approved by the Swedish Ethical Review Authority (permit numbers 2007–483 and 2011–263), in accordance with the Act Concerning the Ethical Review of Human Research. The islets were cultured in CMRL-1066 (ICN Biomedicals, Costa Mesa, California, USA) supplemented with 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 2 mM L-glutamine, 50 mg/mL gentamycin, 0.25 mg/mL fungizone (Gibco BRL; Invitrogen, Paisley, UK), 20 mg/mL ciprofloxacin (Bayer Healthcare AG, Leverkusen, Germany), and 10% heat-inactivated human serum at 37°C in 5% carbon dioxide and humidified air for 1–7 days before single cell suspension preparation.

Dissociation of islet cells

Human pancreatic islets (1500–10 000 islet equivalents) were centrifuged at 150×*g* for 1 min, and the supernatant was carefully aspirated. The pellet was washed three times with 12 mL of phosphate-buffered saline (PBS 1×), each time centrifuging at 150×*g* for 1 min. After the final wash, PBS was aspirated, and the pellet was gently loosened. Islets were dissociated into single-cell suspensions by adding 500 µL of Accutase (Becton Dickinson, Franklin Lakes, New Jersey, USA) and incubating at 37°C for 8–10 min, with gentle pipetting every 2 min. The enzymatic reaction was stopped by adding 10 mL of CMRL 1066 medium, followed by a 5–10 min resting period. The sample was centrifuged at 320×*g* for 8 min, and the resulting pellet was resuspended in 200–500 µL of CMRL 1066.

Insulin and glucagon staining

To analyze endocrine cell content, approximately 50 000 dissociated cells were fixed and permeabilized by adding 25 µL Fix/Perm (Thermo Fisher Scientific, Waltham, Massachusetts, USA) to a 50 µL cell suspension. The mixture was vortexed and incubated at room temperature for 15 min. Subsequently, 125 µL of Perm Buffer containing antibodies specific for insulin (rabbit anti-human IgG, Cell Signaling Technology, Danvers, Massachusetts, USA) and glucagon (mouse anti-human IgG2a, clone 181402, R&D Systems, Minneapolis, Minnesota, USA) was added. These antibodies were conjugated with phycoerythrin and allophycocyanin, respectively, using Lightning-Link technology (Innova Bioscience, Cambridge, UK). After incubation for 1 hour at room temperature, cells were washed with 2 mL PBS,

