ACE Collaborative Projects – Public Summaries

November 2015

Contents:

Aim 1. Characterize peripheral adaptive immunity in autoimmune disease subjects in the context of disease flares/relapses compared to periods of disease quiescence and before/after cell depleting or other immunosuppressive therapies.

1A. Pathogenic B cells: Colorado (Leader: J. Bennett), MGH, Emory, Stanford, OMRF.

1A: How do AQP4-specific B cells vary in the peripheral blood of NMO patients during relapse and quiescence?

An understanding of the genesis and fluctuation of pathogenic B cell populations in human autoimmune disease should benefit from tools to purify and characterize antigen-specific populations in relation to disease activity. Towards this goal, we have been developing protocols to efficiently select AQP4-specific B cells from patients with neuromyelitis optica (NMO). NMO is a severe demyelinating disease in which pathogenic antibodies against the water channel AQP4 selectively destroy astrocytes ultimately leading to death of the myelin-producing oligodendrocytes. We have generated a cell line expressing AQP4 fused to green fluorescent protein that permits the efficient selection of cells engineered to express a surface IgG antibody against AQP4. We have also developed protocols to purify and cryopreserve purified human B cell populations. These methods will now be used to investigate the repertoires of

antigen-specific pathogenic and regulatory B cell populations in NMO patients during disease activity and quiescence.

1B. B cells and T cells: Stanford, OMRF, Michigan Basic (Leaders: P. J. Utz, J. James, A. Sawalha), Colorado, Baylor, Feinstein, Emory.

Summary:

Through this specific subaim the collaborative projects have made significant progress in characterizing peripheral adaptive immunity in systemic lupus erythematosus, neuromyelitis optica and related disorders over the past reporting period. The Michigan Basic ACE program is collaboratively addressing the hypothesis that the size of the demethylated CD4+CD28+KIR+CD11ahi T cell subset interacts with total genetic risk to determine disease activity in lupus, and that this subset can serve as a biomarker for disease progression and remission in lupus patients. Data collected over the interim period has confirmed this association and further demonstrated correlation between the size of this demethylated CD4+CD28+KIR+CD11ahi T cell subset and SLE disease activity as measured by the SELENA-SLEDAI. The size of this demethylated T cell subset was not increased in patients with the antigen-focused autoimmune disease neuromyelitis optica. The Baylor ACE program is focused on understanding roles of various follicular helper T cell subsets (Tfh) in systemic autoimmune disease pathogenesis and has ongoing experiments looking at these subsets and the associated gene expression profiles in pediatric lupus patients and controls. The Oklahoma ACE program has identified a peripheral, plasma-based soluble mediator, comprised of inflammatory and regulatory cytokines, chemokines and soluble TNF-receptor levels, which is associated with SLE disease activity and is significantly elevated in patients before disease flare [Munroe, et al. Arthritis Rheum 2014]. Exosomes are being explored in SLE patients with elevated and suppressed disease as sources or drivers of some of these mediators. In addition, in collaboration with the Stanford ACE, initial mass cytometry and mass cytometry-phophoflow analyses of peripheral blood mononuclear cells from SLE patients and matched autoantibody controls are underway, as well as looking at immune responses in SLE patients after stopping select immunosuppressive medications. The Stanford ACE has also made significant progress in developing technology platforms specifically aimed at the adaptive immune system and characterization of (i.) blood cells such as B cells using CyTOF; (ii.) autoantibodies using large scale protein microarrays, including peptide arrays in collaboration with Shan Wang's lab which is applying Giant MagnetoResistive (GMR) Sensors for rapid measurement of SLE autoantibodies; (iii.) transcriptomics including a meta-analysis of existing transcript profiling experiments that have been deposited in GEO (in collaboration with Purvesh Khatri), then validated the utility of the profile in studying adult and pediatric SLE patients including patients having disease flares; and (iv.) highly multiplexed mass cytometry to perform single-cell analyses to profile the epigenetic landscape in order to identify aberrant epigenetic signatures in lupus and epigenetic marks associated with pathologically important immunomodulatory pathways.

1C. Adaptive Epigenetics: Emory (Leader: J. Boss)

1C. Adaptive Epigenetics (Boss)

