The cross-talk between the duodenum and the pancreas

Profiling the immune system to identify the role of the gut in the pathogenesis and prevention of type 1 diabetes

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1. Purpose

The role of the gut in the pathogenesis of Type 1 Diabetes (T1D) is still not well understood despite extensive research in animal models and studies of the gut microbiome in humans. Similar to other autoimmune diseases, a connection between T1D and an altered intestinal microbiota has been reported in several geographically diverse cohorts. Alterations of the intestinal barrier and increased gut permeability could enhance the exposure of the immune system to pathogens, which is likely to contribute to an already defective tolerance in the context of T1D(1-6).

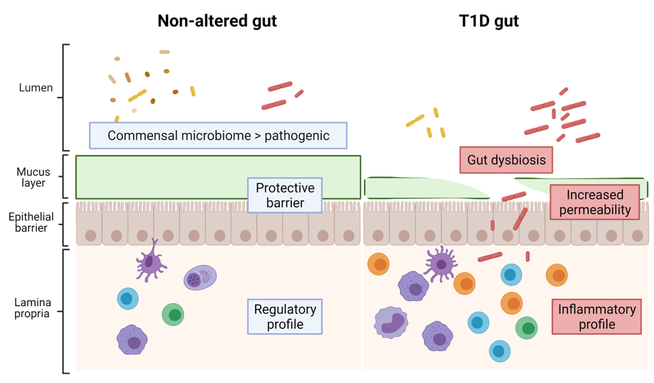

Figure 1:. Overview on the hypothesis that a combination of predisposing genetics, dysregulated intestinal barrier function and aberrant immune responses play an inciting role in type 1 diabetes.
An intact intestinal barrier (left) is composed of a thick mucus layer and continuous epithelial barrier. The immune cells in the underlying lamina propria maintain a regulatory profile. In contrast, a compromised intestinal barrier (right) is characterized by a thin and discontinuous mucus layer and a breached epithelial barrier that allows invasion of microbiota into the lamina propria. This results in activation of immune cells, which acquire an inflammatory profile, and that can induce diabetes after travelling to the pancreas via mesenteric and pancreatic lymph nodes.

2. Methods

Study models for technical optimization

- CaCo-2 cells and PBMCs
- Formalin-fixed paraffin-embedded spleen (donor 6014) and duodenum (donor 6482) from non-diabetic donors

Markers

- 22 antibodies were optimized to characterize the gut epithelial barrier and immune cells
- Markers were subdivided into 7 panels to characterize macrophages/monocytes, dendritic cells, CD3+ T cells, CD4+ T cells, CD8+ T cells, and Tregs

Staining methods

- Duplex immunofluorescence (IF) for cells
- 5-color multiplex IF for tissues

Image analysis

- Tissue slides were scanned with an Axio Scan.Z1 slide scanner (Zeiss)
- QuPath (version 0.3.1)[7] will be used to extract information from cellular features including area, location, perimeter, and intensity
- A detection classifier for automatic assignment of cell classifications using machine learning is in progress

References
[1]Endesfelder et al. 2014 Diabetes. 63(6):2006-14. [2]Mejía-León et al. 2014 Sci Rep. 4:3814. [3]Soyucen et al. 2014 Pediatr Int. 56(3):336-43. [4]Alkani et al. 2015 Diabetes. 64(10):3510-20. [5]Kostic et al. 2015 Cell Host Microbe. 17(2):260-73. [6]Maffeis et al. 2016 Diabetes Metab Res Rev. 32(7):700-9. [7]Bankhead et al. 2017 Sci Rep. 7(1):16878. [8]Apaolaza et al. 2021 Front Mol Biosci. 8:689799.

