## SUMMARY (Intro) 5/23/25 ciT1zen science

### Immune Perturbations in Pancreatic Lymphatic Tissues and Type 1 Diabetes
### ciT1zen science summary

**Source:** Excerpts from "Immune perturbations in human pancreas lymphatic tissues prior to and after type 1 diabetes onset | Nature Communications" (Published: 18 May 2025)
**Overview:**
This study provides a comprehensive characterization of immune cell populations in pancreatic-draining lymph nodes (pLNs), mesenteric lymph nodes (mLNs), and spleens of human organ donors, including non-diabetic control (ND), autoantibody-positive non-diabetic (AAb+), and type 1 diabetes (T1D) individuals. Using high-parameter flow cytometry and CITEseq, the researchers investigated immune cell composition and phenotypic changes across these tissues and disease states. The central finding is that significant, tissue-restricted immune changes occur in the pLNs both before and after the clinical onset of T1D, suggesting these lymphatic tissues play a crucial role in the autoimmune process.
**Main Themes and Key Findings:**

1. **Tissue-Restricted Immune Changes in T1D Development:** The study highlights that immune perturbations associated with T1D are most pronounced in the pLNs, the lymph nodes that drain the pancreas.

- **Quote:** "These results demonstrate tissue-restricted immune changes occur before and after T1D onset."
- **Quote:** "Overall, immune cell phenotypes are modulated in the pLNs and mLNs of AAb+ and T1D donors, with changes predominantly occurring in the pLNs."
- **Quote:** "Importantly, immune alterations were most readily observed in lymphoid tissues that drain the pancreas as opposed to the spleen, implying that disease-related effects may be diluted or influenced by other factors at sites more distal from autoimmune inflammation."

1. **Alterations in CD4+ Regulatory T Cells (Tregs) in pLNs:** A significant observation is the decrease in CD4+ Treg frequency and evidence of their dysfunction in the pLNs of both AAb+ and T1D donors.

- **Quote:** "Compared to ND pancreas-draining lymph nodes (pLN), AAb+ and T1D donor pLNs display decreased CD4+ Treg..."
- **Quote:** "In this regard, we observed a decreased frequency of CD25+CD4+ Tem/Temra in the pLNs of AAb+ donors that was maintained in the pLNs of T1D donors..."
- **Quote:** "Using CITEseq, we further explored alterations in pLNs Tregs within the CD4 Treg/Tcm cluster using a set of genes associated with various Treg signatures...Core Treg genes *FOXP3*, *IKZF4*, and *IL2RA* were significantly reduced in

the CD4 Treg/Tcm cluster in both AAb+ and T1D pLNs, showing that this signature appears prior to T1D onset."
- Evidence suggests impaired Treg stability and function based on reduced expression of key genes like *FOXP1* and *RUNX1* in T1D pLN Tregs.

1. **Increased Stem-Like CD8+ T Cells in AAb+ and T1D pLNs:** The study identifies an increase in less differentiated, stem-like CD8+ T cell populations in the pLNs of AAb+ and T1D individuals. This population is characterized by increased expression of *CXCR3* and decreased expression of *TOX*.

- **Quote:** "...and increased stem-like CD8+ T cell signatures..."
- **Quote:** "The loss of Tregs in AAb+ individuals was accompanied by an increase of a stem-like CD8+ T cell population closely resembling one found in multiple autoimmune contexts, including a population that resides in the pLNs and drives autoimmunity in NOD mice..."
- **Quote:** "In human AAb+ and T1D pLNs, we also observed increased frequencies of less differentiated stem-like *CXCR3*+ memory CD8+ T cells."
- Simultaneously, a *TOX*+ memory CD8+ T cell population decreased in AAb+ individuals.

1. **Decreased Naive T Cell Signatures and Increased Cytotoxic NK Cells in T1D pLNs:** Specifically in T1D donors, the pLNs show a reduction in naive T cells (particularly CD4+) and an increase in cytotoxic CD56dimCD16+ NK cells. These changes are more evident in individuals with longer-standing T1D.

- **Quote:** "...while only T1D donor pLNs exhibit naive T cell and NK cell differentiation."
- **Quote:** "Naive CD4+ T cell frequency decreased in T1D versus ND and AAb+ pLNs..."
- **Quote:** "...we found that there was a proportional increase in the cytotoxic CD56dimCD16+ NK cell frequency in T1D pLNs..."
- Transcriptomic analysis in T1D pLNs reveals increased expression of cytotoxicity-associated genes (*GZMB*, *CRTAM*, *IFNG*) and decreased expression of an inhibitory receptor (*KLRB1*) in NK cells.

1. **Correlation with HLA Genetic Risk:** The study found associations between higher T1D genetic risk (HLA-GRS) and the frequency of certain immune populations in the pLNs, particularly activated CD8+ memory T cells, CD127+ T cells, and CD69+ B and T cells.

- **Quote:** "Further, T cell expression of activation markers and the IL7 receptor correlate with T1D genetic risk."
- **Quote:** "...non-T1D individuals with higher-risk HLA alleles have an increased frequency of activated memory CD8+ T cells in the pLNs."
- **Quote:** "We found that a higher HLA-GRS in AAb+ and T1D donors positively correlates with CD127 surface expression on T cells."

1. **Immune Changes in Single Autoantibody Positive Individuals:** Even individuals with only a single autoantibody, who have a lower risk of developing T1D, show immune perturbations in their pLNs, including decreased CD25+CD4+ Tem frequency, decreased CD38+ T cell subsets, and increased memory B cells.

- **Quote:** "Despite the immune modulations we observed in single AAb+ donor pLNs, it is highly likely these individuals would have maintained β cell tolerance."
- **Quote:** "...even low-risk Single AAb+ donors have immune perturbations in their pLNs, and that certain immune populations tend to gradually change as individuals have autoinflammation for longer periods of time."

**Most Important Ideas/Facts:**

- The pancreas-draining lymph nodes (pLNs) are a key site for immune alterations preceding and during T1D development.
- Loss and dysfunction of regulatory T cells (Tregs) in the pLNs is an early event, observed in autoantibody-positive individuals before overt diabetes.
- The emergence of a stem-like *CXCR3*+CD8+ T cell population in the pLNs is associated with both AAb+ status and T1D.
- Progression to T1D is linked to reduced naive T cell signatures and an increase in cytotoxic NK cells within the pLNs.
- Genetic risk for T1D (HLA-GRS) is associated with specific immune profiles in the pLNs, including markers of T cell activation and IL7 receptor expression.
- Immune changes in the pLNs can be detected even in individuals with low-risk, single autoantibody positivity.

**Limitations Noted by the Authors:**

- Limited number of AAb+ donors with multiple autoantibodies, hindering detailed subanalysis of pre-T1D stages.
- Cross-sectional study design prevents longitudinal tracking of immune changes in individuals.
- Inability to reliably compare results directly to peripheral blood studies due to sample limitations.
- Focus on fresh cell analysis for flow cytometry limits retrospective studies.
- Technical limitations prevented simultaneous assessment of B cell or T cell repertoires in CITEseq analysis.

**Conclusion:**
This study provides valuable insights into the local immune environment of the pancreas in the context of T1D development. By profiling pancreatic-draining lymph nodes, the researchers demonstrate that critical immune shifts, including Treg dysfunction, the emergence of stem-like CD8+ T cells, and changes in naive and NK cell populations, occur in this tissue prior to and after diagnosis. These findings, coupled with the correlation between immune profiles and genetic risk, underscore the pLNs as a crucial site for

understanding T1D pathogenesis and identifying potential targets for immune intervention.