Application Note

PREDICTING DISEASE PROGRESSION:

Sequential pathogenic events in type I diabetes: Recruitment of cytotoxic cells at disease onset

SUMMARY

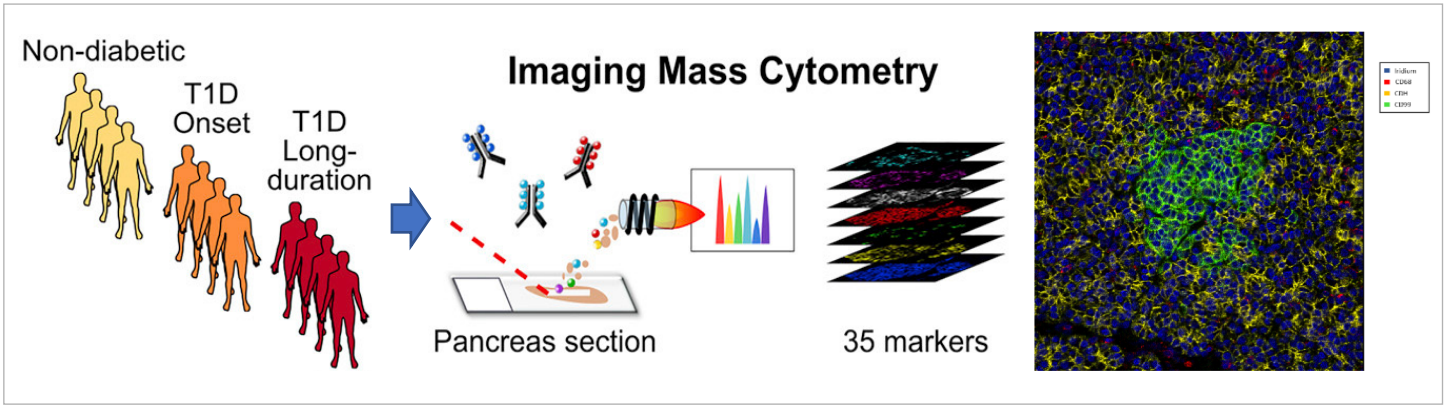
Type 1 diabetes (T1D) results from the autoimmune destruction of insulin producing β cells; however, the complex interactions between islets and cells of the immune system in human patients is poorly described. By performing pseudo time analysis of islets and immune cells using Imaging Mass Cytometry (IMC) thought TD1 progression, we provide snapshots to reconstruct the evolution of β cells loss. Here we described an approach using proprietary spatial data analysis to elucidate the association between the immune cells and islet cells in T1D pathogenesis and provides a rationale for designing new therapeutic to cure this disease.

APPROACH

The pancreas is a highly complex organ consisting of multiple endocrine, exocrine, and stromal cell types and an intricate vascular and neuronal network. In the case of type 1 diabetes (T1D), bidirectional interactions between immune cells and insulin-producing beta cells lead to loss of functional pancreatic beta cell mass and a dependence on exogenous insulin administration for survival. Determining the sequential pathogenic events that take place in the T1D progression in human patients is limited. An understanding of how cell types, cell states, and cell-cell interactions evolve during T1D development is essential to designing strategies to cure or halt this disorder.

In the present study, we characterized the alterations in islet architecture, and immune cell presentation during disease progression. We used Imaging Mass Cytometry (IMC) to investigate complex events on the cellular level and provide new insights on the TD1 pathogenesis. By performing pseudo time analysis, we aim to establish the interaction between the immune system and the endocrine cells and offer a innovative therapeutic approach with a better understanding of events that are critical to the disease pathogenesis.

