Autoimmunity Centers of Excellence
Annual Report 2017

Overview

The Autoimmunity Centers of Excellence (ACE) Program consists of three interrelated research components: (i) basic science projects being conducted by seven U19 sites; (ii) clinical trials led by four UM1 sites; and (iii) collaborative translational projects that arose from the original peer-reviewed grants but that were subsequently integrated to take maximal advantage of the complementary expertise within the ACE group. As described below, these three components are not independent, but rather represent a coherent approach to several of the most important challenges in the field of autoimmune diseases.

Basic Science Component (U19s)

The basic science component of the Autoimmunity Centers of Excellence (ACE) studies different aspects of innate and adaptive immune responses: correlates of disease development and flares, cellular and soluble mediators of disease, genetic and epigenetic determinants of autoimmune responses, diversification and selection of the autoantibody repertoire, and clinical trials design. Funded through multiple U19s more specifically summarized in the following sections, the ACE research exploits a range of cutting-edge technologies including global and single cell RNA-sequencing and epigenetics, high-dimensional flow cytometry and mass cytometry (CyTOF), Next Generation Sequencing of BCR repertoires, antibody repertoire analysis and monoclonal antibody generation through high-throughput single cell interrogation and cloning, *in situ* tissue analysis of cell-cell interactions by cell-distance mapping, and extensive antigen microarrays. These approaches are applied to the understanding of the pathogenesis, natural history and treatment of multiple autoimmune diseases and specifically through the U19s of Systemic Lupus Erythematosus, Rheumatoid Arthritis, Scleroderma, and IgG4-related disorders (IgG4-RD). The ACE basic science programs are highly interactive both among themselves and with the ACE UM1s clinical centers in part through the ACE Collaborative Agenda as well as center-specific collaborations.

Major accomplishment and future goals of the different centers include:

Baylor (V. Pascual, PI)

The Baylor group has continued to study the effects of neutrophil-derived oxidized mitochondrial DNA (Ox mtDNA) on plasmacytoid Dendritic cell (pDC) function and downstream adaptive immunity. In particular, their work shows that activated pDCs skew naïve CD4 T cells towards a unique Type I regulatory T cell (Tr1)-like phenotype. These Tr1-like cells however are not anergic and, rather than being suppressive, provide B cell help in an IL21-independent manner. Furthermore, these cells do not express classic follicular helper T cell (Tfh) markers and both their gene expression and chromatin accessibility profiles differ substantially from those of Tfh. Cells with similar characteristics are expanded in pediatric SLE blood. They also present in tubulointerstitial areas of proliferative (class III and IV) but not in class II or V lupus nephritis sections. The group is currently investigating the potential of these cells as biomarkers of proliferative nephritis and further dissecting the mechanism whereby they provide B cell help to identify potential therapeutic targets.

Chicago (M Clark, PI)

Understanding the balance between immune tolerance and protection: The long-term goal of our research is to understand the pathways that regulate the balance between autoimmune, or specifically in this case polyreactive specificities, and immune tolerance. Our work has found that antigenically novel influenza viruses induce protective and broadly reactive antibody responses derived from memory B cells targeting the highly-conserved stalk region of the viral surface glycoprotein hemagglutinin (HA). The majority of stalk-specific monoclonal antibodies (mAbs) are polyreactive, the property of non-specifically binding to structurally unrelated antigens, including autoantigens. These data suggest that novel influenza strain exposure induces polyreactive antibodies against conserved HA-epitopes. The goal of the proposed research is to investigate the role of polyreactive antibodies in viral binding and protection against infection. We are additionally interested in understanding whether polyreactive antibodies induced by influenza infection or vaccination can induce or contribute to autoimmunity and the immune checkpoints to prevent autoimmunity by polyreactive antibodies.

Analysis of human intestinal plasma cells in the context of inflammatory bowel diseases: Surprisingly, patients deficient in IgA have only a modest increase in rates of intestinal infections. However, they do have an

increased risk for Celiac Disease and Inflammatory Bowel Disease (IBD)' suggesting that plasma cells may play a role in the pathogenesis of intestinal disease. Both IBD and the often-associated autoimmune disease Primary Sclerosing Cholangitis (PSC) have strong HLA-II associations on GWAS, which suggests B cell involvement in pathogenesis. Our preliminary findings show that the plasma cells found in the PSC-IBD colon have a recently-arrived phenotype and are predominantly of the non-IgA isotype. Additionally, we recently identified that this plasma cell phenotype is associated with an IL-17 signaling gene signature. To investigate the significance of these findings we have been sorting plasma cells and T-cells in bulk for transcriptional analysis. This analysis will allow us to identify any repertoire biases within these populations that may exist in diseased individuals. We have also single cell sorted plasma cells from PSC subjects and controls with the intent of performing single-cell transcriptional analysis as well as cloning of the antibody from each cell. These antibodies will be tested for both autoantigen binding and will capture microbiota extracts for 16s sequencing. We expect to see a correlation between microbiota reactivity and IBD syndromes, particularly where the unusual IgG plasmablasts are concerned.

Emory (I. Sanz, PI)

The Emory ACE U19 investigates pathways of B cell activation in SLE. These goals are pursued using a comprehensive B cell Immune-Omics approach that includes B cell profiling by multidimensional flow cytometry; BCR repertoire Next Generation Sequencing; serum autoantibody proteomics; and integrated transcriptome and epigenetic profiling using methylation and chromatin accessibility analysis. These approaches are complemented by ultra-high throughput single cell antibody generation. Antigenic characterization of mAbs is pursued in collaboration with other ACE U19s including the Stanford and Baylor programs. B/T cell interactions *in situ* and *ex vivo* are explored in collaboration with the Chicago, MGH and Baylor ACEs.

Major achievements include:

1. Characterization of the cellular components of the human extrafollicular B cell activation pathway and the magnitude of its participation on active SLE
2. Understanding of the molecular programs underpinning early B cell activation and extrafollicular plasmablast differentiation in flaring SLE.
3. Epigenetic programing of SLE naïve B cell: The collaborative project with Dr. Boss (see Collaborative Agenda), has contributed the first demonstration that transcription factors central to immune-regulatory networks display an open chromatin configuration in resting naïve B cells thereby suggesting they are poised for differentiation before receiving antigenic signaling. In particular, we are identifying genetic modules that are positively and negatively differentially transcribed during extrafollicular B cell activation in SLE.

Publications (including abstracts presented at national and international conferences):

Oleksyn, D., Zhao, J., Vosoughi, A., Zhao, J.C., Misra, R., Pentland, A.P., Ryan, D., Anolik, J., Ritchlin, C., Looney, J*., et al.* (2017). PKK deficiency in B cells prevents lupus development in Sle lupus mice. Immunol Lett.

Sanz, I. (2017). May you live in interesting times: Challenges and opportunities in Lupus research. Arthritis & rheumatology (Hoboken, N.J.).

Tanaka, T., Zhang, W., Sun, Y., Shuai, Z., Chida, A., Kenny, T.P., Yang, G.X., Sanz, I., Ansai, A., Bowlus, C.L.*, et al.* (2017). Autoreactive Monoclonal Antibodies from Patients with Primary Biliary Cholangitis Recognize Environmental Xenobiotics. Hepatology (Baltimore, Md.).

Jenks, S., Margorta, U., Patel, A., Wang, X., Tomar, D., Zumaquero, E., Lim, S.S., Petri, M., Niewold, T., Anolik, J.*, et al.* (2017). Identification of a new effector B cell pathway with defective negative regulation of TLR7 signaling in human SLE. Immunity; Under revision.

Zumaquero, E., Stone, S., Nellore, A., Scharer, C., Jenks, S., Rosal, A., Botta, A., Bradley, J., Wojciechowski, W., Danila, M.*, et al.* (2017). Identification and characterization of human antibody secreting cell precursors – role for the transcription factor T-bet. Immunity; Under revision.

Dale, G.A., Bohannon, C.D., Wilkins, D.J., Dilernia, D., Hunter, E., Bedford, T., Antia, R., Sanz, I., and Jacob, J. (2017). Gene conversion contributes significantly to IgHV somatic hypermutation in mice and humans. The Journal of Immunology *198*, 195.197-195.197.