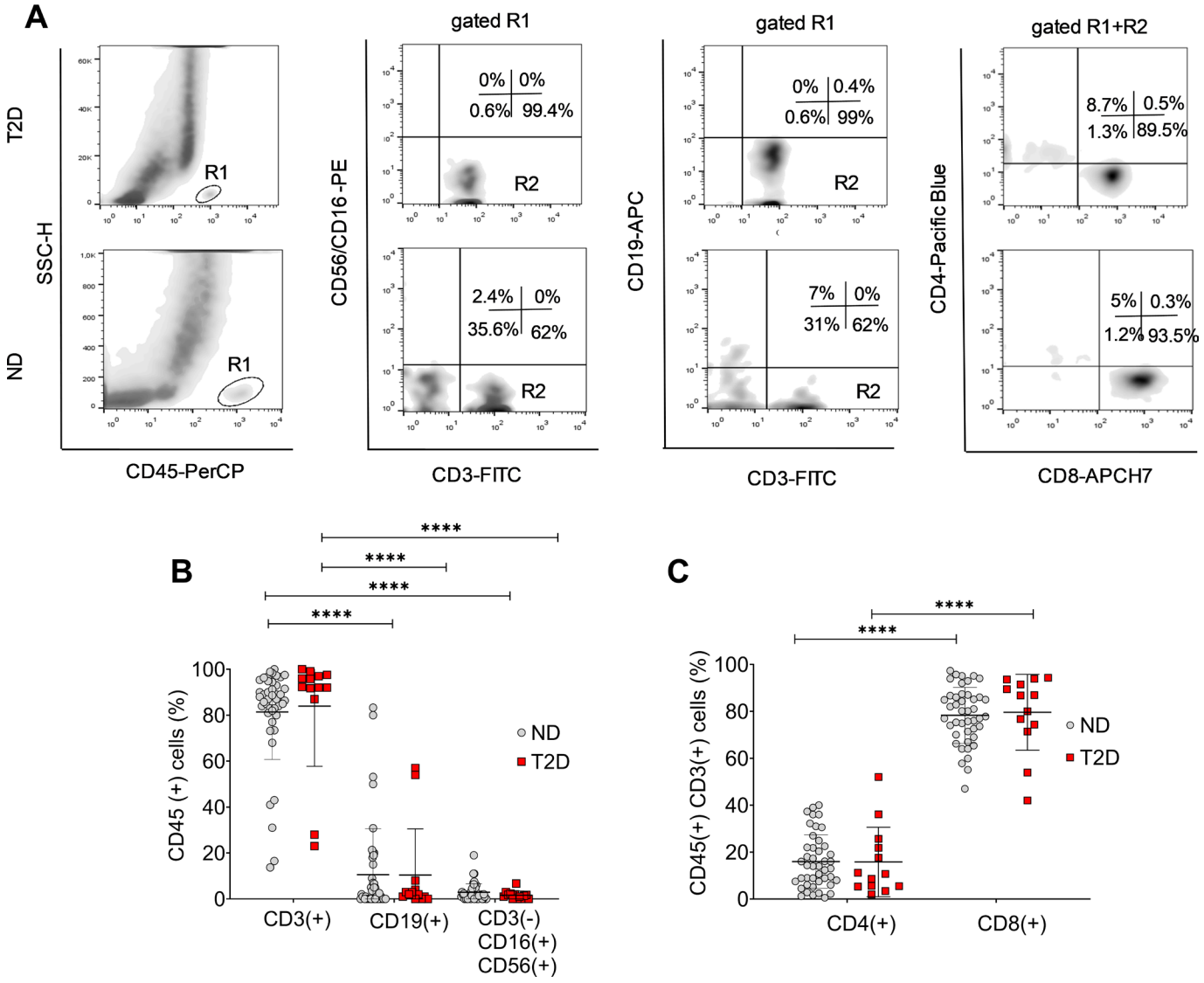

Figure 1 Lymphoid composition in pancreatic islets from ND and T2D organ donors. (A) Representative flow cytometry plots showing the gating strategy used to define the total CD45(+) lymphocyte population by CD45 and side scatter and to identify total CD3(+) T cells, CD3(–)CD16(+)CD56(+) NK cells, and CD19(+) B cells, as well as CD4(+) and CD8(+) T cells in dissociated human pancreatic islets. (B) Cumulative analysis of the proportions of CD3(+) T cells, CD19(+) B cells, and CD3(–)CD16(+)CD56(+) NK from 44 ND and 13 T2D organ donors. (C) Cumulative analysis of the proportions of CD4(+) and CD8(+) T cells within the T cell compartment in dissociated pancreatic islets from 44 ND and 13 T2D organ donors. Data are presented as mean percentages±SD. Each dot represents an individual donor. Differences in the proportion between the groups were compared using the Mann-Whitney test. ****p<0.0001. ND, non-diabetic; NK, natural killer; T2D, type 2 diabetic.

centrifuged at 320×*g* for 5 min, and resuspended in the staining buffer for flow cytometry acquisition.

Lymphocyte staining

For lymphocyte characterization, dissociated cells were preincubated with a 1:20 dilution of FC-block (BD Biosciences, San Jose, California, USA) for 5 min at room temperature. Following this, 25 µL of an antibody mix targeting key lymphocyte markers was added. The mix included antibodies against CD3 (fluorescein isothiocyanate (FITC), clone UCHT1), CD56 (PE-Cy7, clone B159), CD19 (APC, clone SJ25C1), CD8 (APC-Cy7, clone SK1), CD4 (PacBlue, clone RPA-T4), CD25 (FITC, clone M-A251), CD45RO (PE-Cy7, clone UCHL-1), CD69 (FITC, clone FN50), CD103 (PE-Cy7, clone Ber-ACT8), CD27 (APC, clone O323), CD16 (PE, clone 5D2), and CD45 (PerCP, clone HI30). All antibodies were sourced from BD Pharmingen, BioLegend, or eBioscience and optimized for dilution and specificity using isotype controls. After 20 min of incubation in the dark at 4°C, cells were washed with CMRL 1066, centrifuged at 320×*g* for 2 min, and the supernatant was aspirated. The pellet was transferred to FACS tubes containing staining buffer for acquisition.