3. Summary of results

3.1 Phenotypic characterization of the non-altered human gut epithelial barrier and immune cells

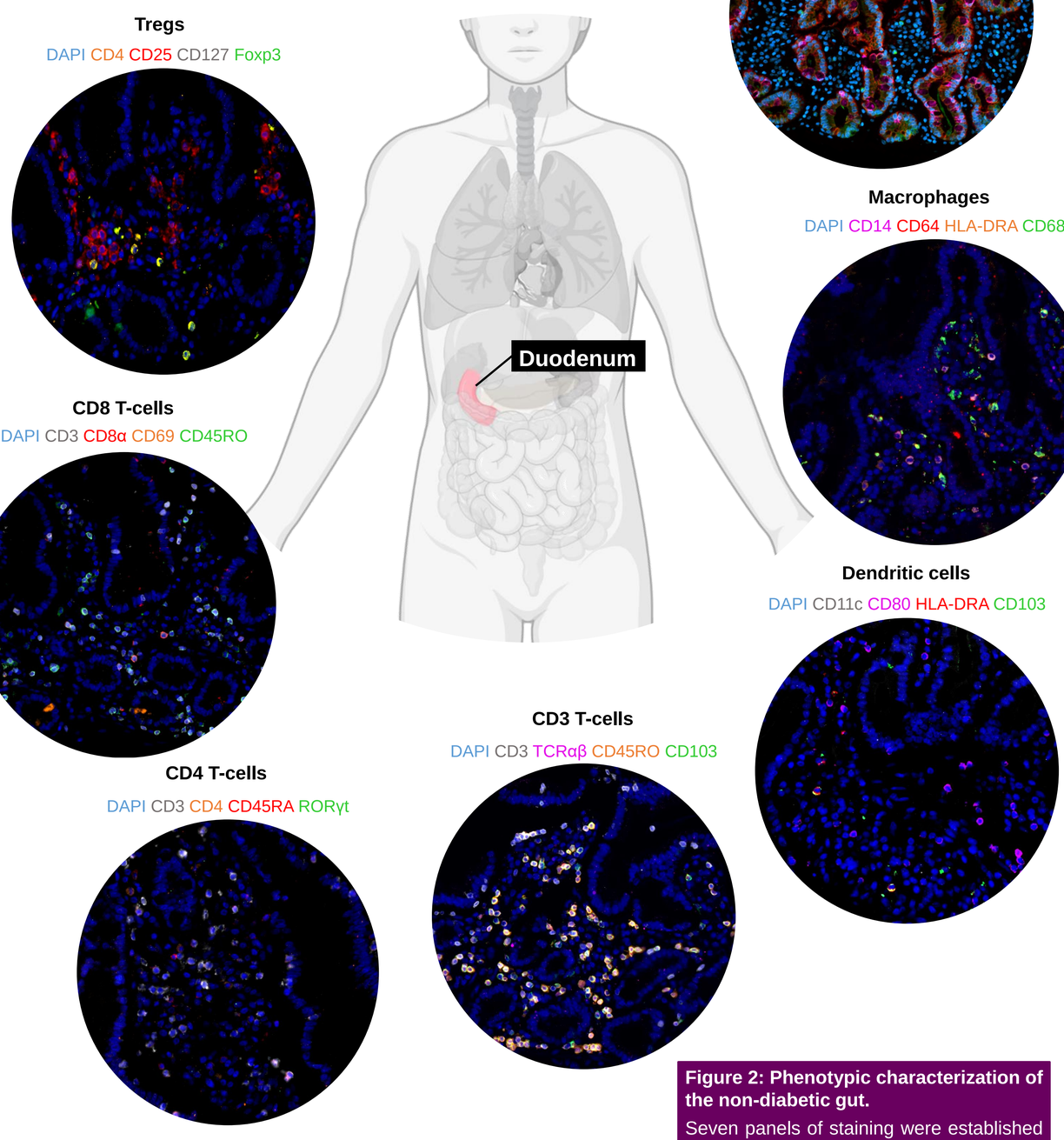

Figure 2: Phenotypic characterization of the non-diabetic gut.
Seven panels of staining were established to characterize different cell populations.

3. 2 Image analysis

With QuPath, we are training a detection classifier to apply machine learning to automatically assign cell classifications[8].

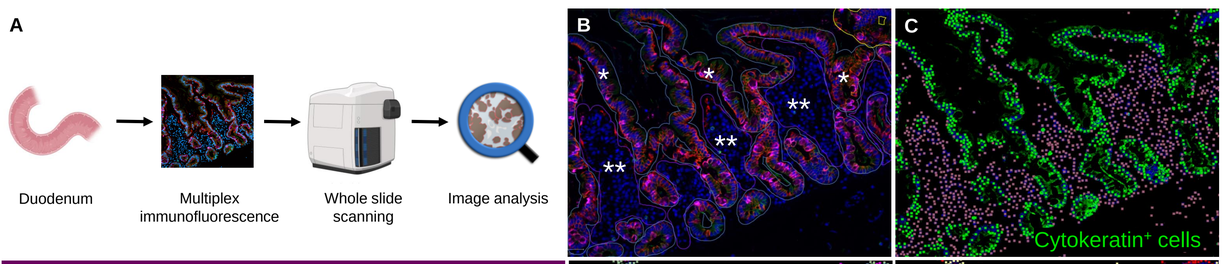

Figure 3: Whole image analysis workflow.
(A) Experimental approach. **(B)** Tissue detection and classification of areas defined as *epithelium* (*) and *lamina propria* (**) were obtained with a pixel classifier. **(C, D, E)** With the single measurement classifier, it was possible to accurately detect epithelial cells stained with Cytokeratin and E-cadherin, and goblet cells stained with Mucin 2. Annotation measurements were exported into a CSV file and processed in Excel spreadsheets.

4. Conclusions

Here we present an experimental overview from the first steps of our project. By its completion we will:

- Provide information about the integrity and composition of duodenum
- Determine if there is an abnormal innate and/or adaptive immune cellular repertoire before and after T1D onset

Outlook

- To understand and define the relationship between the dysregulation of T cell tolerance mechanisms in the duodenum and the destruction of beta cells in the pancreas.

nPOD Network for Pancreatic Organ Donors with Diabetes
JDRF IMPROVING LIVES. CURING TYPE 1 DIABETES.
DZD Deutsches Zentrum für Diabetesforschung
INNODIA