Goldenberg, R., Tipton, C., Jenks, S., Lee, E.-H.F., Sanz, I., and Mehr, R. (2017). Relationships and transitions between B and plasma cell populations in SLE patients. In SCANDINAVIAN JOURNAL OF IMMUNOLOGY (WILEY 111 RIVER ST, HOBOKEN 07030-5774, NJ USA), pp. 325-325.

Hamilton, J., Wu, Q., Yang, P., Luo, B., Liu, S., Li, J., Sanz, I., Chatham, W.W., Hsu, H.-C., and Mountz, J.D. (2017a). Interferon-beta Production By B Cells Promotes B Cell Survival and Is Strongly Associated with Active Disease in African Americans with SLE. In ARTHRITIS & RHEUMATOLOGY (WILEY 111 RIVER ST, HOBOKEN 07030-5774, NJ USA).

Hamilton, J.A., Yang, P., Liu, S., Li, J., Wu, Q., Luo, B., Walter, M.R., Sanz, I., Fish, E.N., Chatham, W.*, et al.* (2017b). Single cell analysis of transitional B cells reveals autocrine IFNβ sustains a dynamic type I IFN network in lupus. The Journal of Immunology *198*, 54.53-54.53.

Khosroshahi, A., Corrado, A., Muraki, T., Kyu, S., Wang, X., SANZ, I., and Lee, E.-H. (2017). Circulating IgG4 Antibody Secreting Cells Are Better Biomarker of Disease Activity Compared to Serum IgG4 Levels in Patients with IgG4-Related Disease. Arthritis & Rheumatology *2016*.

Nguyen, D., Kyu, S., Williams, J., Hong, S.-H., Albizua, I., Galipeau, J., Chiang, K.Y., Waller, E., Garimalla, S., Gibson, G.*, et al.* (2017). Novel in Vitro Cell-free Human Plasma Cell Survival System. Blood *Submitted*.

Scharer, C., Blalock, E., Barwick, B.G., Gandham, B., Neary, B., Sanz, I., and Boss, J.M. (2017). Pathogenic epigenetic programming of expanded B cell subsets in systemic lupus erythematosus. The Journal of Immunology *198*, 54.13-54.13.

Wei, C., Hill, A., Smith, K., Sanz, I., Lim, S.S., and Drenkard, C. (2017). B Cell Abnormalities in Patients with Chronic Cutaneous Lupus Erythematosus. In ARTHRITIS & RHEUMATOLOGY (WILEY 111 RIVER ST, HOBOKEN 07030-5774, NJ USA).

Future goals include

1. Continuing to dissect the molecular pathways that underlie abnormal B cell activation and differentiation of autoreactive B cells in SLE.

2. Completion of studies currently underway using serum autoantibody proteomics and matched cellular NGS of multiple blood, kidney and bone marrow B cell populations to dissect the diversity and stability of SLE autoantibodies as well as the relative contribution of short-lived and long-lived plasma cells.
3. Continue studies of kidney biopsies in patients with Lupus Nephritis to understand the nature and antigenic autoreactivity of activated kidney infiltrating B cells and plasma cells.
4. To continue collaborative efforts with the Stanford ACE (Dr. Utz) to characterize mAbs using antigen microarrays and with the Baylor ACE (Dr. Pascual) to study their reactivity with oxidized mitochondrial DNA.

Massachusetts General Hospital (MGH) (S. Pillai, PI)

Our studies over the past year have focused on the pathogenesis of IgG4-related disease (IgG4-RD) and also of systemic sclerosis (SSc), the latter being in close collaboration with the Michigan ACE. Studies have been conducted on untreated active IgG4-RD tissues as well as on skin biopsies of untreated early diffuse systemic sclerosis subjects from the ASSET trial.

Major accomplishments:

1. Based on our extensive studies on two human diseases we can conclude that these major human fibrotic diseases are linked to the tissue accumulation of cytotoxic CD4+ T cells or CD4+CTLs. These T cells are the dominant T cell population in the tissues of IgG4-RD subjects with active disease, as well as in diffuse systemic sclerosis. SLAMF7/CD319 has been identified as a useful marker for CD4+CTLs. **The discovery of the link between CD4+CTLs and SSc may represent a significant advance in our understanding of the pathogenesis of this disease**.
2. TH2 cells have been quantified in disease tissues and are relatively rare in the tissues of IgG4-RD as well as in SSc.
3. Both IgG4-RD and SSc are linked to CD4+CTLs that express IL-1beta. In SSc, IFN-gamma expression by CD4+CTLs is rare, and this might indicate why fibrosis is so much more severe in SSc than in IGg4-RD (since IFN -gamma has the potential to attenuate IL-1 induced fibrosis and is expressed in IgG4-RD but not in SSc). In collaboration with the Stanford ACE, auto-antibodies against the IL-1RA (antagonist) have been discovered in IgG4-RD, and these antibodies enhance IL-1 signaling. **These studies have strongly suggested that IL-1beta blockade may be a useful therapeutic strategy to consider in both diseases**.
4. Considerable progress has been made using *in vitro* differentiation, single cell RNA seq and ATAC seq in determining drivers and pathways for CD4+CTL differentiation. Validation is being attempted using a CRISPR screen in vitro. A full understanding of the pathway may offer strategies for attenuation CD4+CTL generation and activation.
5. Studies performed in IgG4-RD on T follicular helper cells have helped identify IL-4 expressing BATF+ TFH cells as the major drivers of IgG4 class switching. These cells have been further studied using ATAC seq, RNA seq, single cell RNA seq and whole genome DNA methylation and a detailed molecular understanding of this subset of CD4+CXCR5+ TFH cells (and their counterparts from subjects expressing high levels of IgE) has been obtained.
6. Single cell plasmablast cloning and mass spectrometry has revealed Galectin-3 as an auto-antigen in some subjects with IgG4-RD. Collaborative studies with the Stanford ACE using cytokine arrays has revealed that IL-1RA and CCL23 are also auto-antigens in IgG4-RD.
7. A range of activated B cell populations has been discovered in IgG4--RD subjects especially in the transitional and Double negative pools (pioneered in lupus by the Emory ACE). However, we see a

number of differences between the populations in lupus and IgG4-RD. These are being investigated in detail in collaboration with the Emory ACE.

8. The interaction of activated B cells with CD4+CTLs in IgG4-RD tissues has been demonstrated and quantitated, providing a mechanism to explain why Rituxan is therapeutically useful in this disease.

Michigan (B. Richardson, PI)

The Michigan ACE U19 investigates how T cell DNA methylation defects play an important role in the pathogenesis of systemic lupus erythematosus with the central goal of characterizing the impact of exogenous epigenetic modifiers on the T cell epigenome and gene expression patterns in a novel CD4+CD28+ T cell subset characterized by overexpression of methylation sensitive genes, including CD11a and the KIR gene cluster. The project also aims to identify and test novel therapeutic targets for lupus.

Studies published in 2016 reported that this subset is found in patients with other systemic inflammatory rheumatic diseases including rheumatoid arthritis, systemic sclerosis and Sjogren's Syndrome, and that the size of the subset was directly related to disease activity. Major achievements in the past year include the analysis of the DNA methylome, transcriptome, and chromatin accessibility patters of the cells of interest. Genome-wide DNA methylation analysis has identified 31,019 differentially methylated sites in *in vitro* generated CD4+CD28+KIR+CD11a^{hi} T cells, induced using the DNA methylation inhibitor 5-azacytidine, with >99% being hypomethylated compared to CD4+CD28+KIR-CD11a^{low} T cells. RNA sequencing revealed a clear pro-inflammatory transcriptional profile. Bioinformatics analysis of 718 genes that are hypomethylated and overexpressed in CD4+CD28+KIR+CD11a^{hi} T cells revealed significant enrichment in pro-inflammatory gene ontologies, pathways, and gene meta-groups. T cell receptor repertoire analysis revealed less clonotype diversity in CD4+CD28+KIR+CD11a^{hi} compared to autologous CD4+CD28+KIR-CD11a^{low} T cells. Similarly, primary CD4+CD28+KIR+CD11a^{hi} T cells isolated from lupus patients were hypomethylated and characterized by a pro-inflammatory chromatin structure detected using ATAC-seq. These studies have been published (CD4+CD28+KIR+CD11a^{hi} T cells correlate with disease activity and are characterized by a pro-inflammatory epigenetic and transcriptional profile in lupus patients. Gensterblum E, Renauer P, Coit P, Strickland FM, Kilian NC, Miller S, Ognenovski M, Wren JD, Tsou PS, Lewis EE, Maksimowicz-McKinnon K, McCune WJ, Richardson BC, Sawalha AH. J Autoimmun. 2017 Oct 20.)