Flow cytometric data acquisition and analysis

Flow cytometry was performed on a CyAN ADP system (Beckman Coulter, Brea, California, USA). Fluorescence

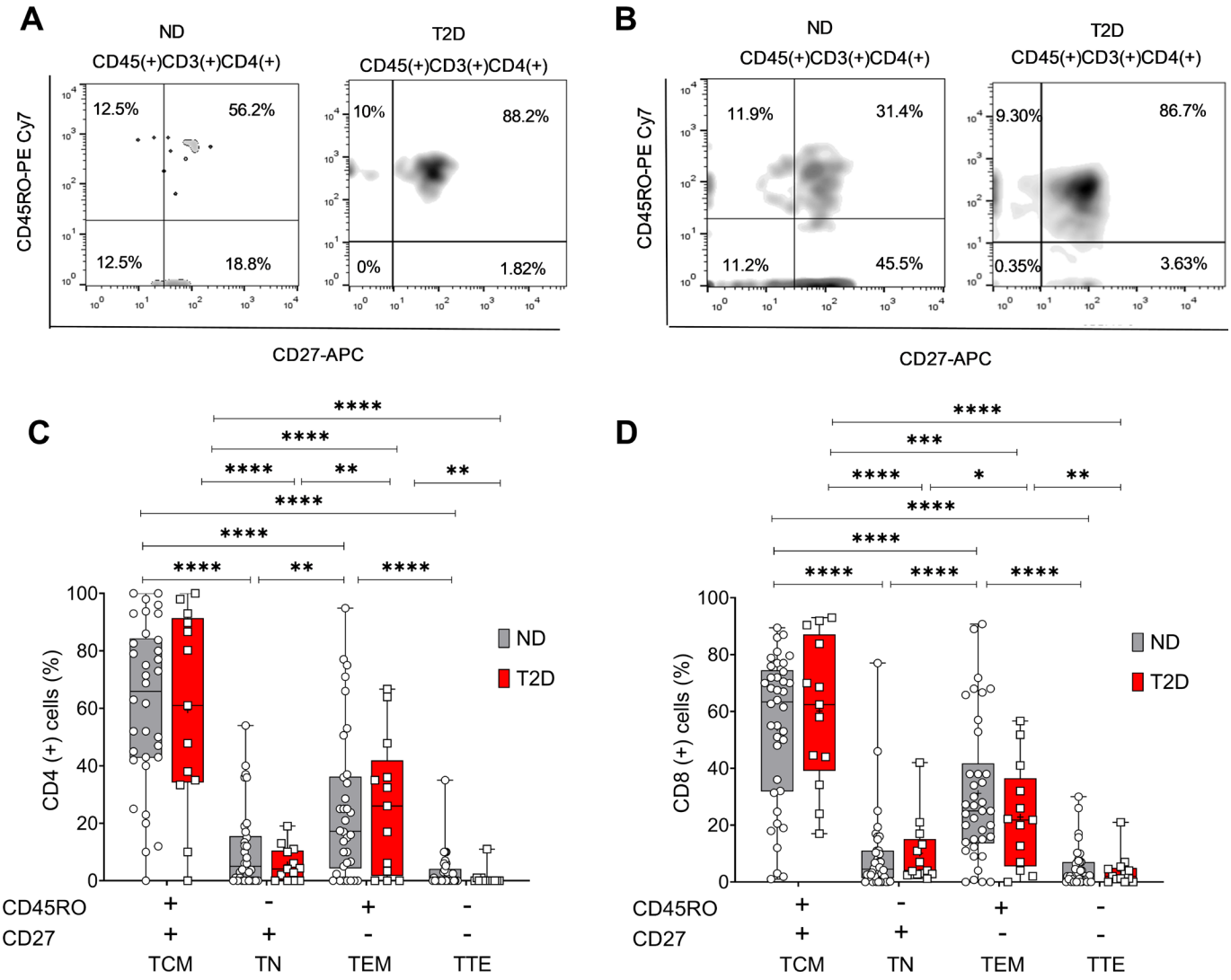

Figure 2 Differentiation stages and memory status of CD45(+)CD3(+) T cell populations in pancreatic islets from ND and T2D organ donors. Representative flow cytometry plots showing naive and memory subpopulations of (A) CD4(+) T cells and (B) CD8(+) T cells. (C) Cumulative analysis of the proportions of CD45RO(+)CD27(+) TCM, CD45RO(–)CD27(+) TN, CD45RO(+) CD27(–) TEM, and CD45RO(–)CD27(–) TTE phenotypes within the CD4(+) populations in dissociated islets from 34 ND and 13 T2D donors. (D) Cumulative analysis of the proportions of CD45RO(+)CD27(+) TCM, CD45RO(–)CD27(+) TN, CD45RO(+) CD27(–) TEM, and CD45RO(–)CD27(–) TTE phenotypes within the CD8(+) populations in dissociated islets from 38 ND and 13 T2D donors. Results are presented as box and whisker plots showing the median. Each dot represents an individual donor. Differences in the proportion between the groups were compared using the Mann-Whitney test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. ND, non-diabetic; T2D, type 2 diabetic; TCM, central memory T cells; TEM, effector memory T cells; TN, naive T cells; TTE, terminal effector T cells.

compensation was carried out using fluorescence-minus-one controls for each antibody panel. Data were analyzed using FlowJo software (V.7.6.5, TreeStar, Ashland, Oregon, USA).

