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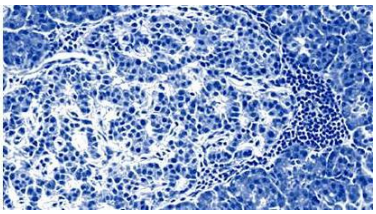

Role of X cell-secreted mAb (x-mAb) in the pathogenesis of T1D

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PURPOSE

We have recently discovered a new cell type that combines characteristics of B and T cells, including expression of TCR and BCR (Ahmed *et al*, Cell, 2019: 177:11583). We refer to this new lymphocyte as X cell to denote its crossover phenotype. Importantly, X cells express a public BCR that also encodes a potent autoantigen in its CDR3 sequence that is about 10-orders more potent than native insulin peptide (InsB:9-23) in binding to DQ8 and activating autologous CD4 T cells. The x-autoantigen cross-activate insulin specific CD4 T cells as a peptide in the context of HLA-DQ8 molecules or as a soluble intact mAb (x-mAb). The goal of this study is to characterize autoreactive CD4 T cells that are responsive to x-mAb to determine their phenotype, cytokine profile and TCR repertoire and whether they express public TCRs.

METHODS

We use an EBV-lymphoblastoid X cell clone as a source of x-mAb (IgM isotype) and a FACS-based protocol to identify x-mAb-reactive CD4 T cells (referred to as (IgM^{pos}) in peripheral blood of T1D patients and HCs. We are characterizing TCR repertoires of sorted IgM^{pos} CD4 T cells using ImmunoSEQ assay, and their functional properties using intracellular cytokine analysis and activation phenotype using surface staining.

SUMMARY OF RESULTS

Our preliminary data show that frequency of IgM^{pos} CD4 T cells is significantly higher in T1D patients as compared to Healthy subjects. In addition, IgM^{pos} CD4 T cells exhibit an activated phenotype as compared to autologous IgM^{neg} CD4 T cells, including expression of CD45RO, CD44, and CD69. In addition, the majority of IgM^{pos} CD4 T cells produce TNFα as compared to IgM^{neg} CD4 T cells. Analysis of TCRVβ repertoire shows that IgM^{pos} CD4 T cells are enriched for public clonally-expanded TCRs as compared to IgM^{neg} counterparts.

CONCLUSIONS

X cells in T1D patients are predominated by a single public BCR and that the secreted version of this BCR (x-mAb) is autoreactive against a specific subset of CD4 T cells that predominated by few clonotypes that express public TCRs. Currently, we examining antigen specificities of IgM^{pos} CD4 T cells and their homing properties. Our results are revealing previously unknown mechanism that appears to be a play critical role in pathogenesis of T1D.