This center is also testing the ability of antibodies to KIR proteins to inhibit B cell stimulation and macrophage killing by the CD4+CD28+KIR+CD11a^{hi} T cells. Using unfractionated CD4+ T cells treated with the DNA methyltransferase inhibitor 5-azacytidine and purified CD4+ T cells from patients with active lupus containing the CD4+CD28+KIR+ T cell subset, these studies found that antibodies to inhibitory KIR block autologous macrophage killing whereas antibodies to stimulatory KIR trigger IFNγ release.

In related studies supported by through other resources, we also found that the same anti-KIR antibodies crossreact with murine Kir proteins and prevent the development of glomerulonephritis in murine lupus. This suggests that the subset may be a target for the treatment of human lupus.

Stanford (PJ Utz, PI)

The Stanford ACE U19 includes a Principal Project (Robinson, PI), Collaborative Project (Utz, PI), an Administrative Core (Utz, PI), and the Human Immune Monitoring Core (Maecker, PI). The Stanford ACE U19 studies B cells and autoantibodies against citrullinated antigens in Rheumatoid Arthritis (RA); antibodies specific to a variety of antigens in connective tissue diseases including systemic lupus erythematosus (SLE) and IgG4-related disease; antigen-specific T and B cells using sorted cells and scRNA-Seq, and epigenetic

marks across multiple different blood cell subsets using a newly-invented technique called EpiCyTOF. Major achievements to date include the demonstration, using single cell RNA-Seq analysis, that anti-citrullinated protein antibody (ACPA) expressing B cells exhibit transcriptional profiles characteristic of germinal center-driven responses, while rheumatoid factor expressing B cells exhibit transcriptional profiles characteristic of innate immune-driven and natural antibody responses. Studies of circulating plasmablasts derived from RA patients using scRNA-Seq demonstrate persistent IgA plasmablast responses in pre-clinical and established RA, and the impact of affinity maturation-mediated epitope spreading within the ACPA response. Collaborative Project studies feature EpiCyTOF characterization of 11 different PBMC subsets in healthy young and old subjects; parallel studies in twin cohorts to determine the differential roles played by genetics and epigenetics in phenotypes; nearly complete EpiCyTOF studies of patients with SSc, SLE, and other autoimmune diseases; and autoantibody profiling in the SCOT transplant trial, using a 288-feature bead-based array.

The Stanford ACE also contributes a state-of-the-art immunological profiling core. This core continues to support mechanistic studies of ACE-sponsored clinical trials including ASC01 (rituximab in systemic sclerosis) through real-time B cell phenotyping, and cryopreservation of PBMC, serum, and plasma for Luminex analysis of cytokines and Ig isotypes; flow cytometry phenotyping of Tregs and Th1/Th2/Th17 cells for Mark Nicolls' sub study; and support of additional ACE studies at several centers including OMRF, Colorado, and MGH. Developmental efforts have included new CyTOF capabilities and adaptation of phenotyping panels to whole blood and to the Smart Tube buffer system, which enables stabilizing blood at the collection site for later shipment to a central lab for CyTOF analysis. Antigen multimers have been developed to isolate ACPA B cells and rheumatoid factor B cells. Additional optimization of single-cell TCRseq assays is also underway. New EpiCyTOF and disease-specific panels are being developed, along with new platforms for single-cell and low-input genomics studies, and liquid handling automation to increase throughput.

Oklahoma Medical Research Foundation (J. James, PI)

The Oklahoma ACE continues to contribute new insights to disease pathogenesis in systemic autoimmune rheumatic diseases, such as systemic lupus erythematosus (SLE), primary Sjgoren's syndrome (pSS), and related disorders. Recent accomplishments have provided new insights to genetic predisposition to SLE and pSS, described gene-environment and gender-biased influences associated with SLE development, identified pathways of impending disease flare in African-American SLE patients, as well as helped identify new pathogenic process in pSS, measures of SLE disease activity and development of a new SLE disease severity index in collaborative work.

As major contributors to the SLE Immunochip Consortium, the Oklahoma ACE has helped to identify 58 distinct non-HLA genetic associated regions in European-American, 9 regions in African-American and 16 regions in Hispanic American, including 24 novel SLE genetic associations. This work has also established a new measure of lupus genetic load which can be used to assess cumulative or cumulative weighted lupus genetic risk (Langefeld, et al. Nat Commun 2017). Additional genetic work in pSS has identified associated functional variants of OAS1 which impact isoform switching, protein expression and interferon response (Li et al. PLoS Genetics 2017). Gene-environment interactions have also been assessed, showing that interactions between vitamin D deficiency and specific vitamin D pathway genetic associations associate with transition to clinical SLE (Young et al. Ann Rheum Dis 2017) and X chromosome dose contributes to gender bias in SLE and pSS (Liu, et al. Arthritis Rheum, 2016). New pathways of impending SLE disease flares have been identified in African-American lupus patients (Munroe, et al. J Autoimmun, 2017).

The Oklahoma ACE has also developed a novel, unbiased strategy for distinguishing the likely functional variants among GWAS-identified SNPs. This genome-wide strategy combines multiple sequencing technologies (ATAC-seq, ChIP-seq, HiChIP) to examine SNPs in the context of the epigenome and genome structure. This provides *a priori* evidence about the possible genetic regulatory functions of the SNPs, including functional relevance in individual cell types or under specific conditions.

The Oklahoma ACE has collaborated with other ACE sites on several studies. Collaborative studies forged through the ACE Consortium have identified mechanisms of clonal lymphoproliferation in pSS (Glauzy et al. Arthritis Rheum. 2017) and unique immune profiles that may regulate autoimmunity in ANA-positive healthy individuals (Slight-Webb, et al. Arthritis Rheum 2016). The Oklahoma ACE assessed 30 soluble mediators and 13 anti-nuclear autoantibodies and helped complete the quality control and analysis of molecular phenotype data from 52 patients in the ASJ02 study (Baminercept in pSS). Expression modules identified three distinct clusters of pSS patients, which differed in interferon and inflammation signatures, which may be informative in selection of patients for clinical trials and to help identify novel pathways to target in pSS therapy.

Clinical Science Component (UM1s)

The clinical science component of the ACE program consists of four programs that reflect distinct, complementary approaches to the treatment of autoimmune diseases. As summarized briefly below, one program is exploring therapeutic strategies designed to inhibit inflammation (Feinstein Institute); one program is exploring strategies designed to inhibit pathologic immune mechanisms (University of Michigan); one program is exploring strategies designed to augment protective immune mechanisms (University of California, San Francisco); and one program is exploring strategies designed to prevent the development of autoimmune disease in people at high risk (University of Colorado).

