

Autoimmunity Centers of Excellence 2014-2019 - Executive Summary

Overview

The Autoimmunity Centers of Excellence (ACE) Program consists of three interrelated research components: (i) basic science projects to be conducted by seven U19 sites; (ii) clinical trials to be led by four UM1 sites; and (iii) the Collaborative Agenda which includes 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.

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U19 Basic Science Components

The Emory University Principal Project (B Cells in Human SLE) will provide a precise understanding of the relative participation of different B cell subsets during acute disease exacerbations which has been previously hampered by disease heterogeneity, imprecise B cell phenotyping and the lack of the high-throughput technologies needed to define the antigenic forces driving the generation and selection of autoreactive B cells and serum autoantibodies. These tools were established during the previous ACE cycle and include multi-dimensional flow cytometry; next generation sequencing (NGS); large scale single cell antibody generation; and bioinformatics platforms for the integrative analysis of high-density immunological, transcriptional and clinical data. In the Principal Project of the Emory ACE U19, we shall take advantage of these technologies to unravel the participation and antigenic selection of different B cell compartments in SLE flares (Aim 1); and the relative contribution of short-lived and long-lived antibody secreting cells to the SLE serum autoantibody proteome (Aim2). In addition, our Principal Project Aim 3 will interface with the Emory component of the ACE Collaborative Agenda led by Dr. Jerry Boss to study the epigenetic and transcriptional control of B cells in SLE.

The work proposed is highly synergistic with the overall ACE Collaborative Agenda. Specifically, the Principal Project will contribute to the B cell and antibody repertoire analysis of different autoimmune diseases proposed under Aim 1 of the Collaborative Agenda. We are also collaborating with Dr. Pascual (Baylor ACE) providing 9G4 monoclonal antibodies for her analysis of anti-mitochondrial DNA reactivity. As part of Aim 1 of the Collaborative Agenda, Dr. Boss will play a leading role in conducting genome-wide epigenetic analysis of different cell types and diseases. We will also collaborate with Dr. Clark (University of Chicago ACE; Collaborative of kidney infiltrating 9G4+ plasma cells in patients with Lupus nephritis Agenda Aim 2) in the analysis of the autoreactivity. Finally, we are collaborating with

Dr. Ueno (Baylor ACE; Collaborative Agenda Aim 2), to provide SLE samples to understand the role of TFH cells in the bone marrow environment.

The Baylor Institute ACE overall goal is to define novel pathogenic amplification loops for type I IFN and autoantibodies in systemic autoimmune diseases. Our studies show that the combination of Type I IFN and autoantibodies against endosomal TLR7 agonists arrest the detoxification of oxidized DNA within the neutrophil mitochondria by blocking its lysosomal degradation. This leads to the extrusion of potent mitochondrial DAMPs that activate plasmacytoid DCs to produce IFN.

The specific AIMS of the project are:

AIM 1. To characterize the basic mechanisms that lead to neutrophil mitochondrial damage and release of mtDAMPs in SLE. We will i) determine how Type I IFN and TLR7 ligands promote neutrophil mtDAMP extrusion, ii) establish the role of SLE-specific autoantibodies in these processes (link to 1A2/1B4), iii) identify specific upstream and downstream mediators that might be the target of therapeutic intervention (explore genetic variants in collaboration with Oklahoma, epigenetic differences with Emory/ Michigan/ Stanford).

AIM 2. To characterize the interferogenic components of mtDAMPs and the mechanisms responsible for their internalization in pDCs. The quantity of mtDNA extruded by SLE neutrophils does not correlate with pDC activation, suggesting that qualitative changes are necessary to convert mtC into mtDAMPs. Indeed, our data show that interferogenic complexes contain heavily oxidized DNA. The role of DNA oxidation and the protein composition of mtDAMPs, as well as their compartmentalization within pDCs will be explored under this aim.

AIM 3. To characterize the effects of SLE mtDAMPs on non-hemopoietic cells that become the target of inflammation in SLE. SLE mtDAMP activation of pDCs can be inhibited by blocking ii) TFAM, a mitochondrial HMG-like protein that binds RAGE and/or ii) TLR9, suggesting that these receptors are important for the interferogenic response to mtDAMPs. RAGE and TLR9 are found expressed in cells that undergo SLE end-organ damage, especially the endothelium. Here, we will study the effects of mtDAMPs on the activation of these cells.

