

Autoimmunity Centers of Excellence Annual Report (2016)

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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

The basic science component of the Autoimmunity Centers of Excellence (ACE) covers broad areas of investigation dealing with different components of the 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 (SLE); 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 used a longitudinal cohort of pediatric SLE patients to investigate descriptors and correlates of disease activity. Using whole blood transcriptomics and sophisticated computational tools, they have unraveled a high degree of disease heterogeneity that allows disease segmentation into 7 groups with separate molecular correlates of disease activity. This study also provides insight into the heterogeneity of lupus nephritis subtypes and therapeutic responses and raises the tantalizing possibility of personalized immunotherapy.

From the point of view of effector mechanisms, this ACE has described the ability of TLR7-mediated neutrophil-released oxidized mitochondrial DNA (Ox mtDNA), to induce massive IFN production by pDC. The investigators have also identified the ability of Ox mtDNA-activated pDCs to skew naïve CD4 T cells towards a unique Type I regulatory T cell (Tr1)-like phenotype characterized by IFN^{high}, IL-10^{high}, IL-2^{low} cytokine profile but lacking regulatory properties.

Future goals for the Baylor ACE include the validation of the genomic algorithm to predict therapeutic responses and to further characterize the significance of extra-follicular, IL10-producing CD4+ T cells in SLE-end target organs.

Chicago (M. Clark, PI)

This center is exploring the mechanistic basis for altered peripheral B cell selection in SLE. Specifically, studies are underway to understand whether healthy relatives of SLE patients, who may have a higher frequency of serum ANA autoantibodies, have: 1) a higher frequency of autoreactive naïve B cells; and 2) whether they respond to influenza immunization with a higher frequency of autoreactive plasmablasts. These studies are based on the initial finding that SLE patients produce qualitatively different autoantibodies within the influenza response. Over the last year, the study has recruited 8 SLE patients and 1-2 close relatives for a total of 41 subjects who were immunized with influenza vaccines and analyzed 7 post-vaccination for the sorting of plasmablasts. To date 73 plasmablast-derived mAbs have been generated and hundreds of additional mAbs are in various stages of production.

Future goals include detailed analysis of the fine specificity, cross-reactivity and disease-related autoreactivity of mAbs produced by SLE and their close relatives in response to influenza vaccination.

Emory (I. Sanz, PI)

The Emory ACE U19 is addressing the pathways of B cell activation that are engaged in SLE both to initiate disease as well as during disease flares. 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.

Major achievements include:

1. Identification of new population of activated naïve cells and their contribution to the emergence of disease-specific autoantibodies in SLE.
2. Cellular origin and properties of antibody secreting cells (ASC) in flaring SLE: published studies document a major contribution from newly differentiated naïve cells and early germinal center reactions. Moreover, monoclonal antibodies expressed from single cells and proteomic sequences demonstrate that pathogenic SLE autoreactivity can be generated in the absence of somatic hypermutation thereby illustrating the pathogenic potential of extra-follicular reactions.
3. Epigenetic programming 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.

Future goals include

1. To dissect the cellular and molecular pathways responsible for the dysregulated activation, differentiation and selection of autoreactive B cells in SLE.
2. To use serum autoantibody proteomics to dissect the diversity and stability of SLE autoantibodies as well as the relative contribution of short-lived and long-lived plasma cells.
3. 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
4. To expand collaborations with the Colorado ACE (Dr. Bennet) to understand abnormal B cell regulation in Neuromyelitis Optica (NMO).

Massachusetts General Hospital (S. Pillai, PI)

The MGH ACE U19 investigates the cellular and molecular basis of IgG4-RD and the role of antibody glycosylation and the microbiome on this disorder.

