Title: Characterization of CD4$^+$ T B Helper Cells in Systemic Lupus Erythematosus

Specific Aim: To define the phenotype of peripheral blood CD4$^+$ T cells in patients with systemic lupus erythematosus (SLE) compared to cells from patients with rheumatoid arthritis (RA) and from healthy controls.

Introduction and Hypothesis: The Craft laboratory has recently shown that the activated CD4$^+$ T cells that drive autoantibody production in vitro and in vivo in lupus-prone mice are markedly expanded, with a T follicular cell (T$\text{FH}$), or B helper cell, phenotype. His lab has also demonstrated that T$\text{FH}$ autoantibody-helping CD4$^+$ T cells in such mice are characterized by unique cell surface markers, identifiable by flow cytometry, among them down regulation of the P-selective glycoprotein ligand-1 (PSGL1, or CD162). In the present work, we hypothesized that T$\text{FH}$ cells would be found in the peripheral blood (PB) of humans with these illnesses, and indeed, in the peripheral blood of mice with lupus. As splenic cells typically escape into the blood, particularly in active lupus, we anticipated that these cells would be expanded in such patients; if so, they would be available for characterization. Hence, we sought them among activated T cells in patients with SLE.

Methods: We analyzed peripheral blood T cells from 9 diseased individuals (6 with SLE and 4 with RA) and 10 healthy controls. Using flow cytometry, activated PB CD4$^+$ T cells were identified by cell surface markers into two groups: central memory cells (activated CD4$^+$ cells that persist in secondary lymphoid organs, defined by downregulation of the marker CD45RA and upregulation of the chemokine-splenic homing receptor CCR7 (CD45RA$^-$CCR7$^+$), and effector memory cells that migrate to the periphery, defined also by downregulation of CD45RA, as well as CCR7 (CD45RA$^-$CCR7$^-$). Naïve (unactivated) cells were also analyzed (CD45RA$^+$CCR7$^+$) as a control. We separated control, central memory and effector memory CD4$^+$ T cells into the following two groups: high level of PSGL1 expression (PSGL1$^{hi}$), and a low level to absent expression of PSGL1 (PSGL1$^{lo/−}$).

Results: We compared the level of PSGL1 expression on naïve CD4$^+$ T cells, central memory CD4$^+$ T cells and effector memory CD4$^+$ T cells among the three groups: SLE, RA and healthy controls. We found no significant differences in the level of PSGL1 expression among groups. There was uniformly high level of expression of PSGL1 in all subjects and CD4$^+$ T cell subtypes. The Craft lab also found uniformly high level of expression of PSGL1 on activated CD4$^+$ T cells in the peripheral blood of lupus prone MRL/Faslpr mice (99.6% of cells were high expressors).

Conclusion: Using PSGL1 expression as a marker, we did not find activated CD4$^+$ B helper T cells (T$\text{FH}$ cells) in the blood of patients with SLE. While such cells are robustly present in the spleens of lupus-prone mice, they were not found in peripheral blood of such animals, suggesting that analogous to our findings in lupus patients, these cells stay "home" in the spleen to help autoantibody-producing B cells. Future studies should focus on the identification and characterization of T$\text{FH}$ cells in the peripheral lymph nodes or tonsils of lupus patients.