Autoreactive B Cells from Spleen and Pancreatic Draining Lymph Nodes from Type 1 Diabetes Subjects

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Purpose: The purpose of this study is to determine the frequency and function of autoreactive B cells from the spleen and pancreatic draining lymph nodes (PLN) from subjects without Type 1 diabetes (T1D), with autoantibody, but without disease and from recent and long-term T1D subjects.

Methods: The analysis of multiple secreted products from single B-cells was detected by microengraving. PLN cells or splenocytes were polyclonally stimulated with BCR cross-linking, soluble CD40L and pokeweed mitogen for 18 hours and then dispersed into the wells (100,000/slide). Glass slides are coated with antigen (rGAD65 or proinsulin) and blocked. The supernatant of the nanowells is exposed to the antigen-coated surface of the glass slide for 2 hours and antibody from the supernatant bound to the antigen is detected with anti-human Ig isotype antibodies labeled with different flurochromes and read in a microarray reader. Cells in the wells are stained with anti-CD20 flurochrome labeled antibody and imaged. Percentages of CD20+ B cells secreting autoreactive antibodies of different isotypes and subisotypes was calculated. In order to detect B-cells secreting cytokines upon polyclonal stimulation, standard intracellular cytokine staining (ICS) and co-staining with CD20+ cells was done with PLN or spleen cells. Standard quantitative PCR for cytokine message expression was done on negatively isolated CD20+ B cells.

Summary of Results: The range of autoreactive B cells was from undetectable to 0.006-0.48% of polyclonally stimulated CD20+ B cells. From 2 PLN and spleen samples from subjects without diabetes, no GAD65- or proinsulin-reactive B cells were detected. nPOD case 6044 and case 6090 are subjects who were positive for GADA in the circulation at the time of demise, but without a history of T1D. From nPOD 6044 PLN, 0.017% of CD20+ B cells secreted IgM antibody reactive with GAD65; no secreted antibody reactive with proinsulin from these two samples or secreted antibody reactive with GAD65 from case 6090 were detected. From 3 subjects with recent onset of T1D (<5 years of diagnosis), secreted antibody reactive with GAD65 (IgM, IgG1 and IgG3) ranging from 0.006 to 0.48% of CD20+ B cells was detected; secreted antibodies reactive with proinsulin were detected from the spleen and PLN from these recent T1D onset subjects. From the spleen from 4 long term (>10 yrs from diagnosis) T1D subjects, frequencies of secreted GAD reactive antibody in the range from undetectable to 0.006% to 0.1% were seen. Polyclonally stimulated CD20+ B-cells from PLN examined for cytokine expression by quantiative PCR and by ICS, a peak of expression of IL-6, IL-10, LTalpha, and TNFalpha at Day 2 post stimulation was seen.

Conclusions: Splenic and PLN CD20+ B-cell secreting antibodies reactive with GAD65 and proinsulin were detected. A trend towards an increased frequency of B cells secreting IgM, IgG1 and IgG3 antibodies reactive with GAD65 was seen from the spleen and PLN from recent onset T1D subjects as compared to the frequencies seen from these tissues from controls, long-term T1D subjects and those with serum autoantibodies, but without disease. Proinflammatory cytokine expression was detected from CD20+ PLN B cells at peak after 48 hrs of polyclonal stimulation. These studies will aid in defining the frequency/function of autoreactive B cells in human T1D and in responder and non-responder patient groups in therapies in which B cell populations are manipulated.