Major achievement to date include:

1. The demonstration that IgG4-RD is linked to tissue infiltration by clonally-expanded antigen - reactivated CD4⁺ CTLs. . The dominant T cells infiltrating a range of inflamed IgG4-RD tissue sites are clonally-expanded CD4⁺CTLs that express SLAMF7, granzyme A, IL-1 β , TGF- β 1 and IFN- γ . Interestingly, clinical remission induced by B cell depletion is associated with a reduction in disease-associated CD4⁺ CTLs. These studies are the first to link CD4⁺ CTLs to any human chronic inflammatory disease.
2. Disease Related Subsets of human CD4⁺ CTL: The MGH center has used both single cell RNA -seq and ATAC seq to describe the differentiation pathway of human disease-related CD4⁺ CTLs. This approach has enabled the identification of a subset that is linked to disease.
3. Identification of a tissue T_{FH} subset linked to disease and IgG4 class switch. The differentiation pathway of these T_{FH} cells has been analyzed using both single cell RNA -seq and ATAC seq.

Future goals include the identification of antigens that drive CD4⁺CTL and T_{FH} cell generation in IgG4-RD.

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.

Major achievements include the analysis of the DNA methylome of the cells of interest. Thus, genome-wide DNA methylation analysis has identified 31,019 differentially methylated sites in *in vitro* generated KIR+CD11a^{hi} T cells, with 99.1% being hypomethylated. RNA sequencing analysis identified 1,620 overexpressed genes including key pro-inflammatory cytokine genes, adhesion molecules, Fc-gamma receptor genes, Toll-like receptor genes, HLA molecules, and metalloproteinases. Of particular interest, and similar to what is known in lupus T cells, the experimentally derived demethylated KIR+CD11a^{hi} T cell subset demonstrates reduced IL-2 mRNA expression (3.3-fold). Bioinformatics analysis of 718 genes that are hypomethylated and overexpressed in KIR+CD11a^{hi} T cells revealed significant enrichment in pro-inflammatory gene ontologies, pathways, and gene meta-groups. Studies to characterize this same T cell subset in lupus patients *ex vivo* are ongoing.

This center is also testing the ability of antibodies to KIR proteins inhibit B cell stimulation and macrophage killing by the CD4⁺CD28⁺KIR⁺ 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, the studies have found that antibodies to inhibitory KIR block autologous macrophage killing whereas antibodies to stimulatory KIR trigger IFN γ release.

Stanford (P J Utz, PI)

Through their Principal Project (Robinson, PI), The Stanford ACE U19 studies the B cell response against citrullinated antigens in Rheumatoid Arthritis (RA).

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.

In addition, this center has performed extensive single cell sequencing of circulating plasmablast antibody repertoire in serial blood samples derived from patients with RA. These studies demonstrate the dynamic nature of this disease-associated autoantibody response as demonstrated by the presence of pruning presumably indicating the impact of antigenic selection of clonal members. At the same time, these studies also document the impact of affinity maturation-mediated epitope spreading within the ACPA response.

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 ASCO1 (rituximab in systemic sclerosis) through real-time B cell phenotyping, and cryopreservation of PBMC, serum, and plasma; flow cytometry phenotyping of Tregs and Th1/Th2/Th17 cells for Mark Nicolls' sub study.

Future goals and developmental efforts include 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 the central lab for CyTOF analysis. Additional optimization of single-cell TCRseq assays is also underway.

Finally, the Stanford ACE Pilot Project (Chang, PI) which ended in 2016 contributed the demonstration of single cell ATAC-Seq in blood and in skin derived from patients with SSc. This accomplishment represents the first description of this method in SSc tissue.

Oklahoma Medical Research Foundation (J. James, PI)

The Oklahoma ACE U19 has contributed new insights into disease pathogenesis in Sjögren's syndrome, systemic lupus erythematosus (SLE), and thrombocytopenic purpura.

Major accomplishments include the first genome-wide association study and large-scale replication study in primary Sjögren's syndrome (pSS). Three thousand Sjögren's syndrome patients and over 12,000 controls were recruited locally and through collaborations, including some with other ACE Centers. The study replicated associations in the HLA region and established *IRF5-TNPO3*, *STAT4*, *IL12A*, *FAM167A-BLK*, *DDX-6-CXCR5*, and *TNIP1* as risk loci for pSS. Suggestive associations were found in 29 other regions.

The OMRF ACE U19 also performed an innovative analysis of the preclinical events that lead to SLE. Studies of samples spanning preclinical periods established temporal relationships between dysregulation of various immune pathways, ANA positivity, and SLE classification (otherwise considered the first clinical flare).