Glucose-stimulated insulin secretion in human pancreatic islets

Islets were handpicked at room temperature and preincubated for 30 min at 37°C in Krebs-Ringer bicarbonate (KREBS) buffer, pH 7.4, containing (mmol/L): 120 sodium chloride, 25 sodium bicarbonate, 4.7 potassium chloride, 1.2 magnesium sulfate, 2.5 calcium chloride, and 1.2 potassium dihydrogen phosphate. The buffer was bubbled with a mixture of 95% oxygen and 5% carbon dioxide and supplemented with 1 mmol/L glucose and 1 mg/mL bovine serum albumin (Fraction V). Thereafter, batches of 10–12 size-matched islets were incubated for 60 min at 37°C in 1 mL of KREBS buffer containing either 1 mM glucose (for basal secretion) or 16.7 mM glucose (for stimulated secretion). At the end of the incubation period, insulin levels were measured by ELISA (#10-1113-01, Mercodia, Uppsala, Sweden). Glucose-stimulated insulin secretion was expressed as ng/islet/60 min.

Statistical analysis

Statistical analyses were performed using Prism V.9.1.1 (GraphPad Software, San Diego, California, USA) and

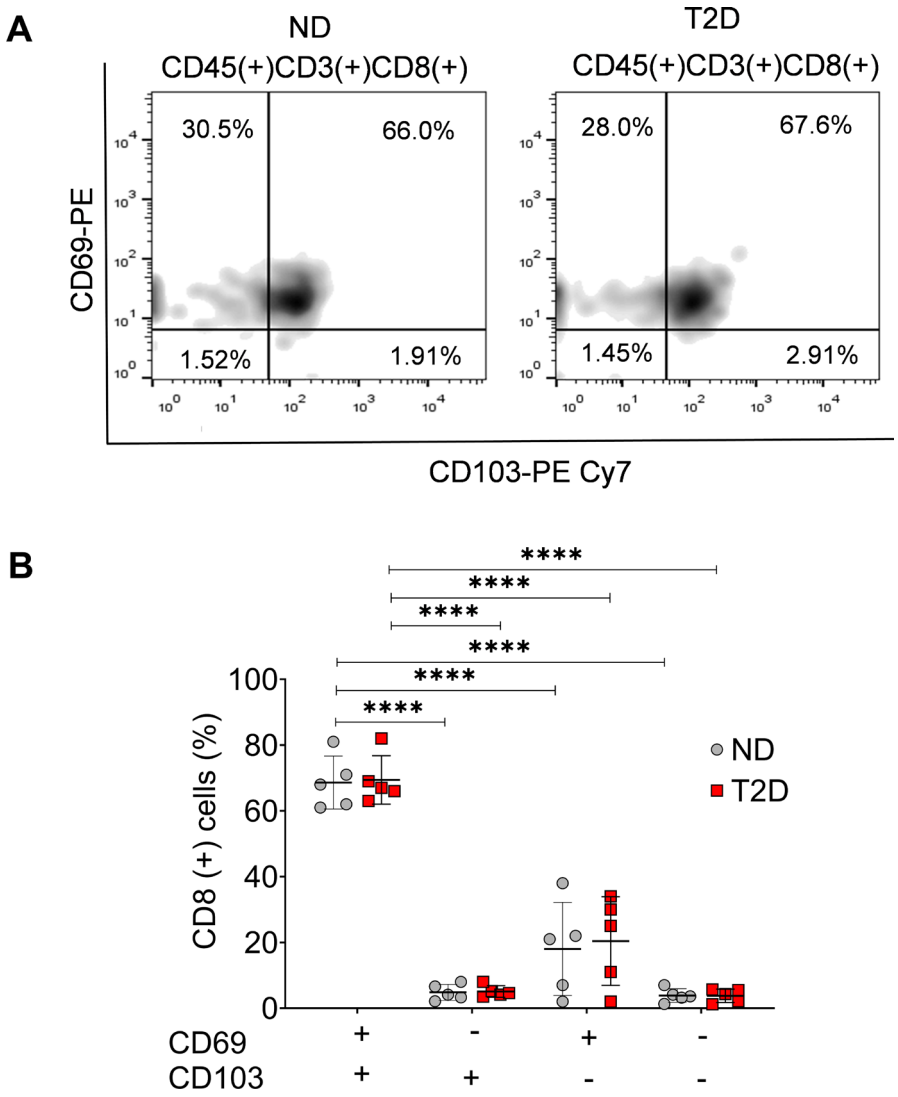

Figure 3 Characterization of resident CD8(+) T cells in pancreatic islets from ND and T2D organ donors. (A) Representative flow cytometry plots showing the expression of CD69 and CD103 on CD8(+) T cells in islet cell suspensions. (B) Cumulative analysis of CD8(+) T cells expressing the CD69 and CD103 from five ND and five T2D organ donors. Data are presented as mean percentages±SD. Each dot represents an individual donor. Differences in the proportion between the groups were compared using the Mann-Whitney test. ****p<0.0001. ND, non-diabetic; T2D, type 2 diabetic.

