# Immunological Characterization of the Pancreas and Secondary Lymphoid Organs of Diabetic and Autoantibody Positive Organ Donors

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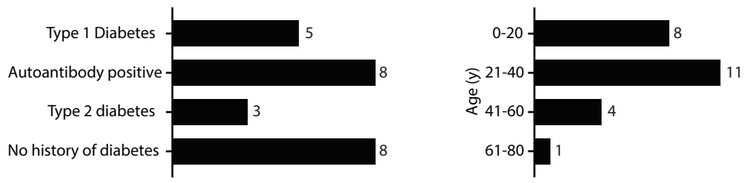
## Introduction - The Human Pancreas Analysis Program (HPAP)

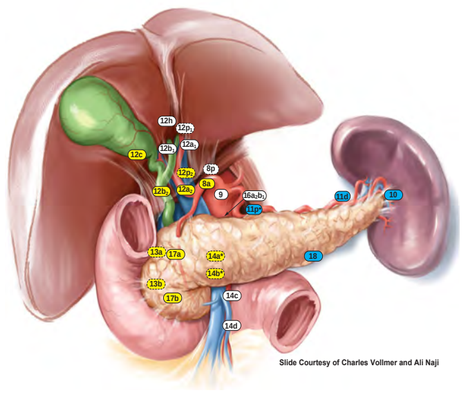
Type 1 diabetes (T1D) is characterized by the autoimmune destruction of insulin-producing beta cells in the pancreatic islets of Langerhans, resulting in dependency on exogenous insulin for glycaemia control. Auto-reactive cytotoxic CD8$^+$ T cells have been directly implicated as drivers of this destruction, but the in vivo functional and phenotypic characteristics of T1D-specific CD8$^+$ T cells within the pancreas itself, or its draining lymph nodes, remain to be clarified. Here, we provide an immunological characterization of the spleen, pancreas-draining lymph nodes and islets obtained by the HPAP consortium, with a focus on the detection of islet-specific CD8$^+$ T cells by peptide-MHC tetramers.

### Cohort




Samples obtained:

- Islets
- Blood
- Spleen
- Pancreatic LN (Head, Body, Tail)
- Mesenteric LN
- Superior Mesenteric Artery LN



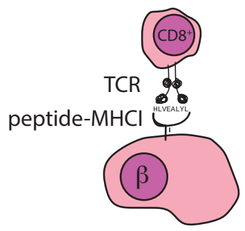

### Immunological characterization

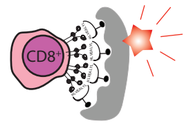
| Lineage | | Homing | |
|---|---|---|---|
| CD3 | CD16 | CCR4 | CXCR3 |
| CD4 | CD19 | CCR6 | CXCR5 |
| CD8 | CD34 | CCR7 | |
| CD11c | CD45 | | |
| CD14 | CD56 | | |
| CD15 | CD123 | | |

| Phenotype | |
|---|---|
| CD45RA | PD1 |
| CD27 | ICOS |
| CD25 | CD69 |
| CD127 | CD103 |
| HLA-DR | Ki67 |
| CD38 | GrzB |
| CD95 | Perforin |
| CD21 | Tbet |
| IgM | Eomes |
| IgD | Foxp3 |
| Bcl6 | |