Similarly, the center studied early predictors of SLE flares and devised a soluble mediator score (SMS) that summarizes the status of the immune system. By integrating plasma levels of 52 cytokines, chemokines and shed receptors, the SMS accurately identifies impending SLE disease flare in 90-99% of patients.

Finally, the Oklahoma ACE has performed a proof-of-concept study for a novel, alternative clinical trial design. In the Biomarkers of Lupus Disease (BOLD) study, 41 SLE patients with active but non-organ threatening disease withdrew their background immunosuppressants and received steroid injections to transiently suppress disease. Forty patients (98%) exhibited flare within six months, including 21 (51%) with early flare (≤ 60 days). Early flare patients had more frequent aCD11b^{hi} monocytes and CD86^{hi} naïve B cells compared to late flare patients (90-165 days, n=13). Early flare patients had elevated IFN-related chemokine plasma levels

and reduced IL-1RA. This novel study design and associated mechanistic findings are being used in ongoing and planned therapeutic SLE clinical trials.

Clinical Trials Component

The clinical trials 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, since tissue injury in autoimmune disease is the end result of multiple and often redundant inflammatory pathways and mediators, an anti-inflammatory approach that modulates multiple inflammatory mediators will have broad applications and may be safer and better tolerated than current therapeutic options. The primary project envisioned a phase II trial in patients with rheumatoid arthritis (RA) 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. This project has been slowed by unanticipated and unresolved problems obtaining the agent. Consequently, the alternate project is being implemented and is proceeding rapidly through protocol development. This project will evaluate the efficacy, safety, and tolerability of ajulemic acid/JBT-101 in patients with systemic lupus erythematosus (SLE) in a phase 2 double-blind, randomized, placebo-controlled multicenter study. Ajulemic acid is a synthetic, non-psychotropic cannabinoid that possesses multiple anti-inflammatory properties. The full proposal has been finalized and an IND has been submitted to the FDA. Additionally, sites for this study have been selected and formal site feasibility assessments are ongoing. Processes to facilitate the procurement of Schedule 1 licenses (a requirement for participating sites) are also underway.

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 SPMS. Thirty-three SPMS patients in the EXPAND trial were recruited for the ACE study. PBMCs were isolated from the patients before and after 6 months and 12 months of treatment, and immunological profile changes were investigated by flow cytometry. Analyses of blinded specimens have already been performed. Interpretation of the data will be available after unblinding of the trial. ClinicalTrials.gov Identifier: NCT02330965

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; the mechanistic studies are supported by the ACE. Thus far, this project has recruited 69 of the target 86 patients, and 52 have completed month 6 of treatment. PBMC and skin biopsies are obtained at 1, 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. Data analysis awaits study completion and unblinding. ClinicalTrials.gov Identifier: NCT02161406

University of California San Francisco (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. Two complementary projects are being conducted. One project is examining Treg therapy in an antigen-non-specific autoimmune disease (SLE)(ClinicalTrials.gov Identifier: NCT02428309), and one is examining Treg therapy in an antigen-specific autoimmune disease (pemphigus). The selection of these two diseases provides an opportunity: (i) to examine whether this therapeutic approach is generally applicable across clinically distinct autoimmune diseases; (ii) to compare safety, efficacy, and mechanism of action across both antigen-specific and antigen-non-specific autoimmune diseases; and (iii) to examine the same target tissue (skin) in both diseases.

Enrollment in the SLE trial has been challenging, but early analyses have provided a tantalizing finding. Twelve weeks after the Treg infusion in the first subject, 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. We are seeking to confirm this result through continued enrollment of subjects with cutaneous lupus. At the same time, the companion pemphigus protocol is being initiated as a multi-site study to determine whether similar mechanisms apply across these two autoimmune diseases.