SPSS V.20 for Windows (SPSS, Chicago, Illinois, USA). Data are presented as mean±SD. Clinical characteristics between T2D and non-diabetic controls were compared using two-sample, two-tailed t-tests. Mann-Whitney tests were used for experiments involving two groups. For experiments with four groups, the Kruskal-Wallis test was employed, followed by pairwise comparisons using Dunn's method. Statistical significance was set at p<0.05, with significance levels indicated as follows: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

RESULTS

CD8(+) T cells, primarily with a central memory phenotype, are a major component of the lymphoid immune cell population in islets, with no significant differences between type 2 diabetic and non-diabetic donors.

We initially analyzed the lymphoid composition of human pancreatic islets using multicolor flow cytometry on CD45(+) T cells from dispersed islets of 44 non-diabetic and 13 T2D organ donors. The results revealed that CD3(+) T cells were the predominant lymphocyte population in both T2D (84.73±26.45) and non-diabetic islets (81.65±21.40). These CD3(+) T cells were significantly more frequent (p<0.0001) compared with CD19(+) B cells and CD3(−) CD16(+) CD56(+) NK cells in both groups (figure 1A,B). CD19(+) B cells represented a minor fraction of the lymphocyte population, comprising 10.38±20.14 in T2D and 10.58±20.6 in non-diabetic donors. Similarly, CD3(−) CD16(+) CD56(+) NK cells were present in minimal amounts, accounting for 1.61±1.81 in T2D and 2.88±3.75 in non-diabetic donors (figure 1A,B). Importantly, there were no significant differences in the proportions of these cell types between