Potential collaborations within ACE:

1. Test how unique SLE monoclonal autoantibodies activate this pathway: Emory, Chicago, Stanford, MGH, Oklahoma (1A2/1B4)
2. Establish the role of epigenetics in the propensity of lupus neutrophils to respond to TLR7 agonistic autoantibodies with the release of interferogenic DNA: Emory, Stanford, Michigan
3. Determine if exosomes containing TLR agonist autoantigens activate this pathway (1B5)
4. Establish if oxidized neutrophil DNA can be found in lupus tissues: Chicago, UCSF, Feinstein
5. Test the role of common genetic alleles involved in TLR downstream pathways/molecules in predisposing neutrophils to respond to TLR7 agonistic autoantibodies with interferogenic DNA release (TNFAIP, Oklahoma)

6. Test neutrophil interferogenicity in lupus clinical trials: All clinical sites.

7. Determine if this pathway is involved in other IFN-mediated autoimmune diseases (Sjogren's, Myopathies etc.): All sites.

The University of Chicago Autoimmune Center of Excellence' central theme is tolerance and adaptive immunity in autoimmune diseases. This theme will be pursued in two distinct projects. In the Collaborative Project, we will determine how adaptive autoimmunity evolves and is propagated *in situ* in autoimmune diseases with specific end organ involvement. We will focus on lupus nephritis (LuN) which is the most common severe manifestation of systemic lupus erythematosus (SLE). Progression to renal failure correlates with tubulointerstitial inflammation (TII) and that the immunological processes associated with TII are intrinsic to the kidney. These processes are not fully reflected in the peripheral blood and murine models of SLE do not mimic the *in situ* adaptive immune responses of human lupus TII. Therefore, animal models cannot substitute for primary studies in humans. During the last cycle of the ACE, we developed novel methods to study *in situ* immunity in human tissue. We can now clone *in situ* expressed antibodies, express these antibodies and characterize the antigens they bind. However, identifying the antigens recognized *in situ* is not sufficient to understand how those B cells are being selected *in situ*. As described, we have developed novel computational tools to identify both cognate cell:cell interactions and global patterns of cellular organization in human inflammation. We will extend these studies to other disease states to establish specific and global mechanisms by which *in situ* tolerance fails in autoimmunity.

The goal of the Primary Project is to examine the consequences of a loss of tolerance, and autoimmunity, in the development of protective immunity to infection. Surprisingly, SLE patients mount more effective humoral immune responses to influenza vaccination than normal controls. In the Primary Project, we will determine if enhanced protective immunity is a consequence of the broader immunoglobulin repertoire associated with SLE and/or if the cytokine milieu of SLE enables better protective immunity.

The University of Michigan Basic ACE studies an epigenetically altered CD4+CD28+ T cell subset characterized by aberrant overexpression of genes normally suppressed by DNA methylation, including those encoding CD11a, CD70, CD40L and the KIR gene family. This subset develops following exposure to environmental agents that inhibit DNA methylation, such as oxidative stress, and the epigenetically altered T cells are sufficient to cause lupus-like autoimmunity in mice genetically predisposed to lupus, but only anti-DNA antibodies in mice lacking a sufficient genetic predisposition. Importantly, this subset is found in patients with active lupus and in patients with other autoimmune diseases including rheumatoid arthritis, scleroderma and Sjogren's Syndrome, and may similarly contribute to the development of these and possibly other autoimmune diseases such as neuromyelitis optica (NMO) in genetically predisposed people.

The goals of the Michigan Basic ACE are to identify the genes that are demethylated and overexpressed within this subset, and determine the relationship between subset size and oxidative stress, disease activity and genetic predisposition to autoimmunity in patients with active autoimmune diseases. The overarching goal is to identify new diagnostic and therapeutic approaches to these diseases. Over the next 5 years this ACE will collaborate with other ACEs at the University of Michigan, University of Colorado, Oklahoma Medical Research Foundation, Stanford University,

Feinstein Institute for Medical Research and Emory University to obtain blood samples from patients with lupus and NMO.