A robust pipeline using minimal numbers of primary human cells has been developed/optimized to determine the epigenetic programming of autoimmune disease causing cells. The assays include reduced representation bisulfite sequencing (RRBS), which assess the DNA methylation status of ~18% of human CpGs; Assay for Transposase Accessible Chromatin-sequencing (ATAC-seq), which provides a readout of chromatin accessibility and the influence of epigenetic processes; and RNA-seq. Each of the above assays has been optimized for 2,000 cells or fewer. Additionally, a ChIP-seq protocol was optimized for histone H3K4me3 that uses 10,000 cells. Additionally, comparisons of biobanked materials for ATAC-seq were made to increase access to subject samples and to improve the workflow. In collaboration with the Sanz lab (Emory ACE), each of these assays was employed on SLE patient and healthy control samples. Five B cell subsets, including those that represent the expanded and potentially pathogenic B cells from 4 SLE and 4 HC were examined by RRBS, ATAC-seq, and RNA-seq. Although the analyses of these datasets is underway, the data point to increased proliferation potential of the expanded SLE subsets compared to their HCs, as well as the influence of estrogen and inflammatory pathways as contributors to the cell fate programming of SLE B cells. DNA methylation is not sufficient to explain all of the epigenetic data, and that it is likely that the histone code is also critical in controlling the cell fate of SLE vs HC B cell subsets. Over the next 12-18 months: additional subjects will be recruited and analyzed above; ChIP-seq will be optimized for additional antibodies (H3K4me1, H3K27ac, and H3K27me3); and Phase II bioinformatics that compares complementary data sets (e.g., RNA-seq vs. RRBS, etc.) will be performed. Additionally, optimized ChIP-seq assays will be employed. Lastly, this set of assays is planned to be applied to SLE patients before and after B cell depleting therapies. The results of such experiments will define whether new B cells emerge following treatments or whether the B cells that repopulate patients represent the same pathogenic cells from before therapy. This collaborative subaim is poised to be expanded to include other aspects of the ACE consortium.

1D. Microbiome: MGH (Leader: S. Pillai)

1D Aim 1D. To determine the nature of the intestinal microbiome of patients with an initial presentation of active autoimmunity or with a disease flare (including patients with IgG4-RD and systemic sclerosis) and to determine if the onset of disease or flare can be linked to distinct bacterial community memberships.

The driving hypothesis of these studies is that autoimmune disease initiation and flares of disease are initiated by changes in the gut (or oral) microbiome. There is evidence in support of such a view in Type I diabetes and more recently in asthma and allergic disorders.

In these studies 54 IgG4-RD subjects from MGH out of a four-year target of 200 have been recruited for microbiome studies. The first five samples from systemic

sclerosis patients from Dr. Dinesh Khanna at the University of Michigan have been received at the Broad Institute (initial target 80). Intestinal microbial communities are being assessed before and after therapy. **Initial sequencing results on the initial 21 patients with IgG4-RD have revealed a striking alteration in microbial diversity that separates IgG4-RD subjects dramatically from controls. It remains to be seen if this striking difference will hold up in a larger study. If it does, this alteration may hold the key to the striking CD4+ CTL T cell clonal expansions seen in IgG4-RD.**

Aim 2. Characterize *in situ* adaptive immunity in autoimmune disease.

2A. *In situ* TFH cell function in inflammation: Chicago, Baylor (Leaders: M. Clark, H. Ueno), Michigan Clinical.

2B. Non-conventional *in situ* antigen presenting cells: Michigan Clinical (Leader: D. Fox), Chicago.

Summary

The overall hypothesis for Collaborative Aim 2 is that the mechanisms of adaptive selection *in situ*, at sites of inflammation and organ damage, will be different than that which occurs in secondary lymphoid organs or that which predominate in the periphery. In Aim 2A, we proposed to examine how collaboration between T follicular helper (TFH) cells and B cells in situ contributes to inflammation. In Aim 2B, we will examine how T cells receive cognate help in situ from both conventional (example: dendritic cells) and non-conventional (example: fibrocytes in rheumatoid arthritis synovium) antigen presenting cells. In Aim 2A, a major focus is on developing protocols for isolating TFH cells and B cells from tissue and performing RNA-Seq to understand how both populations are selected in normal and inflammatory contexts. Initial studies on TFH cells from tonsil and blood have been performed and the analysis is pending. Protocols have been established for isolating cells efficiently from clinical renal biopsies. For single cell RNA-Seq, much of the sequencing will be done in collaboration with the Harvard ACE. In Aim 2B, we have made substantial progress in developing the computational tools to analyze spatial relationships between dendritic cells and T cells. These new cell distance mapping (CDM) tools use both distance and T cell morphology to infer functional relationships. In preliminary studies, CDM analysis indicates that the presence and frequency of plasmacytoid dendritic cells (pDCs) in renal biopsies is associated with severe tubulointerstitial disease. Furthermore, these pDCs appear to be competent antigen presenting cells in situ.

We have also made progress in assessing novel roles of synovial fibroblasts (FLS) in rheumatoid arthritis (RA). These cells have been imaged using high-resolution fluorescence microscopy in contact with CD4+ T cells, and algorithms to calculate cell distance measurements are being developed. FLS also express AIRE (the autoimmune regulator protein, that has a well-defined role in negative selection of autoreactive T cells in the thymus), and ongoing studies will determine the program of gene expression

regulated by AIRE in these cells. Comparison of gene expression between a patient with a dominant-negative AIRE mutation and multi-organ autoimmunty and the patient's healthy first-degree relative reveals that AIRE in fibrocytes regulates a broad program of gene expression that governs interactions between fibrocytes and T cells, as well as various aspects of fibrocyte activation and differentiation.