## 1. CD8$^+$ T cells specific for islet epitopes can be detected in secondary lymphoid organs

### A. Peptide-MHC class I technology



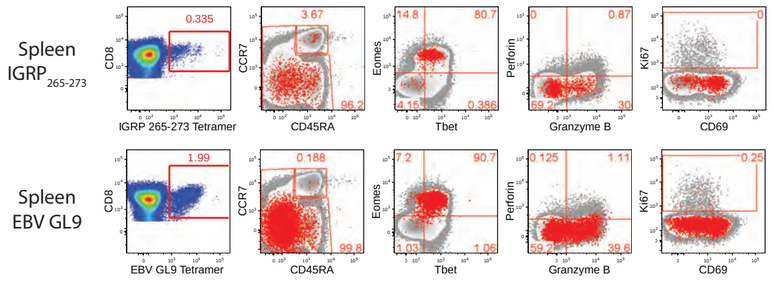




peptide-MHCI tetramer conjugated to fluorophore

| Epitope | Sequence |
|---|---|
| GAD65$_{114-122}$ | VMNILLQYV |
| InsB$_{10-18}$ | HLVEALYLV |
| IGRP$_{228-236}$ | LNIDLLWSV |
| IGRP$_{265-273}$ | VLFGLGFAI |
| IAPP$_{5-13}$ | KLQVFLIVL |
| EBV GL9 | GLCTLVAML |

### B. Islet-specific CD8$^+$ T cells have an antigen-experienced phenotype

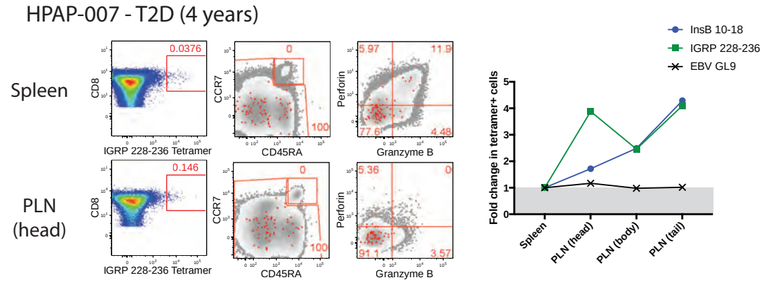
HPAP003 - Autoantibody positive (GAD65)



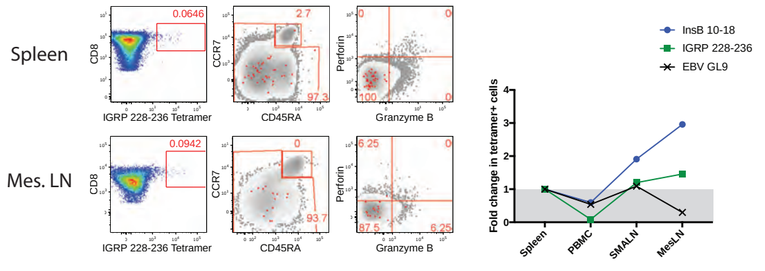

*Single-cell suspensions from the spleen of HPAP-003 were stained with tetramers and cell surface markers before being fixed, permeabilized and stained for intracellular markers. Data were acquired with a BD LSR II flow cytometer. Overlay plots show tetramer-positive cells in red and total CD8-positive cells in gray.*

### C. Islet-specific CD8$^+$ T cells are found at higher frequencies in LN

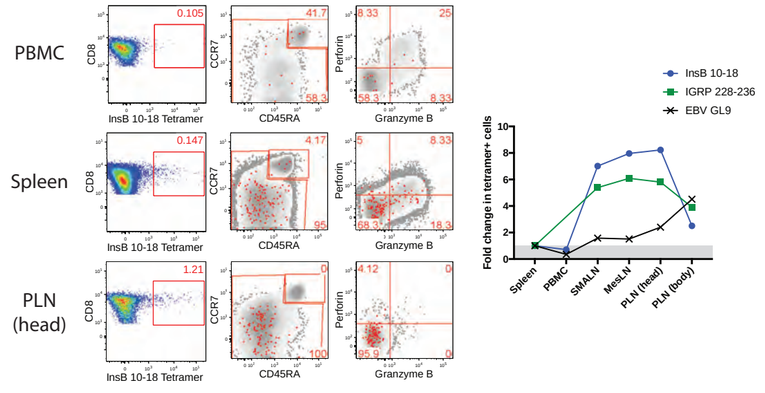
HPAP-007 - T2D (4 years)




HPAP-016 - Autoantibody positive (GAD65, IA-2, ZnT8)