The Oklahoma Medical Research Foundation (OMRF) Principal Project identified TNFAIP3, a potent negative regulator of NF- κ B signaling, as an SLE risk gene. In addition to SLE, genetic variants in the region of TNFAIP3 are associated with rheumatoid arthritis, psoriasis, Crohn's disease, celiac disease, type 1 diabetes, Sjogren's syndrome, systemic sclerosis and juvenile inflammatory arthritis. Therefore, clarifying the mechanisms that regulate TNFAIP3 expression and function are likely to have broad impact in autoimmunity. By fine mapping in SLE cohorts of multiple ethnicities and deep sequencing of TNFAIP3 risk haplotypes, this OMRF laboratory isolated two functional variants (rs148314165, rs200820567) responsible for association with SLE in the region of TNFAIP3. Preliminary data confirm that rs148314165 and rs200820567 (referred to as the TT>A variants) reside in an enhancer element that binds NF- κ B and SATB1 enabling the interaction of the enhancer with the *TNFAIP3* promoter through long-range DNA looping. Impaired binding of NF- κ B to the enhancer harboring the TT>A risk allele, inhibits interaction of the enhancer with the *TNFAIP3* promoter resulting in reduced A20 expression. These results elucidate a novel functional mechanism by which rs148314165 and rs200820567 attenuate A20 expression and support a causal role for these variants in the predisposition to autoimmune disease.

The primary scientific objective of this OMRF Principle Project for the next 5 years will be to elucidate mechanisms of TNFAIP3 transcriptional control that influence autoimmune disease risk. Resources available through the ACE collaborative program will support efforts to define the dynamic chromatin state of enhancers that harbor SLE associated risk variants, including the TT>A enhancer, in B cells isolated from SLE patients before and after flare using ChIP-sequencing and gene expression approaches. These studies will serve to clarify the functional mechanisms that regulate TNFAIP3 expression and lay the groundwork for developing of rational therapeutics aimed at restoring homeostatic potency of TNFAIP3 in autoimmune disease. Furthermore, in concert with the ACE collaborative program, these Principle Project studies will provide an initial preview into changes in regulatory element function genome-wide in the setting of lupus flare. Additional studies of other disease states where this variant may be important in mechanisms of disease flare, such as in rheumatoid arthritis, may also be explore in work with the ACE collaborative program.

The Stanford Principal Project will characterize the B cell repertoire in healthy and autoimmune subjects by using a DNA barcoding technology recently developed in his lab. The approach takes advantage of DNA barcoding and next-generation sequencing to characterize genes co-expressed by individual cells, including the paired heavy and light chain immunoglobulin genes from single-cell sorted plasmablasts or antigen-specific B cells. The resulting sequencing datasets enable generation of "phylogenetic trees" of the antibody repertoire on the basis of similarities and differences between the genes encoding receptor pairs. The power of this approach is that human monoclonal antibodies can be cloned, expressed and purified for further studies, including antigen binding or even autoantigen discovery. This project is important for the ACE mission because it will enable characterization of the B and T cell repertoire over time, including during transition from asymptomatic to symptomatic phases; during disease flare; and following therapeutic interventions.

The Stanford Pilot Project will develop, validate, and employ a novel sequencing method (ATAC-Seq) that enables rapid and comprehensive characterization of transcription factor occupancy in purified populations of immune cells. Rapid profiling of an epigenome is made possible by direct transposition of sequencing adapters into native chromatin using a transposase. The method enables rapid (~30 minutes) capture of open chromatin sites from ~1,000 to 100,000-fold fewer cells than conventional methods. Preliminary studies have demonstrated the method's value in studying B and T cells, and monocytes. The Chang lab has also measured and interpreted the serial daily epigenomes of resting human T cells from a proband via standard blood draws, demonstrating the feasibility of reading personal epigenomes in real time for monitoring health and disease. The pilot phase of this project includes identifying the lower limit of cell number to obtain useful data; to compare healthy cells with cell populations derived from SLE patients; to develop protocols for skin cells obtained from biopsies; and to explore changes that occur following therapeutic interventions. Success in the pilot phase will enable this to become an ATAC-Seq ACE Core in later years of funding.