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 project, entitled “Strategy for the Prevention of Rheumatoid Arthritis (StopRA)”, is led by Dr. Kevin Deane. This project is testing hydroxychloroquine in subjects at high-risk for developing RA. Hydroxychloroquine has multiple immunomodulatory effects that may contribute to its proven ability to reduce the incidence of diabetes in people with RA. Thus far, the following steps have been accomplished: 1) Fifteen of the proposed 18 sites have been activated, with the remaining three sites approaching activation; 2) Since the protocol has been active (4/20/2016), approximately 1300 pre-screenings have been accomplished, and screening efforts are expanding; and 3) 11 subjects have been randomized, one of whom has developed RA. Recruiting efforts are currently expanding, with a lead article in Rheumatology News (Oct 2016) and new recruiting approaches being identified and utilized locally. The latter include blood donor prescreening (OMRF), increased health-fair activities (multiple sites), and expanding clinic referral networks (multiple sites). ClinicalTrials.gov Identifier: NCT02603146

The second project is entitled “Efficacy of Methyldopa to Preserve Beta-cell Function in New Onset Type 1 Diabetes” (T1D01)”. The protocol has been drafted and is being finalized by the study team. The objective of this multicenter, two-arm, double-blind, placebo-controlled, 2:1 randomly assigned, phase II clinical trial is to determine whether methyldopa will slow the autoimmune destruction of insulin-producing islet β -cells and

lead to the preservation of C-peptide secretion in individuals with new-onset T1D between ages 8-45 and having a HLA-DQ8 gene. Participants will receive either a 12-month course of methyldopa or placebo. Safety, glucose control, β -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 (eg 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)

Major Accomplishments:

- Recruitment of 8 NMO patients
- Sorting of IL10+ B cells NMO patients with entry into high-throughput sequencing
- Sorting of AQP4-specific CD27+ and DN memory B cells from active NMO patient with novel AQP4eGFP lentivirus expression system with entry into high throughput sequencing
- Engineering of NMO patient-derived AQP4 specific mAbs that express specific glycovariants to assess the influence of glycosylation status on Ig effector functions

We have made steady progress in the translational analysis of B cell and antibody responses in active and quiescent NMO. At mid-year, 8 NMO patients (of 10 planned) have been recruited, including 4 with relapsing and 4 with inactive disease. B and T lymphocytes have been cryopreserved for analysis in experiments 1-3 of this sub-aim, and serum and plasma samples have been stored for experiment 4. Additional aliquots of B and T lymphocytes have been reserved for novel collaborative projects on Transcriptomic and Epigenomic Analysis of Double Negative B Cells (Colorado [Bennett] and Emory [Sanz, Boss]) and on T Follicular Helper Cells (Colorado [Bennet] and Mt. Sinai [Ueno]). To minimize any batch effect, analysis of double negative B cells will proceed following collection of the planned 10 samples. Aliquots are also available for ACE collaborative studies.

We have also made progress in the individual experiments. In experiment 1, we used a new lentiviral M23AQP4-eGFP transduced cell line to initiate AQP4-specific capture of circulating plasmablasts and memory B cells. Plates are beginning high throughput single cell sequencing in the Stanford pipeline (Robinson Principal Project).

In experiment 2, IL-10-producing B10 and B10Pro regulatory cells from NMO patients with active and inactive disease were stimulated. The percentage of circulating B10 and B10Pro cells was compared between active and inactive patients, and individual IL10-secreting B10 cells were collected for entry into single-cell deep sequencing pipeline (Robinson).

In experiment 3, the frequency of CD19⁺IgD⁺CD21^{lo}CD27⁺IgM^{low} anergic B cells will be analyzed by FACS and individual cells collected for single cell analysis at Stanford in the next month.

In experiment 4, AQP4-specific recombinant antibody constructs were delivered to MGH (Dr. Anthony) for production of glycosylation variants of the antibodies. Control experiments have successfully produced specific G0F, G2F, S1F, and S2F glycoforms of IgG1 and IgG4 isotypes. The glycovariant analyses of AQP4-IgG and whole serum IgG from NMO patients is underway.

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

Major Accomplishments:

- Design and construction of EpiTOF panel, validation in healthy subjects
- Initial analysis in SLE identifies a histone deacetylase previously discovered using Meta-Analysis
- Initial analysis suggesting that the CD4+CD28+KIR+CD11a^{hi} 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

This sub-aim involves current collaborations between Stanford, OMRF, Emory, and Michigan, as well as the completion of a long-standing collaboration between Stanford and the Meffre lab (Yale, from previous ACE). New lines of inquiry in this sub-aim have been enabled by technology development. Using the EPICyTOF 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). We have completed pilot experiments with 20 patients from OMRF's SLE cohort and two 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 are converting from a planar array platform to a bead-based array platform from July-November. Using this platform, initial analyses of pooled monoclonal antibodies derived from patients with systemic sclerosis (30 clones) or AIRE deficiency (~200 clones; Yale [Meffre]) show "hits" for IL-17, interferon- α , eotaxin, and MIP3. Samples from the SCOT trial and OMRF (described below) are now being analyzed.

