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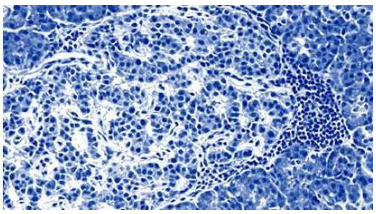
Network for Pancreatic Organ Donors with Diabetes



Analysis of antigen-receptor sequences of a unique lymphocyte reveals a T cell-neoantigen encoded in a public BCR of T1D patients

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PURPOSE

Although it is well accepted that type 1 diabetes (T1D) is an autoimmune disease that is caused by destruction of insulin-producing beta cells by autoreactive T cells, critical gaps and questions in our understanding of how diabetogenic T cells remain elusive despite extensive investigation of the disease process. We hypothesize that difficulty in filling these gaps are due at least in part due to the long-held view that T and B cells are the only adaptive immune cells. We have recently discovered a third adaptive lymphocyte that is a hybrid between B and T cells and clonally expanded in T1D patients as compared to healthy controls, that we referred to as dual expressers, DEs or X cells to denote their crossover phenotype (**Ahmed et al, Cell, 2019: 177:11583**). We hypothesize that investigation of X cells will help answers key outstanding questions in the field and may inform new diagnostic to predict at early age and before antibodies who will likely to develop T1D and eventually new efficacious specific therapeutic interventions that spare depletion of T and B cells

METHODS

We developed a protocol to identify DEs or X cells in peripheral blood using flow cytometry (FACS) and for functional analysis. Visualization of unique phenotype of X cells at single cell level was carried out by AMNIS .We determined the gene make up of X cells by using single cell RNA seq and this method allowed us to determine the genes that are actively transcribed

and expressed in X cells as compared T and B cells. To physically demonstrate co-expression of TCR and BCR in the same cell, we generated immortalized DE cells using EBV-transformation and examined cells from single clone for fully assembled BCR and TCR. We also established their unique functional relevance of X cells in the context of auto-reactivity by showing their structural mimicking as an autoantigen (insulin) presented by MHC-II (HLA-DQ-8) molecules to pathogenic CD4 T cells in T1D cases.

SUMMARY OF RESULTS

Here we describe a rare subset of autoreactive lymphocytes with a hybrid phenotype of T and B cells including coexpression of TCR and BCR and key lineage markers of both cell types (hereafter referred to as dual expressers or DEs). In type 1 diabetes (T1D), DEs are predominated by one clonotype that encodes a potent CD4 T cell epitope in its antigen binding site (referred to as x-idiotype). Molecular dynamics simulations revealed that the x-idiotype (x-Id) peptide has an optimal binding register for diabetogenic HLA-DQ8. In concordance, synthesized x-Id peptide forms stable DQ8 complexes and potently stimulate autoreactive CD4 T cells from T1D, but not healthy controls. Moreover, x-clonotype-bearing mAbs stimulate CD4 T cells and inhibited insulin-tetramer binding to CD4 T cells.

CONCLUSIONS

These results uncovered a widespread existence of a population of lymphocytes ‘X cell’ that are apparently multi-functional as B and T cells, autoreactive and could be an important player in T1D and perhaps autoimmune diseases.