The Stanford Human Immune Monitoring Center will serve as the sample processing unit that supports the Stanford Rheumatic Diseases Registry and Repository (ACE Sample Repository, Utz PI, funded by non-ACE sources). HIMC will perform other companion assays that will be used to fully characterize T and B cells in autoimmune diseases, including Luminex Bead-Based Cytokine and Chemokine Assays; Phosphoflow Cytometry by FACS; and CyTOF characterization of purified cell subsets. ACE Core B will develop and validate CyTOF panels for characterizing B cell subsets, as well as signaling pathways of interest to the overall ACE program.

The Massachusetts General Hospital Principal Project examines the pathogenesis of IgG4-Related Disease. In particular, the role of clonal CD4+ T cell expansions, discovered by Next Gen Sequencing, found in the blood and diseased tissue will be examined in IgG4-RD subjects as well as in other fibrotic diseases including systemic sclerosis. These cells are phenotypically a variant of CD4+ CTLs and their differentiation and induction at the signaling, transcriptional and epigenetic level will be examined. The possibility that plasmablast expansions also found in the disease are required for antigen presentation and activation of these CD4+ CTLs will be examined in situ. Studies are also in progress to use single cell cloning derived human monoclonal antibodies to identify specific antigen/s that drive the disease. We hope to identify the antigenic basis for CD4+ CTL expansion, IgG4 plasmablast expansions, and the induction of T follicular helper cells in this disease. The possible role of genetic variation in this disease will also be studied.

In the MGH Pilot project, the possible role of altered IgG4 glycosylation in contributing to disease pathogenesis will be examined.

The MGH Collaborative project will be a cornerstone of the Collaborative Agenda Aim 1 in studying the possible role of alterations in the intestinal, oral and tissue microbiome and virome in driving the onset of disease.

UM1 Clinical Trials Components

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 will explore therapeutic strategies designed to inhibit inflammation (Feinstein Institute); one program will explore strategies designed to inhibit pathologic immune mechanisms (University of Michigan); one program will explore strategies designed to augment protective immune mechanisms (University of California, San Francisco); and one program will explore strategies designed to prevent the development of autoimmune disease in people at high risk. Although these trials reflect different therapeutic approaches, they will be closely linked to one another by common mechanistic studies. In addition, they are closely linked to the collaborative projects, because they will provide comprehensive clinical trial data that are directly relevant to the central themes of the collaborative projects (next section).

Feinstein Institute – 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 (cytokines), an anti-inflammatory approach that modulates multiple inflammatory mediators will have broad applications and may be safer and better tolerated than current therapeutic options. Toward this end, the principal project consists of a phase II trial in patients with rheumatoid arthritis (RA) using an agent (GTS-21) that activates the cholinergic anti-inflammatory pathway. Specifically, GTS-21 engages the alpha 7 nicotinic receptor, resulting in reduced production of inflammatory cytokines. In a second project, the investigators propose to study ajulemic acid in patients with systemic lupus erythematosus (SLE). Ajulemic acid is a synthetic, non-psychoactive cannabinoid that possesses multiple anti-inflammatory properties.

University of Michigan - This program will conduct mechanistic studies using specimens derived from industry-sponsored trials of novel immunosuppressive agents. The principal project involves a phase III trial of a novel sphingosine-1-phosphate receptor modulator (BAF312) in patients with secondary progressive multiple sclerosis (MS). The proposed mechanistic studies will focus on: (i) pathogenic T cell autoreactivity that is characteristic of MS, and (ii) the ability of the damaged central nervous system to initiate repair and recovery from immune attack. A second project will be conducted in the context of a trial of abatacept in patients with systemic sclerosis. The proposed mechanistic studies for this trial will assess the effects of T cell co-stimulation blockade in scleroderma on: (i) immune cell activation, and (ii) biomarkers of tissue damage and fibrosis in the skin.