Feinstein Institute (C. Aranow, PI)

This program is predicated on the notion that tissue injury in autoimmune disease is the end result of multiple and often redundant inflammatory pathways and mediators. Therefore, an approach modulating multiple inflammatory mediators will have broad applications, and may offer safer and better tolerated interventions than those that are currently available for treatment of inflammatory autoimmune diseases. The primary project of the Feinstein Clinical Center envisioned a phase II trial in patients with rheumatoid arthritis using an agent (GTS-21) that activates the cholinergic anti-inflammatory pathway by engaging the alpha 7 nicotinic receptor, resulting in reduced production of inflammatory cytokines. However, this project was terminated by unanticipated difficulties obtaining the agent. Therefore, the alternate project entered protocol development. This project (ALE09) will evaluate the efficacy, safety, and tolerability of ajulemic acid/JBT-101 in patients with systemic lupus erythematosus (SLE) in a 12-week, phase 2, double-blind, randomized, placebo-controlled multicenter study. Ajulemic acid is a synthetic, non-psychotropic cannabinoid that possesses multiple anti-inflammatory properties. Mechanistic studies evaluating the biologic effects of the agent are an integral component of this ACE supported study. Protocol development is complete and the study is expected to begin enrollment in the first quarter of 2018.

University of Michigan (D. Fox, PI)

This program has two active clinical/translational projects and is also involved in the collaborative projects. The two trials are summarized in this section, and the collaborative work is summarized below in the section devoted to collaborative projects.

Project #1: Immunological Profile Changes in Patients with Secondary Progressive Multiple Sclerosis (SPMS) Treated with BAF312 (AMS04) – PI Yang Mao-Draayer, MD, PhD. This project is designed to evaluate immunological profile changes of SPMS patients before, and 6 months and 12 months post- treatment with BAF312 (Siponimod), a selective sphingosine 1-phosphate receptor modulator. The project is linked to the ongoing Phase III EXPAND trial, which is a multicenter, randomized, double-blind, placebo-controlled study comparing the efficacy and safety of BAF312 to placebo in patients with SPM (ClinicalTrials.gov Identifier: NCT02330965). Thirty-three SPMS patients in the EXPAND trial were recruited for the ACE study. PBMCs were isolated from the patients before and after 6 and 12 months of treatment, and immunological profile changes were investigated by flow cytometry. Following un-blinding of the data, preliminary analysis has shown that the BAF312 treated group has a significantly lower percentage of CD4$^+$ T cells compared to the placebo-treated group. However, the percentage of CD8$^+$ T cells did not change significantly. Though the percentage of B lymphocytes was also significantly reduced, the proportion of regulatory B cells were increased with BAF312 treatment. In addition, the percentage of monocytes and NK cells were also increased with BAF312 treatment compared to placebo group. Studies currently in progress are analyzing the absolute

number changes as well as other in-depth T or B cell subset changes. Further functional analysis and gene expression studies are also on the way.

In addition to the analyses described above, flow cytometry and microarray analysis was performed on 20 SPMS patients from the phase III EXPAND trial at baseline and was compared to 7 benign MS (BMS) patients, 14 relapsing remitting MS (RRMS) patients and 6 healthy controls (HC). SPMS had a significantly lower percentage of CD4$^+$ T cells compared to BMS. Both RRMS and SPMS had a significantly higher percentage of CD8$^+$ T cells compared with BMS. SPMS has a higher percentage of B cells compared with both HC and BMS. Clustering analysis of microarray data identified a series of genes which were differentially expressed in PBMC between BMS and SPMS patients. Functional analysis based on Gene Ontology showed that those regulated genes were enriched in "immune response" and "B cell-mediated immunity" pathways, which may play critical roles in the immune response during MS progression. Further studies will correlate the immune-profiles with MS disease course and treatment response and may lead to future biomarker discovery.

Project #2: ASSET – A Phase 2 Study of Abatacept Versus Placebo in Diffuse Cutaneous Systemic Sclerosis (SSc) – PI Dinesh Khanna, MD, MPH. The clinical protocol upon which this project is based is funded by Bristol-Myers Squibb (ClinicalTrials.gov Identifier: NCT02161406); the mechanistic studies are supported by the ACE. Thus far, this project has recruited 88 subjects, 2 more than the target 86 patients. Sixty percent of subjects have completed month 12 of treatment and have entered the 6-month open label extension portion of the study. PBMC were obtained at 0, 1, 3 and 6 months, and skin biopsies were obtained at 0, 3 and 6 months. All of the planned assays on blood and skin have been successfully validated and implemented, including detailed multi-color flow cytometry, immunohistology and RNA-seq. Hypotheses focus on the potential of abatacept to reduce the numbers of activated pathogenic Th2 cells in blood and skin, and also anticipate a greater response in the subset of patients with an active inflammatory molecular signature in the skin. Results to date have shown a baseline expansion of CD4+CD319+ cells, an unusual cytotoxic CD4+ subset. Further data analysis awaits completion of the double-blind phase and un-blinding in April 2018.

University of California, San Francisco (UCSF) (D Wofsy, PI)

This program is designed to examine the potential for using *ex vivo* expanded autologous regulatory T cells (Tregs) in the treatment of autoimmune diseases. Initially, we sought to examine Treg therapy in patients with cutaneous manifestations of systemic lupus erythematosus (SLE) (ClinicalTrials.gov Identifier: NCT02428309), but enrollment proved to be very challenging due to complicating features of SLE. One patient with SLE underwent the protocol and provided very tantalizing findings. Twelve weeks after the Treg infusion, there was a 75% increase in percent Tregs in affected skin, with documentation that the increase in Tregs reflected the presence of the infused cells. Moreover, there was a profound impact on the local microenvironment, characterized by a selective 75% decline in IFN-γ-producing T cells. RNA-seq analysis confirmed a dramatic decrease in IFN-γ response genes. A similar pattern was observed in the peripheral blood. This work constitutes the first analysis of the effects of Treg therapy at the site of autoimmune tissue injury. However, due to enrollment challenges, we have chosen to focus instead on a different cutaneous autoimmune disease, pemphigus. Preparation for the pemphigus trial is complete and the study was officially opened as a multicenter trial in October 2017.

University of Colorado (M Holers, PI)

This program is testing innovative therapeutic strategies designed to prevent the development of autoimmune disease by administering immunomodulatory agents to individuals who are at high risk for developing disease.

The principal clinical trial project is led by Dr. Kevin Deane and is entitled "Strategy for the Prevention of Rheumatoid Arthritis (StopRA)" (ACE designation 'ARA08') (ClinicalTrials.gov Identifier: NCT02603146). This project is a randomized, placebo-controlled trial to evaluate the efficacy and safety of hydroxychloroquine (HCQ) to prevent or delay future RA in subjects who at high-risk for developing RA based on serum elevations of the anti-cyclic citrullinated protein (anti-CCP) antibody. HCQ has been selected because it has multiple immunomodulatory effects that may contribute to its proven ability to reduce disease activity in people with RA, and because of its safety and tolerability. Thus far, the following steps have been accomplished: 1) 18 of the originally proposed sites have been activated, and 2 more are being considered; 2) Since the first study site was activated in April 2016, approximately 3,000 pre-screenings to identify anti-CCP positive individuals have been performed; 3) Thus far, 50 subjects have been randomized (25% of the randomization goal of 200), and 4) 6 subjects have developed the primary endpoint of Classifiable RA (2010 ACR/EULAR). Because randomization was initially slower than expected, the StopRA budget was increased to improve sites' ability to prescreen, to support the performance of other site activities, and to support novel approaches to identifying anti-CCP positive individuals through activities such as biobank testing and blood donor screening. These changes are having a positive impact on enrollment.

The second project is entitled "Efficacy of Methyldopa to Preserve Beta-cell Function in New Onset Type 1 Diabetes" (T1D01)". The protocol was finalized by the ACE study team. Subsequently, support from TRIALNET was obtained. The current plan is to perform the study in subjects at risk for the future development of T1D. Safety, glucose control, ß-cell function and immune function will be assessed throughout the study.

Collaborative Projects

Aim 1: Peripheral Adaptive Immunity in disease flare, relapse, and quiescence

B and T cells play central roles in most autoimmune diseases. In some diseases, pathogenic B cells secrete autoantibodies that directly mediate disease (e.g. neuromyelitis optica, NMO), while in other diseases the role of antibodies remains poorly understood. Similarly, T cells are required for disease to occur in mouse models of Type I diabetes and are likely to play key roles in many human autoimmune diseases. The goal of Aim 1 is to explore the role of adaptive immune cells and their secreted products in human autoimmune diseases.