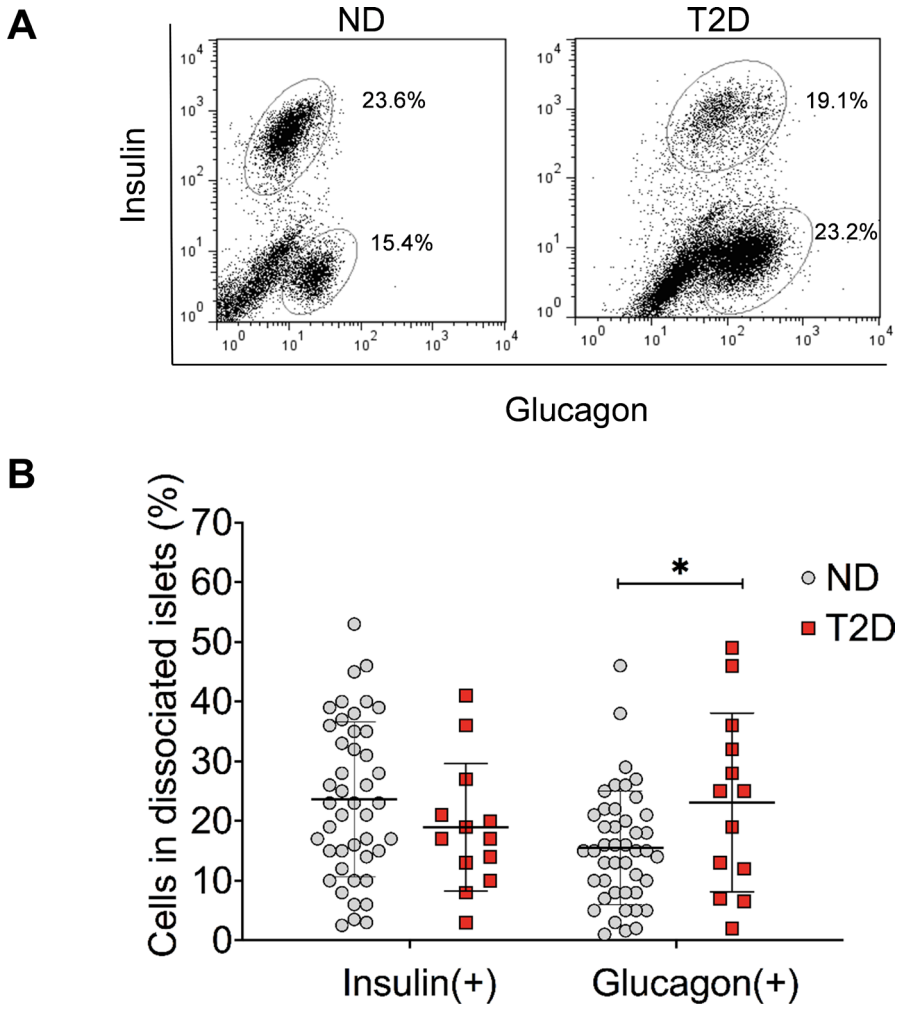

Figure 4 Percentages of pancreatic insulin and glucagon-positive cell populations in pancreatic islets from ND and T2D organ donors. (A) Representative flow cytometry analysis of islets cell suspension depicting the percentage of insulin and glucagon-positive cells. (B) Cumulative analysis of glucagon-producing alpha cells and insulin-producing beta cells from 44 ND and 13 T2D organ donors. Data are presented as mean percentages±SD. Each dot represents an individual donor. Differences in the proportion between the groups were compared using the Mann-Whitney test. *p<0.05. ND, non-diabetic; T2D, type 2 diabetic.

T2D and non-diabetic donors (figure 1B), suggesting that the overall lymphoid composition, with respect to CD3(+) T cells, CD19(+) B cells, and CD3(−)CD16(+) CD56(+) NK cells, remains consistent across diabetic status.

Further analysis of CD45(+) CD3(+) T cells revealed that CD8(+) T cells were the predominant subset in both T2D (79.59±16.16) and non-diabetic donors (78.30±11.94). In contrast, CD4(+) T cells were significantly less prevalent in both groups (T2D: 15.80±14.77, p<0.0001; non-diabetic: 15.96±11.38, p<0.0001) (figure 1C). The comparative analysis of CD8(+) and CD4(+) T cell proportions between T2D and non-diabetic donors showed no significant differences, indicating that the distribution of these T cell subsets is also consistent regardless of diabetic status.

To further assess whether lymphoid composition in pancreatic islets is influenced by obesity, age, or diabetic status, we stratified organ donors by BMI into three distinct categories: normal weight (BMI: 20–24.9; non-diabetic, n=19; T2D, n=6), overweight (BMI: 25–29.9; non-diabetic, n=20; T2D, n=3), and obese (BMI: ≥30; non-diabetic, n=5; T2D, n=4). We analyzed the proportions of CD3(+) T cells, CD19(+) B cells, CD3(−)CD16(+) CD56(+) NK cells, CD4(+) T cells, and CD8(+) T cells across these groups. Notably, our analysis revealed no statistically significant differences in these populations, indicating that lymphoid composition in pancreatic islets is not significantly influenced by BMI or diabetic status (online supplemental figure 1A–E and online supplemental table S4). To further evaluate the potential impact of age, we stratified donors into two age intervals: 45–60 years and 61–81 years. Consistent with our findings from the BMI-stratified analysis, no significant differences in lymphoid cell composition were observed between T2D and non-diabetic donors within these age intervals (data not shown). Collectively, these findings demonstrate that the lymphoid landscape in pancreatic islets remains unaffected by BMI, age, or diabetic status.

