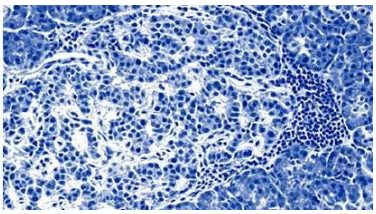
nPOD
Network for Pancreatic Organ Donors with Diabetes



T cell autoreactivity mapping in individual islets with islet biology from donors with type 1 diabetes (T1D)

AUTHORS

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PURPOSE

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In the islets of donors with T1D, lymphocytic infiltration into islets is heterogeneous with non-infiltrated islets in close proximity to infiltrated islets. Our purpose is to examine individual islets from donors with T1D for lymphocytic infiltration, to determine the repertoire of T cell autoreactivity from individual islets, and to determine the phenotype of islet cells in infiltrated and non-infiltrated islets.

METHODS

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From donors with T1D, single islets were handpicked into individual wells of 96 well plates with media for T cell stimulation and expansion. After brief culture, lymphocyte outgrowth from individual was observed. Lymphocytes were recovered from individual wells, further expanded, phenotyped, and tested for T cell autoreactivity. In addition, we used a mid-throughput method of T cell autoreactivity testing. Autologous splenic B-LCL pulsed with pools of peptides (known native and neoepitopes, grouped by HLA class I and class II binding for each donor) were co-cultured with T cell lines derived from individual islets and a CD40 antibody to capture CD40/CD154 complex on the cell surface of activated T cells for detection by flow cytometry. From one donor with T1D, nPOD 6477 (20 year old female with 8 years of T1D duration, $GADA^+$ and $IA\text{-}2A^+$ at time of demise; HLA A*02/29, B*07/13, DR*01/01, DQ*05/05), islets from wells with and without T cell outgrowth were pooled, respectively, labeled with hashtagging antibodies to distinguish all cells in the two pools and then labeled with barcoded antibodies to identify immune cell types by surface expression of markers. Single cell RNAseq was determined using the 10x platform.

SUMMARY OF RESULTS

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From donors with T1D, an average of 25% of individual islets had T cell outgrowth. From an islet from nPOD donor 6342, a $CD8^+$ T cell clone recognized $ppIAPP_{5-13}$ in the context of HLA-A2 and from donor T1D.8 (30 year old male with 20 years duration of T1D), a $CD8^+$ T cell clone recognized $ZnT8_{186-194}$ in the context of HLA-A3. A T cell reactivity map for $CD4^+$ and $CD8^+$ cells was generated for the lines from each donor. As previously reported, a broad range of T cell autoreactivity was seen with focused T cell reactivity seen in some islets and broad T cell reactivity seen in other islets. From nPOD donor 6477, islets with little or no T cell infiltrate showed high levels *INS* mRNA while islets with infiltrate showed fewer *INS* transcripts. I□□□□□ with infiltrate showed upregulated transcripts for *CCL20, CXCL2 and IL-32* as compared to islets with little or no infiltration.

CONCLUSIONS

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As shown by immunohistochemistry/immunofluorescence data on pancreata from donors with T1D, lymphocytic infiltration into individual islets is heterogeneous; our data supports this conclusion. As shown here by mapping the autoreactivity for individual infiltrated islets, the T cell repertoire can be focused or more broad. By examining the transcriptome of islet cells from infiltrated and non-infiltrated islets, a pro-inflammatory environment for macrophages and chemoattractive for lymphocytes and neutrophils was observed in islets with T cell infiltrate coupled with a decrease in *INS* mRNA. Deeper sequencing of individual cell types within infiltrated and non-infiltrated islets will clarify factors within the islets that may promote lymphocytic infiltration. Understanding the heterogeneous pattern of infiltrated islets, both the heterogeneous T cell repertoire and the heterogeneous islet phenotype will promote understanding of the disease process in T1D.