1A. Pathogenic B cells (J. Bennett, Colorado)

The translational analysis of the B cell and antibody responses in active and quiescent NMO has steadily accrued additional NMO patients and moved forward on each of the four project subaims. At mid-year, 16 of 20 NMO patients have been recruited (9 relapsing and 7 quiescent). B and T lymphocytes have been cryopreserved for analysis in Aims 1-3 and additional aliquots have been reserved for collaborative projects with Emory (Transcriptomic and Epigenomic Analysis of Double Negative B Cells, [Sanz, Boss]) and Mt Sinai (T Follicular Helper Cells, [Ueno]). Plasma samples collected from the same 16 patients have been analyzed in Aim 4 to correlate glycovariant abundance with disease activity. Subaim progress is as follows:

Aim 1: We used a lentiviral M23AQP4-eGFP transduced cell line to capture AQP4-specific circulating plasmablasts and memory B cells. Paired VH and VL sequences were recovered from circulating plasmablasts. Paired VH and VL sequences were not recovered efficiently from memory B cells. Recombinant antibodies (rAbs) have been constructed from over-represented single cell VH/VL sequences and their specificity for AQP4 is being tested. Flow cytometric analysis has identified an expanded population of CD19+CD27-CD11c+CXCR5- B cells that correlate with NMO disease activity.

Aim 2: IL-10-producing B10 and B10Pro regulatory cells were induced using defined stimulatory parameters. The percentage of circulating B10 and B10Pro cells were assessed in active and inactive patients: no statistical difference was noted. Individual IL10-secreting B10 cells were collected for single cell deep sequencing using the Stanford pipeline; unfortunately, due to low RNA copy number, IgG from the single cells were not efficiently amplified.

Aim 3: The frequency of $CD19^+IgD^+CD21^{lo}CD27^-IgM^{low}$ anergic B cells were analyzed by FACS. The percentage of anergic B cells were lower in active than quiescent NMO subjects (33.4% vs. 39.3%) but was not statistically significant. Due to low efficiency of single cell sequencing in non-plasmablast populations, anergic B cells were collected for bulk VH sequencing. Patient-specific anergic VH repertoires will be compared with the same subject's repertoires obtained in Aims 1 and 2.

Aim 4: Glycovariants of IgG1 AQP4-specific rAbs were produced at MGH (Dr. Anthony). Agalactosylated and sialylated glycoforms of a lower affinity AQP4 rAb exhibited reduced complement dependent cytotoxicity. The mechanism is being investigated. Additional effects on FcR binding and ADCC will be assessed. We have not observed differences in the abundance of serum IgG glycovariants in active and quiescent NMO patients. Glycovariant analysis of affinity-purified serum AQP4-specific IgG is underway.

1B. B and T cells (A. Sawalha, Michigan; P.J. Utz, Stanford; & J. James, OMRF)

Major Accomplishments:

- Design and construction of EpiTOF panel, validation in 24 healthy subjects (12 elderly and 12 young, half male and half female).
- Analysis of 19 healthy twin pairs, including complete analysis and submission of 2 manuscripts to top tier journals.
- Design and completion of a T cell EpiTOF panel, and design and initial construction of a TReg-specific T cell subpanel.
- Analysis of samples from subjects with juvenile idiopathic arthritis (Stanford pediatric rheumatology, CyTOF run and initial informatics analysis completed); SLE (OMRF, CyTOF run completed, informatics ongoing); SSc (SCOT trial and Stanford rheumatology, CyTOF run completed, informatics ongoing; SSC, Michigan study proposed); and RA (Colorado, samples delivered and CyTOF run scheduled January 2018).
- Construction and validation of 288-plex Luminex autoantigen array, new 384 well Luminex instrument and liquid handler delivered and now on line.
- Initial EPiTOF analysis of samples from Michigan suggesting that the CD4+CD28+KIR+CD11ahi demethylated T cell subset interacts with genetic risk as a marker of disease activity in lupus.
- Identified molecular pathways and predictive marker panels which are enriched in SLE patients who will have a clinical flare within the following six to twelve weeks (Munroe et. al. J. Autoimmun. 2017).
- Screened a large cohort of nearly 800 non-autoimmune disease individuals, identified 7% of individuals with significant levels of SLE or pSS associated autoantibodies and these responses were enriched in American Indian participants. Autoantibody positive individuals had higher levels of a number of inflammatory molecules and enhanced IFN stimulation; however, these individuals also had higher levels of regulatory pathways such as the IL-1 receptor antagonist (Slight-Webb, et al. Arthritis Rheum 2016).
- Tested gene expression modules and soluble mediator profiles in primary Sjogren's syndrome patient samples from individuals participating in the ACE Baminercept in pSS clinical trial. Baseline analyses identify three groups of pSS patients, including one group with minimal interferon or inflammatory responses.
- Completed autoantibody profiling using bead-based arrays in Baminercept trial.
- In collaboration between Stanford and OMRF, completed single-cell analysis of surface markers in PBMCs from 18 SLE patients (9 receiving and 9 not receiving hydroxychloroquine), along with analysis of 51 soluble mediators and 4 phospho-proteins under basal and stimulated conditions. Manuscript has been drafted.
- In other collaborations between Stanford and OMRF, mass cytometry has identified diminished STAT-3 driven cell pathways which characterize SLE patients treated with mycophenolate mofetil (MMF) compared to SLE patients with similar disease activity but not treated with this lupus medication. These experiments have informed mechanistic studies for the ongoing ACE trial of MMF withdrawal in quiescent SLE.
- Ongoing collaborative work within this component of the consortium is expanding their previous study (above) using mass cytometry, stimulated phosphoflow cytometry and associated approaches to understand mechanisms of autoimmune disease suppression in autoantibody- positive healthy individuals.

Sub-aim 1B involves current collaborations between Stanford, OMRF, Emory, and Michigan, as well as the completion of two long-standing collaborations between Stanford and OMRF with the Meffre lab (Yale, from previous ACE). The OMRF-Meffre collaboration identified new mechanisms of B cell compartment lymphoproliferations in pSS patients (Glauzy et al. Arthritis Rheum 2017). The Stanford-Meffre collaboration was completed in 2017 and a manuscript submitted in December 2017 to a top tier journal in which autoantibodies cloned from single cells derived from AIRE-deficient subjects identified novel anti-cytokine reactivity.

New lines of inquiry in this sub-aim have been enabled by technology development. Using the EpiTOF platform invented by Cheung and Kuo, the Stanford group has established a platform for characterizing SLE blood cells. This platform enables large-scale characterization of 22 cell surface markers and 40 histone post-translational modifications. We created and validated all reagents, then completed profiling of 2 cohorts of normal subjects (24 subjects, 12 M/F, and 12 old/young). This has now been submitted for publication as a pair of papers, one on new discoveries of relevance to autoimmunity, and one purely on methods development. We have completed pilot experiments with 20 patients from OMRF's SLE cohort and 20 patients from Michigan. In addition, the Wang lab at Stanford developed a real-time giant magnetoresistive (GMR) sensor capable of detecting 60 SLE autoantigens simultaneously. We are currently measuring reactivity patterns and on/off rates of >40 SLE monoclonal antibodies provided by Sanz (Emory) and Clark (Chicago), using an 80-feature next generation GMR chip. The Utz group at Stanford has also expanded capabilities for anti-cytokine autoantibody profiling. We successfully converted from a planar array platform to a bead-based array platform from July-November 2017. Over 1,000 serum samples and monoclonal antibodies have already been analyzed in 2017, using a custom-designed 288-plex array that is ideally suited to deployment in ACE clinical trials.

This subaim also includes experiments in which the Michigan Basic ACE program is collaboratively addressing the hypothesis that the size of the demethylated CD4+CD28+KIR+CD11a^{hi} 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. The strategy to address these questions includes a cross sectional and a longitudinal approach.