University of California San Francisco (UCSF) - This program will examine the potential for using *ex vivo* expanded autologous regulatory T cells (Tregs) in the treatment of autoimmune diseases. Two complementary projects will be conducted. One project will examine Treg therapy in an antigen-nonspecific autoimmune disease (SLE), and one will examine Treg therapy in an antigen-specific autoimmune disease (pemphigus vulgaris)(PV). The selection of these two diseases will provide 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-nonspecific autoimmune diseases; (iii) to compare polyclonal and antigen specific Treg therapy in the same disease (PV); and (iv) to examine the same target tissue (skin) in both SLE and PV.

University of Colorado – This program will test 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 will test 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. The second project will test methyldopa (aldomet) in patients at high risk for developing type 1 diabetes mellitus (T1DM). Methyldopa interacts with the peptide-binding groove on distinct Class II molecules that have been implicated in T1DM.

Each of the clinical projects is closely linked conceptually to the overarching themes of the collaborative projects (below), in that they involve careful characterizations of adaptive immune mechanisms in the setting of active disease, relapsing/evolving disease, and/or quiescent disease (Aim 1). In addition, the projects from the University of Michigan and UCSF will enhance the second major theme of the collaborative projects by examining mechanisms of autoimmune disease in tissue (Aim 2).

Collaborative Agenda

The ACE Collaborative Projects focus on two large specific aims:

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

Many autoimmune disorders have fluctuating disease courses with periods of increased disease activity (flares or relapses) superimposed on previous periods of relative disease suppression or quiescence. Many critical questions remain in understanding the pathogenic mechanisms of autoimmune disease flares which usually require treatment with toxic immunosuppressive drugs. A few include: Do unique B and T cell populations (not necessarily clonally defined) expand in autoimmune disorders and drive pathogenesis? Do unique B and T cell clones defined by Next Gen Sequencing drive autoimmune flares, decline during quiescence (or after select therapies) and re-appear upon relapse? Do disease related B and T cell clones or populations exhibit altered gene expression patterns compared to similar populations from subjects without disease and how are these changes epigenetically regulated? Additionally, does the epigenetic potential of select cell types reemerging following immune cell depletion mimic the disease or naïve state? Among potential environmental factors that could contribute to flares or relapse - do specific microbiome components or metabolites track with disease initiation and evolution?

To address these critical questions the ACE collaborative projects will characterize mechanisms of disease flare/relapse compared to disease quiescence, initially in patients with two autoimmune diseases, systemic lupus erythematosus (SLE) and neuromyelitis optica (NMO). Both of these diseases are characterized by disease flares or relapse, although NMO patients may be able to have disease-free periods between relapses even while off immunosuppressants in comparison to SLE which usually requires ongoing immunomodulation. Both are also characterized by the presence of autoantibodies, although in NMO these are primarily directed against one primary autoantigen (aquaporin 4) and in SLE autoantibodies are directed against a host of different primarily DNA- or RNA-binding proteins. Both diseases are also areas of clinical and experimental expertise for the current ACE. Lessons learned in these two initial autoimmune diseases will also be tested in other autoimmune

disorders, such as rheumatoid arthritis and systemic sclerosis, as resources allow and scientific rationale dictates.

The first aspect of this aim will focus on understanding antigen-specific autoimmune repertoires in B cells in NMO and SLE through different methods such as antigen-specific B cell capture, sequencing and monoclonal antibody generation, as well as through different deep sequencing and analytic approaches. The unique association of AQP4-specific autoimmunity and NMO allows for a detailed investigation of how changes in antigen-specific pro-inflammatory B cell population govern autoimmune disease activity (relapses) and inactivity (remission). Since neurologic disability in NMO is uniquely associated with relapse activity, understanding the role of pro-inflammatory AQP4-specific B cell functions in disease may have immediate translational impact as prognostic biomarkers. As outlined elsewhere in the document, lessons learned in NMO will be compared and contrasted with SLE- and IgG4 related disease-specific work which is ongoing as part of several principle ACE projects. The other major aspect of this subaim is to examine the relationship between the presence, repertoires and function of regulatory and/or anergic B cells during disease flare or relapse (starting in NMO and potentially expanding to IgG4-RD and SLE) compared to quiescent disease. Many key questions remain unanswered regarding the role of B10 cells in human autoimmune disease: (1) the nature of antigenic stimuli producing B10 cells; (2) the correlation between regulatory B10 cells and autoimmune disease activity; (3) the phenotype of antigen-specific B10 cells and the circulating B cell pools they occupy; (4) their ability to suppress antigen-specific T cell and innate immune responses; and (5) impairment of B10 function in human autoimmune disorders. Through analyzing the numbers and function of regulatory B cells in flare compared to suppressed disease, initially in NMO and SLE and then moving to other autoimmune disorders, the ACE Collaborative project will provide answers to many of these important questions.