FIGURE 1. Acquisition of single-cell data by IMC

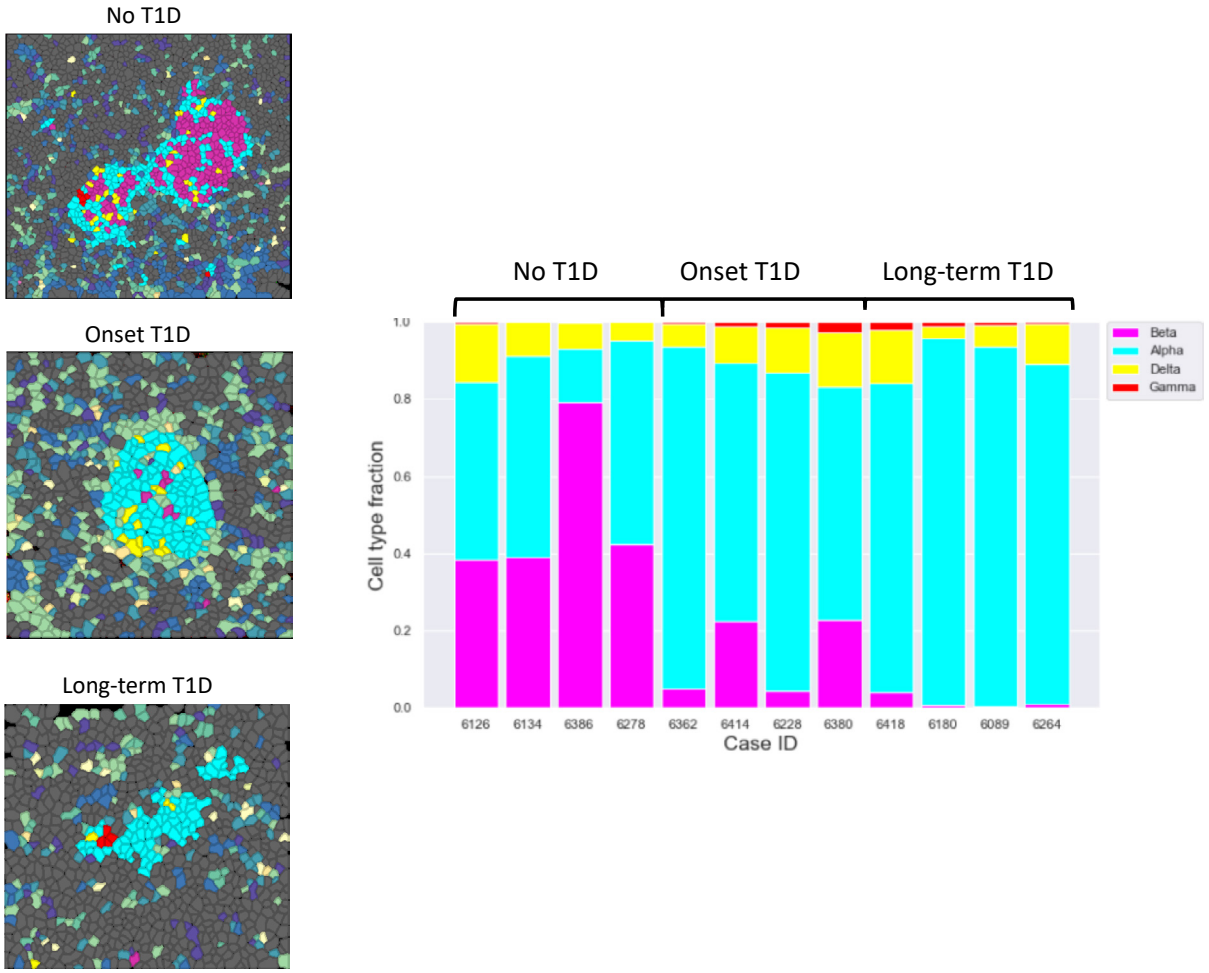


EVOLUTION OF ISLET CELLULAR COMPOSITION

Our patient cohort consisted of twelve (12) human donors to represent recent-onset T1D (<0,5 year, N=4), long standing TD1 duration (≥8 years, N = 4), and controls without diabetes (N = 4) and for each donor analyzed two sections originating from different anatomical regions of the pancreas (tail & body).

We used machine learning algorithms to generate cell segmentation masks, which represent pixels belonging to the same cell respectively. Applying these masks over high-dimensional pictures allowed retrieval of phenotypic and functional marker expression (images below). We then sought to determine how islets cell type composition change with T1D progresses. We observed large inter-donor variations however as compared to non-diabetic controls, β cell fraction was dramatically reduced in donors with recent-onset TD1 (Fig. 2). As expected, pacreata from donors with prolonged disease duration were almost entirely devoid of β cells.

FIGURE 2. Average islet cell type fraction for each of the 12 donors in our dataset.