Samples from 105 female lupus patients for the cross-sectional arm of our study have been recruited (68 European-American, 31 African-American, 3 Asian, and 3 from other ethnicities). The size of the CD4+CD28+KIR+CD11a^{hi} T cell subset was determined by flow cytometry in each patient, and total genetic risk for lupus was calculated using 43 confirmed genetic susceptibility loci. We showed that the genetic risk for lupus was significantly higher in African-American compared to European-American lupus patients for the first time. We also showed that the demethylated CD4+CD28+KIR+CD11a^{hi} T cell subset size was a better predictor of disease activity in young (age ≤ 40) European-American patients independent of genetic risk. The Michigan Basic ACE is also collaborating with the Stanford ACE in using CyTOF to further characterize the epigenetic profile of these demethylated T cells. An EPICyTOF experiment (n=10 patients/20 samples) demonstrated significant differences in the histone modification landscape between KIR+CD11a^{hi} and KIR-CD11a^{low} cells isolated from the same lupus patients. Validation and functional consequences of these differences will be performed.

The Michigan Basic ACE has, to date, recruited 91 lupus patients for the longitudinal arm of the study. Flow cytometric assessment of the demethylated CD4+CD28+KIR+CD11a^{hi} T cell subset has been performed in all 91 patients at baseline, and at two time points in 62 of the 91 patients, three time points in 33, four time points in 15, five time points in 7, six time points in 3, seven time points in 2, eight time points in 1 patient, and nine

time points in 1 patient. The disease activity of each lupus patient, at each time point as assessed by SLEDAI score, was recorded at the time of blood draw.

The Michigan Basic ACE is also collaborating with the Clinical ACE program to determine if demethylated T cells in peripheral blood correlate with response to therapy in the Study of Subcutaneous Abatacept to Treat Diffuse Cutaneous Systemic Sclerosis (ASSET) trial. Samples have been collected and processed from 50 patients at multiple time points to date. Analysis of these data will be possible when the trial is unblinded.

To evaluate more deeply the roles of B cells, T cells, and other immune cells in SLE disease flares, a collaboration between OMRF, Michigan, and Stanford has defined cellular immune mechanisms associated with SLE flares. PBMCs from African American SLE patients with high or low disease activity were stimulated with T cell, B cell, and Toll-like receptor (TLR) ligands. Stimulated and unstimulated cells were analyzed by mass cytometry for phospho-proteins, and culture supernatants were analyzed by 37-plex assay and ELISA for cytokines. Compared to patients with low disease activity, patients with elevated disease activity had increased T cell responses (sCD40L, IL-2, IL-13) to simulation with TLR4, TLR 7/8, and PMA-ionomycin. In general, SLE patients had a decreased fold change in response to stimulation compared to healthy controls. Patients with high and low disease activity showed no significant differences in signaling by CD4+ T cells or CD8+ T cells after CD3/CD28 signaling. Granulocytes of patients with high disease activity showed significantly elevated phosphorylation of STAT5 and PLCg$_2$ following Poly I:C, LPS, or CpG stimulation, compared to granulocytes of patients with low disease activity. In contrast, high disease activity was associated with reduced dendritic cells responses to stimulation with TLR7/8, TLR9, and BCR stimulation, indicated by lower pSTAT3 (TLR7/8), pp38 (TLR9), Syk (BCR), pSTAT1 (BCR), and pSTAT5 (BCR). High disease activity was also associated with a reduced p38 phosphorylation response in B cells stimulated by TLR4.

In addition, the OMRF ACE has collaborated with Stanford to examine immune cell phenotypes in SLE patients taking (n=9) or not taking (n=9) hydroxychloroquine. Before and after anti-IgG and IGM stimulation, PBMC surface markers were analyzed by mass cytometry, and phosphoproteins (pERK, pPLCγ2, pSTAT5, phospho-p38) by phospho-specific flow cytometry. Plasma soluble mediators were assessed by 51-plex assay and ELISA. Compared to patients not taking hydroxychloroquine, PBMCs of those taking hydroxychloroquine showed a small, non-significant increase in total number of cells per mL, along with significant reductions in the numbers of B cells, CD4+ T cells, transitional B cells, and effector T cells (Th1, Th17, and Tfh). Hydroxychloroquine use was also associated with reduced cell activation, reflected by lower expression of HLA-DR and CD38 on B cells, IL2 receptor and CCR6 on T cells, and CD86 expression on dendritic cells. Patients using hydroxychloroquine also had reduced levels of plasma soluble mediators produced primarily by monocytes, platelets, and endothelial cells, including PDGF-BB, MCP-1, PAI-1, sEselectin, resistin, and IL7. No significant differences were observed in TCR or BCR signaling after stimulation, but BCR-stimulated CD4+ effector T cells showed lower levels of PLCγ in SLE patients taking hydroxychloroquine.

As outlined above, the OMRF SLE cohort will be the source of samples for Stanford to develop, validate, and then interrogate epigenetic landscapes in TReg cells using a TReg-specific EpiTOF panel being developed in the coming year. A collaboration between OMRF, Stanford, Baylor, and Michigan will use this resource to assess disease flare signature modules, predictive algorithms, and mechanisms.

In parallel studies to the pediatric SLE studies of the Pascual ACE, the OMRF ACE has identified 200 adult SLE patients, including samples for suppressed and elevated disease activity. Gene expression modules, autoantibody profiles, soluble mediator responses and clinical information have been gathered. Through

bioinformatics analyses, seven clusters of SLE patients with distinct molecular profiles were identified. These clusters differed in IFN, T cell and inflammation module scores. Other clusters showed neither elevation of IFN nor inflammation modules, but were distinguished by monocyte, neutrophil, plasma cell and T cell module scores. All three IFN expression modules strongly correlated with circulating IFN-related mediators, including IFNα, IFNγ, IP10, MCP1, MIG, MIP1α, and MIP1β. The clusters with high IFN and inflammation scores had elevated IL-10, as did Cluster 6, which had the highest plasma cell module score and had only moderate IFN and inflammation module scores. Clinically, Clusters 1 and 4 were similar, displaying the highest SLEDAI scores, as well as increased rates of low complement, DNA binding antibodies, proteinuria and hematuria. Cluster 2 showed slight elevations in IFN and inflammation modules, but had low SLEDAI scores and reduced prevalence of low complement and DNA binding antibodies. Clusters 3, 5, 6, and 7 had higher rates of rash, alopecia and arthritis. Molecular profiles encompassing interferon, T cell, neutrophil, plasma cell, and inflammation signatures distinguish groups of SLE patients and reveal multiple potential pathways of clinical disease. These profiles can potentially contribute to clinical trial design and individualized treatment. Ongoing and planned collaborative experiments within this subcomponent focus on further evaluation of anti-cytokine responses, genetic evaluation within each cluster and collaborative analyses of pediatric and adult SLE patients across ACE programs.

1C. Adaptive epigenetics (J. Boss, Emory)
Major Accomplishments:

1 – Developed protocols for epigenetic analyses on low numbers of cells and biobanked samples.

2 – Determined lineage relationships between HC and SLE subject B cell subsets.

3 – Showed that the activated naive (aN) and double negative 2 (DN2) subsets display similar programming in SLE patients compared to HC.

4 – Identified AP-1 family member signatures associated with SLE and that naïve B cells in SLE patients are already epigenetically programmed towards a disease bias.

The goal of this program is to elucidate the epigenetic programming that controls cell fate decisions and phenotypes associated with autoimmune disease. To achieve this goal, we developed a series of assays that require low cell numbers that could be obtained following the sorting of peripheral cells. Three assays were developed that include reduced representation bisulfite sequencing (RRBS) to determine the landscape of DNA methylation events; ATAC-seq to determine chromatin accessibility and serve as a readout of epigenetic marks and regulatory element potential; and RNA-seq to assess the transcriptome of cells and link the potential role that epigenetic modifications may have on gene expression. In a project with Dr. Inaki Sanz, we have applied these assays to 5 B cell subsets from 9 SLE patients and 8 healthy controls. A limited number of plasma cell subsets were also processed as these cell types provide the terminal differentiation effector stage of the B cell program and function. Sequence library construction and deep sequencing is now complete on these samples and two phases of detailed informatic analyses is nearly completed.