This sub-aim also addresses the hypotheses that the size of the demethylated CD4+CD28+KIR+CD11a^{hi} T cell subset interacts with total genetic risk to determine disease activity in SLE, and that this subset can serve as a biomarker for disease progression and remission in lupus patients. These questions are being addressed with both a cross sectional and a longitudinal approach. To date we have recruited and processed samples from 105 SLE patients for the cross sectional study and 23 patients for the longitudinal study. For the longitudinal study, we have already collected two time-point samples from 16 patients, and three time-point samples from 7 patients.

An interim analysis was performed with 49 European-American patients from the cross sectional study who were genotyped across 43 confirmed lupus susceptibility loci. The CD4+CD28+KIR+CD11a^{hi} T cell subset size correlated with disease activity in lupus patients as measured by SLEDAI score ($r=0.36$, $P=0.012$). Linear regression models suggested that the subset size is a better predictor of disease activity when normalized for total genetic risk in each individual ($r=0.42$, $P=0.003$), further suggesting that the relationship between KIR+CD11a^{hi} T cell subset size and disease activity in lupus is influenced by genetic risk. We have recently demonstrated that the CD4+CD28+KIR+CD11a^{hi} T cells are polyclonal, demethylated, and characterized by a pro-inflammatory transcriptional profile.

The Stanford ACE and the Michigan Basic ACE are using CyTOF to further characterize the epigenetic profile of these cells. An initial EPICyTOF experiment (n=2 patients/4 samples) demonstrated significant differences in the histone modification landscape between KIR+CD11a^{hi} and KIR-CD11a^{low} cells isolated from the same lupus patients. The penetrance of epigenetic modifications associated with immune cell populations, serum cytokine/chemokine levels, and immune stimulations is being explored. In addition, we identified two elevated chromatin marks in KIR+/CD4+ SLE T cells, and gene expression changes linked to these marks were consistent with trends in the RNA-seq data. Using these initial data, we estimate that 10 samples will provide sufficient power to detect differences between the two T cell subsets; sample collection is ongoing and experiments completed in Year 3 of funding.

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 46 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 flares in European-American and African-American patients. PBMCs from 34 patients with variable disease activity were analyzed by CyTOF for 33 cell surface lineage markers and activation/regulatory markers, and plasma was tested for 52 soluble mediators by bead-based assays. European-American SLE patients with increased disease activity had more CD4+ T cells and fewer CD56+ NK cells. Patients with elevated disease activity also had decreased expression of the inhibitory receptor CD85j on monocytes and B cells, which associated with elevated Th1, Th2 and Th17-type plasma cytokines. In African-American SLE patients with elevated disease activity, frequencies of effector memory CD4+ T cells were increased, and CD4+ T cells had an activated phenotype with higher frequencies of CCR6+, CD25+, CD127+, and CXCR3+ CD4+ T cells. This activated CD4+ T cell phenotype correlated with elevated ICAM-1 and diminished TGF- β plasma levels. Pro-inflammatory cytokines were increased and correlated with increased disease activity across races.

Ongoing experiments with these subjects are assessing epigenetic profiles in the same cell populations with EPICyTOF, and testing the anti-cytokine profiles in these plasma samples. Preliminary analyses have identified prominent changes of chromatin modification marks across multiple cell types. Expanded replication cohorts are currently being recruited and will be used to confirm and extend the current results. In addition, the single cell epigenetic results will be refined and correlated with expression data both from sorted cell populations and from single-cell RNA-Seq. OMRF has already collected data for genotyping, whole blood gene expression, and plasma biomarker activities on a longitudinal adult lupus cohort (n=200). A collaboration between OMRF, Stanford, Baylor, and Michigan will use this resource to assess disease flare signature modules, predictive algorithms, and mechanisms associated with transitions between quiescent disease and flare in adult SLE patients.