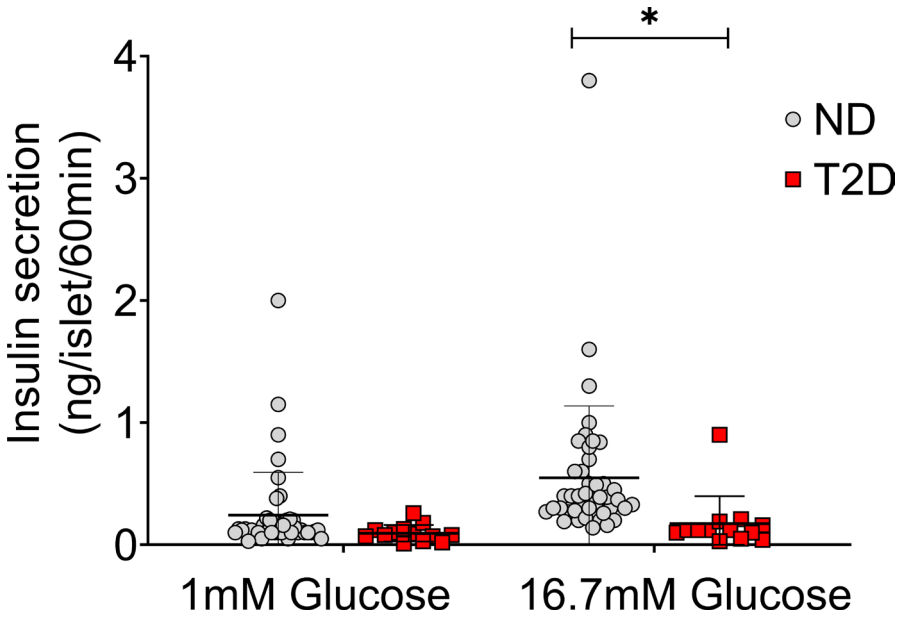

Figure 5 Insulin secretion in pancreatic islets from 44 ND and 13 T2D organ donors after stimulation with 1 mM glucose (basal secretion) and 16.7 mM glucose (stimulated secretion). Data are presented as mean±SD. Each dot represents an individual donor. Differences in the proportion between the groups were compared using the Mann-Whitney test. *p<0.05. ND, non-diabetic; T2D, type 2 diabetic.

To characterize the differentiation stages and memory status of CD45(+) CD3(+) T cell populations within pancreatic islets, we performed a detailed analysis of CD45RO and CD27 surface expression in dispersed islets from T2D donors and non-diabetic controls. Cells were classified into four distinct subsets: CD45RO(−) CD27(+) naive (TN), CD45RO(+)CD27(+) central memory (TCM), CD45RO(+)CD27(−) effector memory (TEM), and CD45RO(−)CD27(−) terminal effector (TTE). We quantified their proportions among total CD4(+) and CD8(+) T cells. Specifically, islets from 34 non-diabetic and 13 T2D donors were analyzed for the CD4(+) T cell memory compartment, while islets from 38 non-diabetic and 13 T2D donors were evaluated for the CD8(+) memory compartment. Our analysis revealed that the majority of CD4(+) and CD8(+) T cells exhibited a TCM and TEM phenotype in both T2D and non-diabetic donors, with TCM populations being predominant. In contrast, the frequencies of naive and terminal effector cells were significantly lower (figure 2A–D). There were no significant differences in the frequencies of TCM, TN, TEM, and TTE populations between T2D and non-diabetic donors (online supplemental tables S5 and S6).

CD8(+) T cells in T2D donors have phenotypic characteristics of resident cells

To further characterize CD8(+) T cells in dispersed islets, we analyzed tissue-specific resident memory cell markers CD69 and CD103 in samples from five non-diabetic and five T2D donors. Our findings revealed that all T2D donors exhibited significantly higher expression of CD69(+)CD103(+) markers (69.4±7.37) compared with CD69(+)CD103(−) (20.4±13.4, p<0.0001), CD69(−) CD103(+) (5.08±1.78, p<0.0001), and CD69(−)CD103(−) (3.76±2.02, p<0.0001). This indicates that most CD8(+) T cells in the pancreatic islets of T2D donors display a TRM phenotype. Similar proportions of non-circulating CD8(+) T cells expressing the canonical residency markers CD69(+)CD103(+) (68.6±8.08, p=0.87) were also observed in the islets of non-diabetic donors (figure 3A,B).

T2D donors exhibit a higher proportion of glucagon-producing alpha cells but maintain similar proportions of insulin-producing beta cells compared with non-diabetic donors

Quantitative assessment of the composition of endocrine cells within the pancreatic islets showed that the percentage of glucagon-producing alpha cells was significantly higher in T2D donors (23.11%±14.98%, n=13) compared with non-diabetic donors (15.49%±9.51%, n=44, p=0.03). Conversely, the proportion of insulin-producing beta cells did not differ significantly between T2D donors (18.92%±10.68%, n=13) and non-diabetic donors (23.61%±12.99%, n=44, p=0.24) (figure 4A,B).

Decreased glucose-stimulated insulin secretion in type 2 diabetic islets

To evaluate islet functionality, we assessed insulin secretion in response to glucose stimulation in islets from 44 non-diabetic and 13 T2D organ donors. At a basal glucose concentration of 1 mM, T2D islets exhibited insulin release levels comparable to non-diabetic islets, indicating that basal insulin secretion is not significantly impaired in T2D islets. However, upon stimulation with a high glucose concentration of 16.7 mM, T2D islets released significantly less insulin than non-diabetic (p=0.02) (figure 5), indicating that glucose-stimulated insulin secretion is significantly impaired in T2D islets.

DISCUSSION

Current evidence underscores the critical role of myeloid cell recruitment, particularly monocytes and macrophages, as key pathological factors in T2D.[2 21] However, the composition of lymphoid cells in pancreatic islets of patients with T2D remains poorly understood. To address this gap, our study used multicolor flow cytometry to characterize the presence, phenotype, and frequency of islet-associated lymphocytes in patients with T2D compared with non-diabetic donors.