In the analysis where like data sets are compared (i.e., RRBS vs RRBS) several new findings arise. 1) From a cell subset perspective, the data suggest a progression of cell differentiation that begins with the resting naive B cells that is closely followed by activated naive (aN) and transitional 3 (T3) B cells. By their DNA methylomes, the B cell subset that is most enriched in SLE patients and believed to be pathogenic – termed DN2 – are closer to a switched memory (SM) phenotype and plasma cells then they are to the above cell types, placing them far along the differentiation pathway. This is the first clear molecular description of a

potential order to the differentiation process and provides a molecular relationship between these cells. 2) From a disease perspective, DNA methylation fully separates cells isolated from HC and SLE subjects when normalizing for cell type differences. 3) Chromatin accessibility comparisons of HC by principle component analyses showed substantial overlap between all cell types except the DN2, suggesting that these are distinct even in healthy individuals. In contrast, the transcriptome data sets showed that each cell type had a distinct set of specific genes, with the exception of the T3 cells, which overlapped with the aN subset, reinforcing the relationship between these cells and intermediaries in the differentiation process. It should be noted that like DN2, aN cells are greatly increased in SLE patients. 4) Chromatin accessibility at distal regulatory elements of SLE patient cells showed that DN2 and act B cells share similar chromatin configurations. This observation was also supported by an analysis of the transcriptomes within these cells. 5) Among the intriguing genes dysregulated in SLE aN and DN2 B cells are the immunoinhibitory cell surface receptor PD-1 and the integrin CD11c, which is typically a marker of dendritic cells. 6) The AP-1/ATF transcription factor family binding motifs are greatly enriched in SLE open and accessible chromatin by a number of bioinformatics programs. The factors ATF3, FosL1, and a few others are display increased expression in SLE cells, suggesting that they may play a role in modulating disease. Together, these data support the concept that there is a clear epigenetic footprint associated with SLE that affects all B cell subsets, including the naive subset, with the DN2 and actB being most pronouncedly distinct.

Collaboration with the Bluestone group was initiated to develop an epigenetic/molecular diagnostic tool to determine the quality of *ex vivo* expanded Tregs and predict through the detection of molecular biomarkers their ultimate efficacy for use in T1D patients. We have completed the first round of comparisons between ex vivo expanded Tregs from HC and patient samples.

In collaboration with the Sanz group, manuscripts are in revision, and a manuscript describing the results of our studies is in preparation.

1D. Microbiome (R. Xavier, MGH)
Whole genome shotgun sequencing of the fecal microbial DNA/RNA to detail the metagenome and metatranscriptome has been undertaken in subjects with IgG4-RD, Systemic sclerosis and with CVID (common variable immunodeficiency), the latter as a control cohort. Community gene functional characteristics (metagenomes) and expressed functional profiles (metatranscriptomes) have been compared with disease status and genotype. Over a hundred subjects were included in each group.

Interestingly, microbial communities in IgG4-RD and SSc are very similar and the overall patterns look like the reciprocal mirror image of the microbial community seen in CVID.

Other MGH Collaborative studies

We are working closely with the Michigan ACE to analyze the pathogenesis of SSc. We have developed staining protocols for a number of B, T and innate immune cell subsets, that we apply on sections from formalin fixed paraffin embedded tissues. These data are being extended to subjects with IgG4RD, Systemic sclerosis (Michigan ACE) and lupus nephritis (Chicago ACE). Distance-mapping studies will be performed by the Chicago ACE Studies are being undertaken of activated B cell populations in IgG4-RD following the lead of the Emory ACE and we will collaborate actively with Emory to delineate differences in these population between IgG4-RD and lupus subjects.

We are working closely with the Stanford ACE for some of our studies on antigens that bind to auto-antibodies in IgG4-RD

Aim 2: Examination of *in situ* adaptive immunity in autoimmune diseases

The original proposal for Aim 2 was a joint effort between the University of Chicago, the University of Michigan and Mt. Sinai in New York. Since the initial proposal, Massachusetts General Hospital has joined the effort and is now integral to the effort.

Major Accomplishments:

1. GC Tfh1 cells in tonsils exclusively help memory B cells, suggesting that pathogenic Tfh1 cells in lupus mainly act on memory B cells.

2. Developing a strategy to detect rare AQP4-specific CD4+ T cells in NMO blood samples

3. Demonstrated that *in vitro* immunoglobulin polyreactivity can reflect mechanisms that increase both affinity and specificity *in vivo*.

4. Serum anti-vimentin antibody titers predict treatment resistance in human lupus nephritis.

5. DR3^{+} sarcoid lung patients are characterized by a coordinated (T and B cell) *in situ* adaptive immune response to vimentin.

6. Identified two molecules secreted by synovial fibroblasts, CD13 and Id1, as mediators of joint inflammation in rheumatoid arthritis.

7. Molecular identification of two G-protein-coupled receptors as CD13 ligands.

8. Discovery of a novel ligand for CD6, CD318 which is expressed by synovial fibrocytes in rheumatoid arthritis.

9. Developed novel computational tools to analyze human tissue and infer functional relationships between different populations of T cells and antigen presenting cells.

10. Discovery of link between CD4+CTLs and both IgG4-related disease (IgG4-RD) and systemic sclerosis (SSc) may represent a significant advance in our understanding of the pathogenesis of fibrosis in autoimmunity.

11. Identification of IL-1b as a new potential therapeutic target in both IgG4-RD and SSc.

Narrative Summary:

Aim 2A1: *In situ* TFH cell function in inflammation (H. Ueno, Mt. Sinai)
1. *Peripheral Tfh cells in lupus*. We have found that the frequency of CD57+ circulating T follicular helper 1 (cTfh1) cells showed a strong positive correlation with SLEDAI score and a negative correlation with serum C3 complement level in pediatric lupus cohort. This observation suggests the involvement of Tfh1 cells (which express T-bet, and produce IL-21 and IFN-gamma in lupus pathogenesis. This is somewhat surprising, given the fact that cTfh1 cells are the least efficient helpers among human cTfh subsets (Ueno. Curr Opin Immunol. 2016). How Tfh1 cells contribute to lupus pathogenesis is completely unknown.

Therefore, we sought to characterize the function of Tfh1 cells in germinal centers of human tonsils. We identified a GC Tfh population that co-express T-bet and Bcl-6, and co-produce IFN-gamma and IL-21. Similar to ICOS+ activated cTfh1 cells (Bentebibel et al, Sci Transl Med, 2013), these GC Tfh1 cells were capable of inducing memory B cells to differentiate into CD138+ plasma cells. However, GC Tfh1 cells were incapable of helping GC B cells, partly due to the expression of Fas ligand (Bentebibel et al, under revision). These

observations suggest that the target of Tfh1 cells in lupus is likely memory B cells. In collaboration with Chicago ACE and Emory ACE, we will investigate which memory B cell subset (including CD27-IgD- double-negative cells) are efficiently helped by cTfh1 cells, and whether this B cell subsets are in proximity of Tfh1 cells in inflammatory tissue in lupus patients.

2. AQP4-specific T cells in NMO (Associated to Aim 1B)

Together with Colorado ACE, we are analyzing AQP4-specific CD4+ T cells in NMO patients. As in other autoimmune diseases, detection of AQP4-specific CD4+ T cells is extremely difficult in NMO patients, due to low frequency in blood. My lab is aiming at establishing a novel platform to detect and isolate these rare autoreactive T cells. We have found that pre-removal of CD25+ Tregs by magnetic beads from PBMC yielded better detection of AQP4-specific CD4+ T cells in a CD154 assay (unpublished). We are currently developing a panel for CyTOF to fully characterize AQP4-specific CD4+ T cells in NMO patients.