The ACE collaborative program has a unique opportunity to address B and T cell activation mechanisms of disease flare in systemic autoimmunity through samples available from ongoing ACE clinical trials, select stored samples from longitudinal historical collections, datasets already assembled from well-phenotyped collections, cutting-edge technology/ associated informatics and novel hypotheses to be tested, focusing initially in SLE or in NMO. The first aspect of this subaim will use CyTOF, gene expression profiling and serologic multiplex profiling of soluble mediators to understand mechanisms of SLE disease flare using primarily samples from the ALE06 ACE trial studying withdrawal of mycophenolate mofetil in stable lupus or in longitudinal SLE cohorts. The second aspect of this subaim will test a specific hypothesis that a select subset of hypomethylated T cells are enriched in SLE disease flare and contribute to disease pathogenesis and will also determine if this T cell subset is functionally relevant in other autoimmune diseases. The third will test a specific hypothesis that a specific follicular helper T cell subset is involved in SLE disease flare and NMO relapse compared to quiescent disease. Identification of autoimmunity to histones, cytokines, chemokines, growth factors, and cell surface receptors has also been implicated in autoimmune disease pathogenesis and the fourth of these subaims will test whether specific autoantibody subsets are enriched in disease flare. The final part of this subaim will focus on testing whether exosomes encapsulate autoantigens and inflammatory mediators which functionally stimulate autoreactive responses and correlate with disease flare or relapse across autoimmune diseases.

Epigenetic modulation of adaptive immune cells may also be important in driving autoimmune disease pathogenesis and this aspect of the ACE Collaborative Program will examine these responses in cell-specific ways in the context of disease flare or relapse. Comparative analyses of epigenetic and gene expression profiles are predicted to define the programming and cellular potential or dysfunction of autoimmune cells and identify novel biomarkers that could be used to measure or predict disease onset/disease flare, as well as to assess efficacy of therapeutic interventions. Moreover, the combined global analyses described below will identify the pathways that are important to autoimmune cell function and provide numerous targets for future therapies. This subproject hypothesizes that the epigenetic determinants of autoimmune B and T cells will: a) be distinct from healthy B and T cells and that some of these determinants will be shared across multiple autoimmune diseases; b) identify novel biomarkers of autoimmunity and immune dysfunction that are prognostic of disease severity and predictive of treatment response; and c) identify genes that are poised for expression and mediate disease. To test these hypotheses, four parameters will be integrated to derive the epigenetic program of a cell: DNA methylation, histone modifications, accessible chromatin, and gene expression profiling. The final component of this large Aim of the ACE Collaborative Agenda focuses on examining the impact of the intestinal microbiome and microbial metabolome on autoimmune disease activity or disease flare through 16S and shotgun sequencing of gut microbiome and profiling of the gut metabolome.

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

Our overall hypothesis is that the mechanisms of adaptive selection *in situ*, at sites of inflammation and organ damage, will be different than that which occurs in secondary lymphoid organs or that which predominate in the periphery.

Rationale: It has become increasingly apparent that in lupus nephritis, and other autoimmune diseases, that *in situ* adaptive immunity and inflammation, are pathogenic and substantially contribute to progressive organ destruction. However, because of severe limitations in the number of cells that can be obtained from clinical samples, it has not been practical to directly study *in situ* immunity in human autoimmune diseases. For many autoimmune diseases, such as lupus nephritis, attempts to identify surrogates of disease activity, or prognostic biomarkers, in peripheral blood have been disappointing. Furthermore, because we have lacked the tools to study *in situ* immunity, the mechanistic relationships between organ-intrinsic immune responses and immune perturbations measured in the blood are unknown. Recent technological advances in many fields have now, for the first time, made a mechanistic dissection of *in situ* immunity and inflammation in human clinical biopsies feasible. Remarkably, all of the necessary critical technologies are available within the ACE. Therefore, we propose to use the technical (single cell RNA-Seq, ATAC-Seq, antibody expression from single cells, high-throughput protein arrays, sensitive RNA-FISH, and Cell Distance Mapping, CDM), intellectual, clinical, and organizational resources of the ACE to dissect how *in situ* adaptive immunity arises in different autoimmune diseases and to compare these to immunity propagated in secondary lymphoid organs.

