Identification of GAD65 and Proinsulin-specific CD4+ and CD8+ T Cells in Pancreatic Lymph Nodes, Spleen, and PBMC from Subjects with T1D and Islet Autoantibody Positivity

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Purpose: The development and progression of type 1 diabetes (T1D) is mediated by antigen-driven T cells leading to the destruction of pancreatic islet beta cells, resulting in the loss of insulin production. Antigen specific central and effector memory T cells generated in the secondary lymphoid tissues have migratory properties that allow tissue specific homing. CC chemokine receptor 7 (CCR7) and C-X-C chemokine receptor 4 (CXCR4) have been previously shown in the diabetes murine model to be important in this process. In type 1 diabetes islet autoantigen specific T cells can be identified in peripheral blood, but whether these T cells truly represent the pathogenic T cell repertoire in the pancreatic islets is still unclear. Access to pancreatic lymph nodes, spleen and PBMC from the same subjects before or around the time of onset of T1D has facilitated studies on islet specific CD4+ and CD8+ T cells that are present at the site of autoimmune inflammation. We investigated the presence of islet autoantigen specific CD4+ and CD8+ T cells in the tissue and PBMC samples from nPOD cases at different stages of disease progession: T1D (variable duration of the disease), autoantibody positive subjects (islet autoimmunity with no clinical T1D) and normal individuals (islet autoantibody negative). Cryopreserved samples from the pancreatic lymph nodes, spleen and PBMC were requested.

Methods: A comparative analysis of T cell specificities and phenotypes was performed to elucidate the differences between the T cells derived from the site of autoimmune inflammation (PLN, spleen) and periphery (PBMC) in those subjects from whom samples were available. In ex vivo tetramer assays, cells from PLN, spleen and PBMC were stained with PE labeled DR4 (DR401, 402, 404, or 405), DRB4 0101, DR301 tetramers or HLA-A2 pentamers containing GAD65, PPI and insulin peptides followed by staining with antibodies specific for exclusion and viability markers (CD14, CD19, CD56 = “dump gate”), CD3, CD4, CD8 , CD45RA/RO, CXCR4, and CCR7. Tetramer binding of the CD4+ or CD8+ T cells was analyzed by FACS-LSRII flow cytometer.

Summary of Results: GAD65 and insulin specific CD8+ and CD4+ T cells were detected in the spleen and PLN from theT1D patients and the autoantibody positive subjects and also in the PBMC from the T1D patients. Tetramer binding T cells were not detected in the tissue samples obtained from the normal individuals. All tetramer positive T cells displayed memory (CD45RO+) phenotypes, and variably expressed chemokine receptors CCR7 and CXCR4.

Conclusions: Identification of islet autoantigen specific T cells by direct tetramer staining offers an advantage in further characterizing phenotypes of T cells responding to a single, known antigen mediated by a defined MHC restriction. nPOD samples offer a unique opportunity to understand the functional and lineage specific features of migrating T cells to specific tissues in subjects at different stages of the autoimmune process. Our findings also suggest potential variability in the localization and dynamics of the islet homing T cells which remains to be further investigated.