The major finding of this study is the predominance of CD3(+) T cells with central memory phenotypes in T2D islets, highlighting a significant dominance of CD8(+) T cells over CD4(+) T cells, CD19(+) B cells, and CD3(−)CD16(+)CD56(+) NK cells. Additionally, we identified a previously unrecognized subtype of T cells within the islets of patients with T2D using markers for the memory compartment (CD45RO and CD27) and canonical tissue-resident T cells. These findings support earlier studies that have shown a higher prevalence of CD8(+) T cells in T2D compared with other immune cell types[11–13] and advance our understanding by being the first to investigate their memory differentiation states thoroughly.

Interestingly, the consistency in lymphoid composition, with respect to the relative frequencies of CD3(+) T cells, CD19(+) B cells, CD3(−)CD16(+)CD56(+) NK cells, and the memory status of CD45(+)CD3(+) T cell populations within pancreatic islets between patients with T2D and non-diabetic donors suggests that diabetic status, BMI, or age does not significantly alter the lymphoid landscape of pancreatic islets. Further analysis of memory CD8(+) T cells, using integrin αE (CD103) and glycoprotein CD69 as markers of tissue-resident memory cells, revealed that CD8(+) T cells with a resident memory phenotype constitute the major subset of lymphoid cells in the pancreatic islets of T2D donors. Notably, there were no significant differences in the CD8(+) TRM cell proportion between islets from non-diabetic and T2D donors, suggesting that this specific population of non-circulating memory T cells persists in the islets of patients with T2D as part of a protective immune response against infectious agents rather than contributing to inflammation or cytotoxicity.

Moreover, our study provides additional insights into the composition of endocrine cells within the pancreatic islets of T2D and non-diabetic donors, complementing recent findings in the field.[22 23] One of the most striking findings was the significantly higher proportion of glucagon-producing alpha cells in T2D donors. In contrast, the proportion of insulin-producing beta cells remained relatively stable between T2D and non-diabetic donors. Despite this stability, T2D islets exhibited significantly reduced insulin secretion upon stimulation, suggesting that beta cell dysfunction in T2D occurs without a corresponding decrease in the relative abundance of beta cells within the islets.[24] These findings raise important questions about the role of alpha cells in T2D, particularly considering their increased proportion. However, the role of alpha cells remains controversial, with existing studies presenting conflicting findings.[25–27] The limited research on the fraction and mass of pancreatic alpha cells has led to inconsistent conclusions when comparing patients with T2D to non-diabetic subjects, possibly due to variations in study populations, analytical methods, or clinical factors. Further investigations are needed to elucidate the exact role and impact of alpha cells in the pathophysiology of T2D.

The study is limited to patients with T2D aged 45–81 years, with an average age of 62 years, which may not fully capture the progression of T2D, particularly in its early stages. During the initial phases of T2D development, the numbers of CD8(+) TRM cells may vary. Investigating the dynamics of CD8(+) TRM cells in the early stages of T2D could provide valuable insights into their role in establishing low-grade inflammation as the disease progresses. Additionally, the scarcity of islets recovered from T2D donors in our study may restrict the generalizability of our findings, highlighting the need for larger-scale studies to validate and extend our observations.

In conclusion, our findings indicate that, similar to non-diabetic controls, the majority of T cells in the pancreatic islets of T2D donors are CD8(+) TRM cells exhibiting a central memory phenotype. Although our study did not identify differences in the proportion of CD8(+) TRM cells between T2D individuals and non-diabetic donors, the results raise the possibility that the immunological environment of pancreatic tissue in T2D may be characterized by qualitative rather than quantitative changes. However, this hypothesis remains speculative and warrants further investigation through functional studies.

While contributing to tissue immune surveillance, these pancreatic resident leucocytes might also respond to adverse local microenvironmental conditions, leading to subsequent beta-cell dysfunction. Therefore, further research should focus on elucidating the functional roles and activation states of these cells to better understand whether this specific population of resident leucocytes differs in activation status and specific antigen responses between healthy and T2D conditions.

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Contributors MR: data curation, formal analysis, investigation, methodology, validation, writing the original draft, and writing the review and editing. JA: data curation, investigation, methodology and validation. LS: conceptualization, formal analysis, supervision, writing the original draft, and writing the review and editing. CMC: conceptualization, formal analysis, supervision, funding, writing the original draft, and writing the review and editing. LS and CMC 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 work. LS and CMC are joint last authors.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants. All procedures were approved by the Swedish Ethical Review Authority (Permit numbers 2007-483 and 2011-263), in accordance with the Act Concerning the Ethical Review of Human Research. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available in a public, open access repository. Not applicable.

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