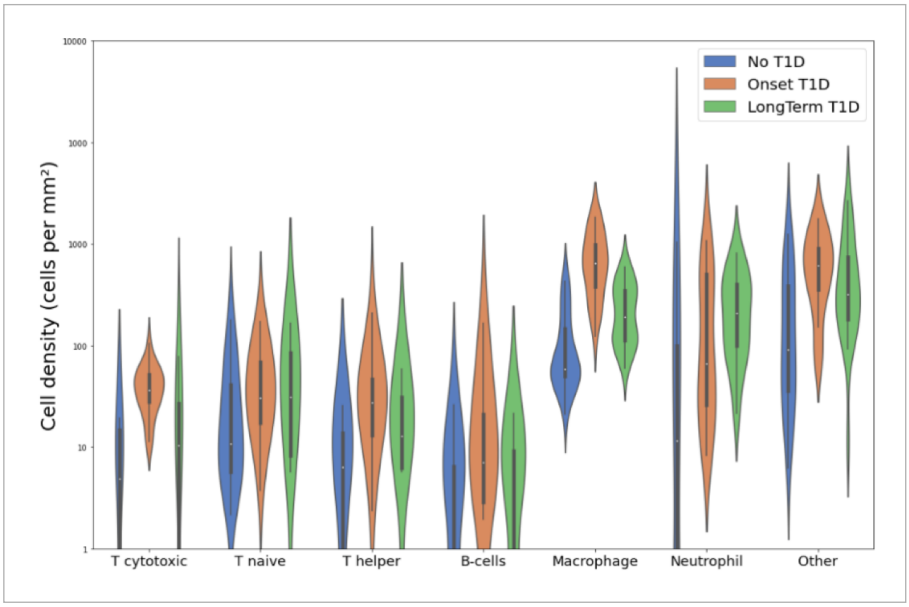


ALTERATIONS IN IMMUNE CELLS COMPOSITIONS

To better understand the immune cells expression profiles change with disease progression, we measured the density of each immune cell type (i.e. number of cells per mm2) across TD1 duration.

All measured immune cell types were more abundant in recent onset than control donors (Fig.3). In long-duration TD1 donors, Neutrophils densities were elevated relative to donors without T1D, whereas T cytotoxic cell , T helper cell and Macrophages abundances were decreased compared to recent-onset donors but higher
than in patients without TD1.

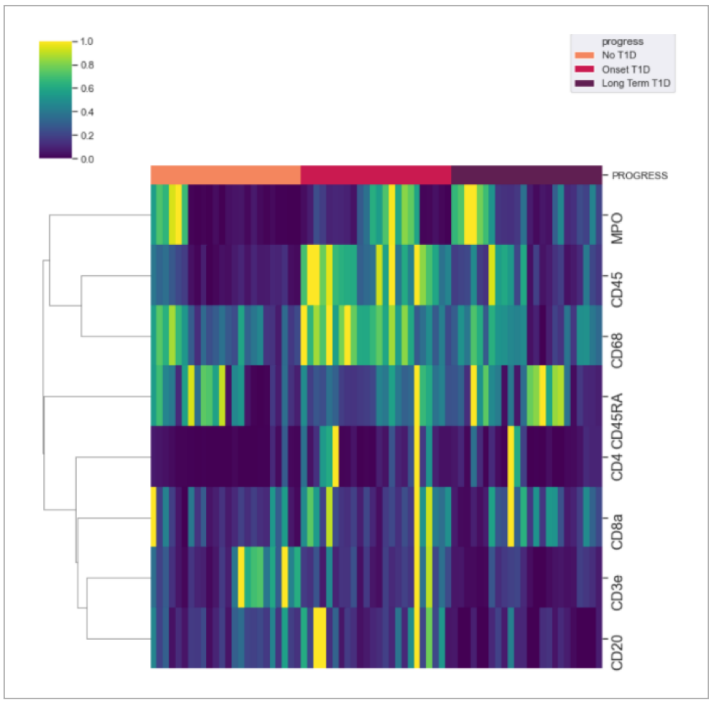
FIGURE 3. Density (cell per mm2) of immune cell types in pancreata from controls and donors with TD1



MOLECULAR CHANGES IN T1D

To analyze this observation in more detail and better understand how immune cell types change with disease progression, we performed supervised hierarchical clustering across TD1 duration using the main identified immune cell types in our dataset: cytotoxic T cells (CD3+ CD8+CD45RA-), Helper T cells (CD3+CD4+), B cells (CD20+), macrophages (CD45+CD68+) and neutrophils (MPO+). Hierarchical clustering revealed a clear separation between immune markers contained in pseudo stage no TD1 and immunes markers in pseudo stage onset- and long-term TD1. Overall, T cells (cytotoxic and helper) and macrophages were more abundant in donors with recent-onset T1D and the number of B cells was very constant across disease progression and remained very low.

FIGURE 4. Heatmap showing expression of immune markers with columns arranged to pseudo time progression



