Foundation for Diabetes Research

Semi-Annual Progress Report (07/01/2019 - 12/12/2019)

IMMUNE CHANGES IN THE HONEYMOON PERIOD AT THE SINGLE CELL LEVEL
PI: Howard Davidson, PhD.

Goals: The overall goal of our project is to identify novel biomarkers that can be used clinically to more accurately define patient specific disease trajectories during the first 1-5y following diagnosis of clinical T1D. To accomplish this goal we are using innovative single cell RNA sequencing technologies and assays to analyze functional changes in human T cells donated by patients attending the Barbara Davis Center. The results we obtain will then be used to define novel immune signatures associated with the "honeymoon" - a period of spontaneous complete or partial remission from disease. If successful our study has the potential to generate new tests that can be used to enhance the efficiency of future clinical trials, and thus accelerate a successful outcome to the search for a true cure for T1D.

Accomplishments: In the first six months of the project our primary focus has been on recruiting subjects to our study, and processing and storing the blood that they are generously donating. We have also calculated their insulin dose adjusted HbA1c (IDAA1C) values from their clinical charts, and conducted an initial genetic analysis of their DNA to identify carriers of common variants that may influence the metrics we are studying. To date we have recruited 16 subjects who have each donated at least 1 blood draw during their honeymoon phase that is of sufficient size to allow our analyses to be performed. This comprises 10 subjects who had previously provided archived samples, and 6 subjects recruited after the commencement of funding. Our goal is to compare samples from each donor at 2 longitudinal time points - the first (HM) during their honeymoon, and the second (PHM) between 6mo and 3y after their honeymoon has ended. To date at least 3 of our enrollees have exited their honeymoon, although only 1 (AV11) has so far donated a PHM specimen. The progression of disease in this subject is shown in Fig 1.

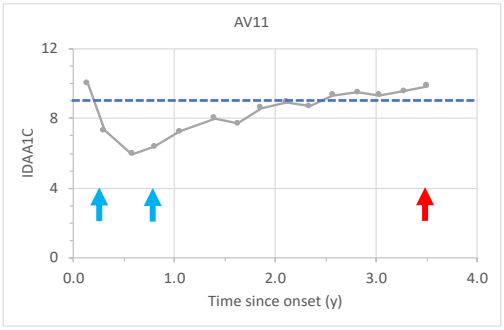

Figure 1. Time dependent changes in IDAA1C in subject AV11. Donor AV11 is an 18y old male who developed T1D in November 2015. We already had archived samples obtained at 2 time points during his honeymoon (blue arrows), and banked a 3rd blood draw at the commencement of this project approximately 1y after he had exited his honeymoon period (red arrow). The graph shows his progression into and out of partial remission, defined by an IDAA1C value ≤ 9 (dotted line).

An example of the results we are obtaining from our single cell analyses is shown in Fig 2.

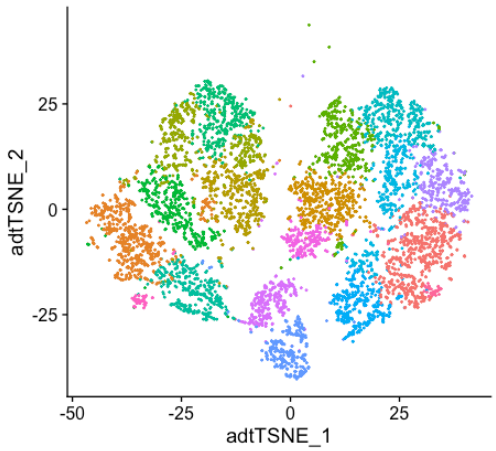

Figure 2 Identification of T cell subsets by CITE-Seq. T cells from donor AV117 were activated with APC avatar "OKT181" for 16h then stained with a panel of 8 DNA barcoded antibodies and analyzed by CITE-seq. A nearest-neighbor based unsupervised clustering algorithm was used to define sub-groups of cells based upon differential expression of the chosen phenotypic markers. Eighteen distinct populations were identified that ranged in size from 0.7% to 10% of the total cells present. Our hypothesis is that the frequency of these various sub-groups, and the spectrum of genes that they express, will vary with the "activity" and "endotype" of T1D in that subject, and that these features can be used to augment current models designed to predict the future rate of loss of pancreas function in an individual.

Goals for next funding period: In the next six months we aim to bank PHM samples from at least 2 additional donors, to complete the analysis of the HM and PHM samples from donor AV11, and the data collection and initial analysis of the HM samples from at least 4 other donors.