Aim 2A3: Antibody repertoire selection in inflammation (M. Clark, Chicago and S. Pillai, MGH)
1. *Selection and function of anti-vimentin antibodies (AVAs) in inflammation*

a. Origin and selection of AVAs *in situ*. Originally, we used laser capture microscopy to clone expanded expressed immunoglobulin repertoires from the tubulointerstitium of patients with lupus nephritis (Arth Rheum, 2014). Eleven of these 25 initially characterized antibodies were reactive with the cytoplasmic intermediate filament vimentin. In contrast, none of the antibodies cloned from the tubulointerstitum were reactive with dsDNA or ribonucleoproteins. We have now reverted the anti-vimentin antibodies and have extensively compared their binding characteristics. These studies have revealed that *in vivo*, there is a selection for both polyreactivity (as measured by *in vitro* assays) and affinity to vimentin. However, binding studies to vimentin in tissue indicate that *in vitro* polyreactivity is associated with both increased binding and specificity *in vivo*. These data suggest that current interpretations of *in vitro* assays of polyreactivity or autoreactivity are incorrect. *In vitro* polyreactivity does not necessarily confer risk for autoimmunity. Rather, it reflects a mechanism for increasing both affinity and specificity *in vivo* (manuscript in preparation).

b. Prognostic significance of AVAs in lupus. In collaboration with Genentech, we have done an extensive analysis of the lupus nephritis LUNAR cohort, characterizing the relationships between serum vimentin titers and prognosis. These studies demonstrated that high anti-vimentin antibody titers were an independent indicator of poor response to therapy, regardless of treatment group (manuscript in preparation).

c. Selection of AVAs in the lung of sarcoidosis patients. *In situ* autoimmunity to vimentin is not a specific feature of lupus. In DR3+ sarcoidosis patients, lung resident antigen presenting cells display vimentin peptides what are recognized by T cells expressing the Vα2.3β22 TCR. These T cell populations are commonly expanded in the lungs of DR3+ sarcoidosis patients. In collaboration with Dr. Johan Grunewald at the Karolinskia Institutet, we have now characterized *in sit*u humoral immunity to vimentin in sarcoidosis patients. These studies demonstrated that DR3+ patients had high titers of AVAs in broncheoalveolar lavage fluid (BALF) and that titers correlated with the frequency of CD4+ T cells expressing the Vα2.3β22 TCR. The strongest correlations were with BALF C-terminal AVAs. These and other data demonstrate a coordinated adaptive immune response to vimentin in the lungs of sarcoidosis patients. Interestingly, high BALF AVAs were not reflected in the serum. This suggests that the immune response in the lung is remarkably self-contained and is not in equilibrium with that in the periphery (manuscript submitted).

d. Single cell RNA-seq of *in situ* B cell populations in inflammation. This project has been a full collaboration between U Chicago with MGH who has performed the single cell RNA-seq. Using protocols similar to those

used by the Accelerating Medical Partnership (AMP) consortium, we have developed and validated protocols to isolate single B cells from renal biopsies and subject them to single cell RNA-seq. Preliminary studies have been done on renal allograft rejection as these biopsies are readily available. Initial studies of B cells from five renal biopsies and two human tonsils have demonstrated that the transcriptome of infiltrating B cells is both broad and very different than that observed in tonsil B cell populations. Most notably, renal B cells express many more inflammatory cytokines as well as molecules involved in TLR signaling. Analysis of immunoglobulin repertoire has revealed some intra-renal clonality but less than that observed in tonsil. We are currently cloning representative antibody variable regions for expression and characterization of antigenic specificity.

Aim 2B: Non-conventional *in situ* antigen presenting cells (D. Fox, Michigan and M. Clark, Chicago)
1. *Fibrocytes and tissue fibroblasts as important antigen presenting cells in organ specific autoimmunity (D. Fox, Michigan).* Accomplishments to date include: 1- Establishment of AIRE as a molecule that controls the expression of the TSH-receptor by fibrocytes in thyroid eye disease (TED) and possibly in RA. Activation of circulating fibrocytes by autoantibody to the TSH-R leads to the eye inflammation that accompanies Graves' hyperthyroidism, while engagement of the TSH-R by TSH induces IL-6 production by RA synovial fibroblasts; 2- Development of high resolution multi-color confocal immunofluorescent methods to study interactions between lymphocytes and accessory myeloid and fibroblastic cells in RA synovium; 3- Definition of 2 new secreted and shed molecules from synovial fibroblasts – CD13 and Id1 – as inducers of joint inflammation; 4- Identification of citrullinated Id1 as a novel autoantigen in RA; 5- Molecular identification of 2 G-protein-coupled receptors as receptors for CD13; 6- Discovery of a novel CD6 ligand, CD318, that is expressed by synovial fibroblasts both as a cell surface glycoprotein and as a shed mediator with chemotactic properties.

2. *Cell distance mapping, version 3 (CDM^3)(M. Clark and M. Giger, Chicago).* Previously, we demonstrated that quantitative analysis of human frozen tissue samples imaged by multicolor confocal microscopy could be used to characterize interactions between T follicular helper (TFH) cell populations and B cells (Sci Trans Med, 2014). In these investigations, we demonstrated that when TFH cells formed a cognate, or antigen specific, interaction with B cells, the nuclei of each cell became tightly apposed. This was associated with extensive entanglement of cytoplasmic processes with apposition of T cell receptor (TCR) and MHC class II respectively. These data indicated that distances between nuclear borders could discriminate between cognate interactions and when T and B cells were just in close proximity. Therefore, by mapping relative distances between T and B cells in tissue (cell distance mapping or CDM) we could infer functional relationships.

However, CDM had several limitations. Most notably, it used fixed filters and algorithms to segment nuclear and other stains within tissue. These tools were insufficient for defining boundaries and position of larger complex objects such as stains associated with dendritic cells. Therefore, CDM could not be applied to all potential antigen presenting cells (APCs). Furthermore, CDM did not accurately capture the shape of objects. We postulated that this might be important as T cells adopt different shapes when scanning for antigen and after recognizing peptides in the context of MHC. In the latter case, T cells flatten against the APC to increase surface contact area.

To circumvent the limitations of CDM, we developed convolutional neural networks (CNNs) to both better segment large objects and to capture object shape. We then developed additional CNNs that assessed both distances between cells, and changes in T cell shape as a function of distance, to develop predictive models capable of discriminating cognate from non-cognate T cell:APC interactions. These set of analyses we refer to as CDM^3. The CDM^3 CNNs were then trained on a mouse data set (provided by R. Germain, NIH) in which cognate and non-cognate T cell interactions with dendritic cells (DCs) had been visualized using two-photon microscopy. Surprising, in a direct comparison, CDM^3 was better at discriminating cognate from non-cognate T

cell:DC interactions than two-photon microscopy. The better discrimination afforded by CDM^3 was primarily due to higher throughput and because CDM^3 provided richer data sets than two-photon microscopy. Most recently, we have applied CDM^3 to human lupus nephritis. These studies indicate that plasmacytoid DCs, and not myeloid DCs, are major APCs in severe tubulointerstitial nephritis. Therefore, we have developed computational tools that provide, for the first time, a quantitative understanding of adaptive cell networks in human inflammation. These results are being prepared for publication.

Aim 2C: Mechanisms of fibrosis in autoimmune diseases including IgG4-RD and SSc (S Pillai, MGH and Dinesh Khannah, U Michigan).

This is an exciting and potentially important new subaim in the portion of the collaborative agenda focused on *in situ* adaptive immunity. The initial studies are focused on classic fibrotic diseases. However, the elucidated mechanisms might be of relevance to other diseases which manifest fibrosis including lupus nephritis and rheumatoid arthritis. We have proposed to pursue this possibility in an amendment to the collaborative agenda.

a. Based on our extensive studies on IgG4-RD and SSc, we can conclude that these major human fibrotic diseases are linked to the tissue accumulation of cytotoxic CD4+ T cells or CD4+CTLs. These T cells are the dominant T cell population in the tissues of IgG4-RD subjects with active disease, as well as in diffuse systemic sclerosis. SLAMF7/CD319 has been identified as a useful marker for CD4+CTLs.

b. Both IgG4-RD and scleroderma are linked to CD4+CTLs that express IL-1b. In SSc IFN-g expression by CD4+CTLs is rare. In collaboration with the Stanford ACE auto-antibodies against the IL-1RA (antagonist) have been discovered in IgG4-RD, and these antibodies enhance IL-1 signaling.