1C. Adaptive epigenetics (J. Boss, Emory)

Major Accomplishments:

1. Developed protocols for epigenetic analyses on low numbers of cells and biobanked samples.
2. Performed epigenetic analyses on pathogenic B cell populations associated with SLE and compared those data to healthy control samples
3. Identified NF- κ B, AP-1 family member signatures associated with SLE and that naïve B cells in SLE patients are already epigenetically programmed towards a disease bias.

To understand the epigenetic basis for SLE we developed a series of robust assays that use limited numbers of cells to measure DNA methylation (RRBS), chromatin accessibility (ATAC-seq), and transcription (RNA-seq). In collaboration with the Sanz group at Emory, the ATAC-seq assay was optimized for biobanked samples (Scharer et al. *Scientific Reports* 2016) and allowed for the profiling of high quality subjects collected over the last three years.

We have applied these assays to distinct B cell subsets that the Sanz group at Emory has found to be expanded and associated with SLE. To date, we have completed epigenetic and transcriptional profiling of five B cell subsets from eight health controls (HC) and nine subjects with SLE. In total, there 199 unique sequence data sets (85 RRBS; 35 ATAC-seq; and 79 RNA-seq) representing 12.7×10^9 unique sequence reads. Informatic analysis is progressing through two distinct phases. Phase I analyses are comparing like data sets (i.e. RNA-seq vs RNA-seq, ATAC-seq vs ATAC-seq) to answer questions about differences between pathogenic cell types and between B cell subsets of SLE patients and HC. Preliminary results suggest a distinct programming difference between normal and expanded pathogenic B cell subsets. Across all B cell subsets, a pathogenic signature was evident in the DNA methylome, chromatin accessibility pattern, and transcriptome. Astonishingly, resting naïve B cells in SLE patients exhibited epigenetic marks of the disease, despite not being exposed to antigen. The preliminary results identified enhanced type I IFN activity, growth factor signaling, NFkB, and responses to viruses that are manifested across the SLE data sets. We have now begun Phase II analyses to integrate the data from each technology (i.e. RNA-seq vs ATAC-seq vs RRBS) to define epigenetic processes that regulate transcription modules. Preliminary results identifying unique regulatory modules in pathogenic cell types indicate that SLE and HC B cell subsets progress differently through differentiation. For example, there is a concordance of loss of DNA methylation, opening of chromatin, and enhanced gene expression regulated by factors in the AP-1 pathway, including ATF3 and BATF.

Additionally, collaborations with the Bluestone group were initiated to understand the epigenetic and gene regulatory pathways that occur in Tregs associated with T1D patients. Through this collaboration, we have begun profiling therapeutic Treg cells from T1D and HC subjects. The goal of these studies is to develop an epigenetic/molecular diagnostic tool to determine the quality of *ex vivo* Tregs and predict efficacy.

1D. Microbiome (R. Xavier, MGH)

The goals of this subaim are to determine the nature of the intestinal microbiome of patients with IgG4-RD and systemic sclerosis with an initial presentation of active autoimmunity or with a disease flare, and to determine if the onset of disease or flare can be linked to distinct bacterial community memberships. To date, we have examined intestinal microbial community profiles after total B cell depletion and clinical remission has occurred in IgG4-RD subjects. Our ongoing and future work will evaluate intestinal microbial community profiles in IgG4-RD subjects at the MGH ACE before therapy with Rituxan, and compare these profiles to those in healthy controls and patients with other immunological disorders, particularly rheumatoid arthritis (a cohort analyzed at the Broad Institute as part of HMP-2) and systemic sclerosis, from the Michigan ACE. In addition, intestinal microbial community profiles in systemic sclerosis patients (from the Michigan ACE, before therapy) will be compared with those of healthy controls and patients with IgG4-RD at MGH. We anticipate that these experiments will both operationally define the patient-associated microbial communities that promote or suppress inflammatory states and refine understanding of how different clinical and immunological phenotypes can shape these microbial communities. The project has the potential to contribute to fundamental knowledge regarding human CD4+ T cell polarization, the pathogenesis and /or course of IgG4-Related Disease and systemic sclerosis, as well as potential targets for therapeutic intervention.