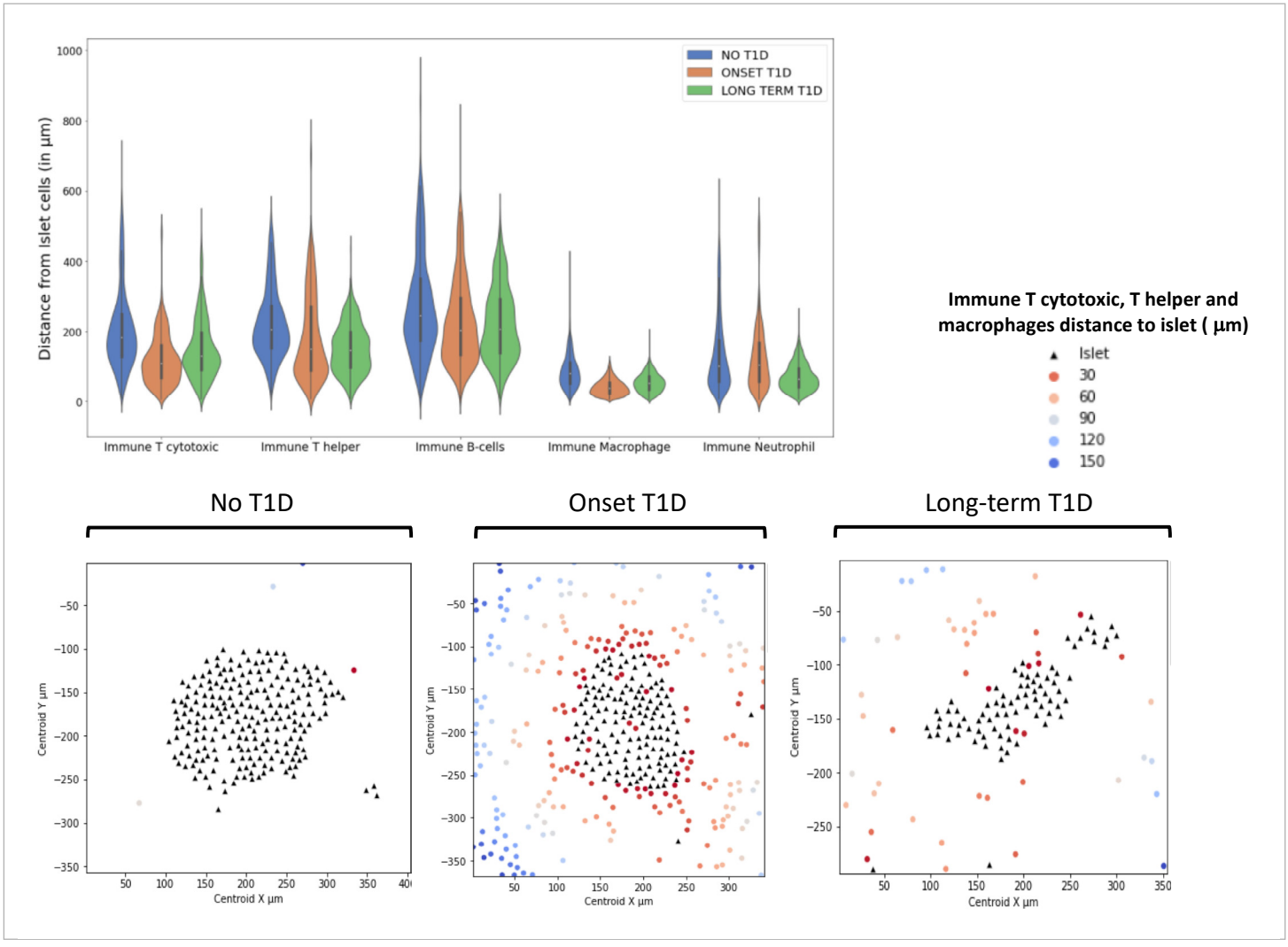
DYNAMIC CHANGES IN CELL-CELL INTERACTIONS IN TD1

Tissue function depends on interaction between different cell type and this is also the case in the pancreas: TD1 progression is determined by interaction between immune and β cells.

Understanding how cell-cell interactions change from one disease stage to the other is, therefore, key to a better understanding of T1D. To systematically probe interactions between cell types we performed a spatial analysis. We measured for each image the significant association according to spatial proximity of all cell type pairs in our dataset.

Cytotoxic and helper T cells are thought to be directly involved in the destruction of β cells in T1D. Our global spatial analysis detected association between T cells, macrophages and β cells in donors with recent-onset T1D, where the rate of β cell death is maximal (Fig.5). T cells and macrophages were significantly enriched in the islet proximal area (within and up to 30 µm to islets) in the regions with remnant β cells compared with those containing no β cells. Together with the results of our immune analysis, these results suggested that not only that onset TD1 stage islets have more associated T cells, but also that, in those islets, T cells contact β cells more frequently.

FIGURE 5. Distance of islet cells with immune cells.



CONCLUSION

Thanks to Aliri's platform and proprietary image analysis tools and the development of a new workflow, we have been able to segment cells and to identity cell population for further classification and spatial analysis in diabetes with a high accuracy and precision.

With an array of protein data, we were able to identify major pancreatic and immune cell types and quantify complex cellular interactions during TD1 progression. We found dramatic decrease of β cells in islets with recent-onset TD1. We observed simultaneous recruitment of T cytotic, T helper and macrophages and β cells destruction . This results could confirm that immune cells form a destructive motif that plays a role in β cell demise.

The imaging techniques and deep data analysis workflow described in this white paper open new avenues for the exploration of TD1 pancreas pathology and help to address the questions that surround the disorder.

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