HPAP-024 - Autoantibody positive (GAD65)



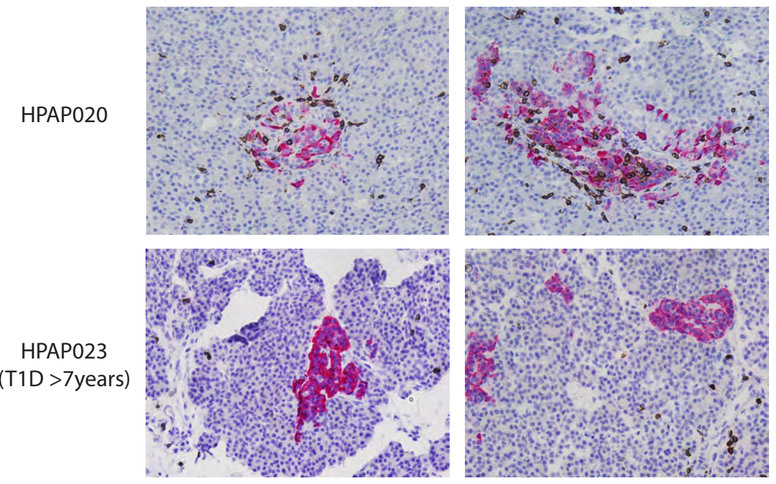

*Cells were stained with tetramers and phenotypical markers as described in 1B.*
*Fold-changes were calculated for each tetramer-positive population by dividing the frequencies in the tissues by the freqency in the spleen.*

## 2. HPAP-020: a 14-year old with unsuspected T1D

### A. Case History

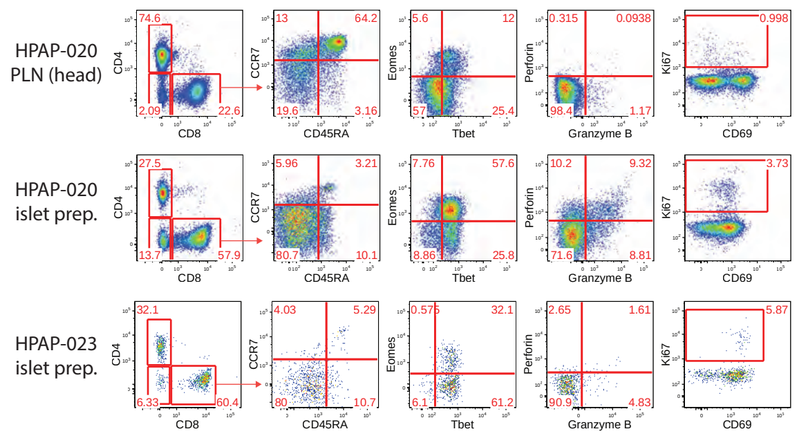
Here we present the case of an unfortunate 14-year old boy with unsuspected type 1 diabetes who died after a brief illness. Upon admission, the subject had a BMI of 13.32 and a glucose level greater than 700mg/dL. The C-peptide level was 0.37 and autoantibodies were detected against GAD65, IA-2, ZnT8 and insulin.
Tissues were recovered by HPAP and subjected to pathological, physiological, genetic and immunological analyses.

### B. Insulitis in HPAP-020 pancreas




*FFPE sections of the pancreas were stained by immunohistochemistry for the detection of CD8* (brown) *and glucagon* (pink)*, followed by a counterstaining with hematoxylin. Images shown at 20x magnification.*

### C. Cytotoxic CD8$^+$ T cells in the HPAP-020 pancreas




*For the isolation of purified islets the pancreas tissue was disaggregated with collagenase in the presence of trypsin inhibitors. Islets were isolated by density gradient centrifugation and cultured at 22 degrees Celsius. Supernatants containing leukocytes were collected and analyzed by flow cytometry as described in 1B.*

### D. Islet-specific CD8$^+$ T cells in the HPAP-020 pancreas and SLO