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

Major Accomplishments:

1. TLR7-dependent OX40L expression drives Tfh development in SLE.
2. Downstream of the TCR, IRF4 instructs Th1 versus Tfh differentiation.
3. Synovial fibrocytes can induce inflammation by shedding CD13 and Id1.
4. AIRE regulates fibrocyte function in thyroid eye disease and possibly in RA.
5. Bcl-2 is a potential therapeutic target in lupus nephritis.
6. Convolutional neural networks resolve cognate from non-cognate T cell:DC interactions.

Narrative Summary:

Aim 2A1: *In situ* TFH cell function in inflammation (H. Ueno, Mt. Sinai).

1. Identifying the role of the TLR7-OX40L axis in autoantibody production in systemic lupus erythematosus (SLE). We have demonstrated that 1) OX40 ligand (OX40L) was overexpressed by myeloid APCs in blood and in inflammatory tissues of SLE patients, 2) OX40 signal promoted human naive and memory CD4⁺ T cells to become Tfh-like cells, and 3) RNA-containing ICs present in SLE sera induced monocytes to express OX40L in a TLR7-dependent manner (*Immunity*, 2015). We propose that RNA-containing ICs in SLE patients activates the TLR7-OX40L axis, and promote generation of autoantibodies by promoting Tfh cell response.

2. cTfh biomarker correlating with disease activity and severity in pediatric SLE. Through an extensive analysis of the expression of Tfh markers on circulating Tfh cells in pediatric SLE patients and a comprehensive analysis of the correlations between the flow data and clinical parameters, we have found that the frequency of CD57⁺ cTfh1 cells showed a strong positive correlation with SLEDAI score and a negative correlation with serum C3 complement level.

3. IRF4 as a key transcription factor defining the early differentiation pathway between Th1 and Tfh cells. We have found that high IRF4 expression was required to promote early CD4⁺ T cell differentiation programs towards Tfh and away from Th1 cells (*Cell Reports*, 2016). Low IRF4 promoted the expression of Eomes, and accordingly the population producing IFN- γ . This suggests that factors regulating the expression levels of IRF4 in CD4⁺ T cells, for example the strength of TCR signaling, affects the fate of CD4⁺ T cells in inflammatory sites in autoimmune diseases.

Aim 2A3: Antibody repertoire selection in inflammation (M. Clark, Chicago)

1. Selection and function of anti-vimentin antibodies in inflammation. Previous studies had indicated that vimentin, a molecular pattern of inflammation, was commonly targeted *in situ* (*Arth Rheum*, 2014). Reversion of anti-vimentin antibodies (AVAs) to germline revealed selection *in situ* for both higher affinity as well as poly-reactivity. Analysis of repertoire in naïve and anergic B cells from normal individuals revealed that 5% of cells in each population expressed AVAs. In the LUNAR trial neither rituxan nor mycophenolate treatment was effective in suppressing IgG AVA titers. However, those patients with high AVA titers at enrollment were more likely to respond to therapy. These data indicate that AVAs are common in the normal repertoire, are selected in inflammation and may have a role in ameliorating renal damage (manuscript in preparation).

Aim 2B: Non-conventional *in situ* antigen presenting cells. (D. Fox, Michigan; M. Clark, Chicago)

1. Fibrocytes as important antigen presenting cells in organ specific autoimmunity (D. Fox,) as 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.

2. Robust bioinformatic approaches to understand cell:cell interactions *in situ* (M. Clark). Initial approaches could discriminate cognate from non-cognate T:B cell interactions (*STM*, 2014). However, cell distance mapping (CDM) was unsuitable for studying larger antigen presenting (ex., dendritic cells, DCs). Subsequently, we improved our segmentation algorithms to better identify cell subtypes in confocal images. Using this approach, we identified Bcl-2 as a potential therapeutic target in tubulointerstitial inflammation (*Arth Rheum*, *in press*). We have now used convolutional neural network theory to develop a third generation of CDM (CDM³) which can define both cell:cell distances and cell shape. The latter allows identification of cognate T cells interacting with DC cytoplasmic processes (manuscript in preparation).