Aim 2A. *In situ* T_{FH} cell function in inflammation (Baylor, Chicago, Michigan).

We will determine the relationships between peripheral and *in situ* adaptive immunity for the specific case of B cell activation in the presence or absence of T cell help.

2A1a. Understand the relationships between traditional and inflammatory T_{FH}, and competent versus incompetent inflammatory T_{FH} cell populations (Baylor and Chicago). Hypothesis: Competent inflammatory T_{FH} cells will have unique expression signatures (RNA-Seq), and genomic landscape profiles (ATAC-Seq), reflective of mechanisms of differentiation and maintenance in the context of inflammation and possibly autoimmunity.

2A1b. Understand the relationships between tissue and peripheral T_{FH} cells (Baylor, Michigan and Chicago). Hypothesis: Peripheral T_{FH}-like populations will have phenotype and molecular signatures reflective of corresponding tissue inflammatory T_{FH} populations. Same methods as 2A1a.

2A1c. Determine the mechanisms driving T_{FH} activation at inflammatory sites (Baylor and Michigan). Hypothesis: The T_{FH} subsets dominant in inflamed tissues might differ among diseases, as signals delivered from microenvironment and APCs will be different. RNA-Seq will provide molecular signatures of inflammatory T_{FH} populations suggestive of specific pathways of differentiation. These will then be explored in *in vitro* differentiation assays.

2A2. *In situ* adaptive immunity in IgG4-associated disease (MGH, Baylor and Chicago). Hypothesis: *In situ* B cells and plasmablasts are selected by T_{FH} cells (CDM).

2A3. Antibody repertoire selection in inflammation (Chicago, Stanford and Emory). Hypothesis: T_{FH} help provided *in situ* in lupus nephritis will give rise to a different repertoire of B cells than that usually sampled in the periphery of patients with lupus nephritis. This hypothesis will be tested using single cell antibody expression followed by unbiased antigen characterization.

2A4. Determine the roles of T_{FH} cells in the regulation of IgG Fc glycan (MGH and Baylor). Hypothesis: The nature of T cell help will determine the glycosylation and therefore functional activities of IgG.

Aim 2B. Non-conventional *in situ* antigen presenting cells.

We will identify both conventional (DCs) and non-conventional APC populations in RA synovium, underlying mechanisms by which they select antigen for presentation as well as the T cell populations they present antigen to.

2B1. Identify conventional and non-conventional *in situ* APCs in inflammation and the T cell populations they present antigen to (Michigan, Chicago and Baylor). Hypothesis: Understanding the *in situ* spatial relationships between different T cell populations and potential conventional (monocytes/macrophages, DCs), and non-conventional (such as fibrocytes, fibroblasts, and endothelial cells) antigen presenting cells, will reveal which populations are likely to be presenting antigen to specific T cell subpopulations *in vivo*. Putative populations will be identified by CDM and single cell RNA-Seq. The functional antigen presenting capacity of putative APC populations will be explored in *in vitro* assays. If AIRE is expressed, the role of AIRE in selecting autoantigens for presentation will be examined.

2B2. Examine the relationships between circulating and *in situ* non-conventional APCs. Hypothesis: APC populations identified *in situ* will be present in the blood. It is anticipated that RNA-Seq experiments in Aim 2B1 will identify markers of specific APC populations that will then be identified in peripheral blood of RA patients.

2B3. Determine the clinical utility of measuring non-conventional APC and associated soluble mediators in peripheral blood (Oklahoma, Stanford, Colorado, Baylor and Michigan). Hypothesis: Circulating non-conventional APCs, expressing specific markers defined in *in situ* populations will be biomarkers of disease activity or specific disease manifestations. Methods as in Aim 2B2.