RHEUMATOLOGY RESEARCH FOUNDATION

SUMMER RESEARCH SERIES

PATHOGENESIS OF SYSTEMIC LUPUS ERYTHEMATOSUS

FRIDAY, JULY 30TH
2:00 - 4:00 PM EDT

Richard Bucala, MD PhD
Yale University

Defining Susceptibility to Lupus Inflammation

Innovative Research Award

The morbidity and mortality of systemic lupus erythematosus (SLE) results from a severe and tissue-destructive inflammatory response. Macrophage Migration Inhibitory Factor (MIF) is an upstream cytokine and genetic determinant of disease severity in SLE. Caucasian and African-American SLE patients with high-expression MIF alleles have significantly increased serositis, nephritis, and CNS disease. A confluence of recent insights into MIF's mechanism of action, which include: 1) its activating role in NFκB and inflammasome activation, 2) identification of the unique transcription factor, ICBP90, that activates MIF's variant promoter microsatellite (-794 MIF CATT5-8), 3) the development of "humanized" MIF mice, and 4) the discovery of a small molecule inhibitor (CMFT) that blocks ICBP90 interaction with the CATT microsatellite, create an opportunity for developing a precision medicine approach to SLE treatment. We will pursue two Specific Aims: 1. Define the relationship between high-genotypic MIF expression and the innate immune response in human lupus monocytes. Our data indicate that MIF upregulates innate responses, including the NLRP3 inflammasome, in response to lupus immune complexes. We will apply sensitive multi-dimensional CyTOF and RNASeq transcriptional profiling to examine the activation responses of high- and low-genotypic MIF expressing human monocytes, and define quantitative relationships between MIF, its receptors, and downstream effector molecules. 2. Define the impact of high-genotypic MIF expression on lupus immunopathology in a novel humanized MIF mouse model. We created two humanized MIF mouse strains by recombinant replacement of the endogenous murine Mif gene with the low-expression MIF CATT5 and the high-expression MIF CATT7 human MIF alleles. We will define the impact of high- versus low-genotypic MIF expression on SLE immunopathology and test the ability of CMFT to reduce MIF-dependent innate responses in humanized Sle1.Yaa mice. The completion of these Aims will help delineate the mechanistic relationship between MIF genotype and tissue-damaging inflammatory responses and provide proof-of-principle for precision-based inhibitors that target high-genotypic MIF expression in SLE.

Alessandra Pernis, MD
Hospital for Special Surgery

Signaling Pathways Regulating ABCs
Innovative Research Award

While expansion of germinal center (GC) B cells and plasma cells (PC) has long been associated with SLE, recent murine studies have uncovered the existence of new B cell subsets that also contribute to disease. Studies in aging mice indeed have identified a B cell subset, termed Age/Autoimmune-associated B cells (ABCs), which exhibits a unique phenotype and preferentially expands in females with age. In addition to classical B cell markers, ABCs also express myeloid markers like CD11c. Formation of ABCs is promoted by TLR7/TLR9 and cytokines like IFN-gamma and IL-21. Although generation of ABCs requires T-bet (hence these cells are also known as CD11c+T-bet+B cells), the molecular pathways that promote their generation, function, and differentiation in autoimmune settings are largely unknown. Multiple lines of evidence have implicated Interferon Regulatory Factors (IRFs) in autoimmunity. Amongst the IRF family members, IRF4 plays a fundamental role in the activation of both T and B cells. In addition to IRF4, genetic studies have highlighted the importance of IRF5 in human SLE. The SWEF family is comprised of only two members, SWAP-70 and DEF6, which has recently been identified as a genetic risk factor for human SLE. The SWEF proteins are multifunctional proteins and regulate T and B cell function by controlling the activity of IRF4. The SWEF proteins play an important immunoregulatory role in vivo and the concomitant lack of DEF6 and SWAP- 70 in C57BL/6 mice (Double-knockout=DKO mice) leads to the development of SLE, which, like human lupus, preferentially affects females. Autoimmunity in DKO mice is associated with increased IL-21 production. We have recently found that the lupus syndrome that develops in DKO female mice is also accompanied by a marked accumulation of ABCs, which is IL-21-dependent. DKO ABCs exhibit a distinctive transcriptome characterized by enhanced expression of proliferative and proinflammatory pathways and increased Ig transcription. DKO ABCs also display a unique chromatin landscape marked by enrichment in IRF binding motifs and depletion of MAF-bound regulatory regions. Notably, we have found that generation of ABCs in DKO mice is controlled by IRF5. Analysis of Blimp1-reporter DKO mice has furthermore revealed a population of CD11c+ B cells that expresses high levels of Blimp1, IRF4, and CD138 suggesting that ABCs can differentiate into CD11c+ PCs. Here we will investigate the hypothesis that the SWEF proteins regulate ABC generation and function by a dual mechanism, which involves restraining IRF5 activity as well as preventing the downregulation of MAF proteins and, thus, the loss of MAF-containing inhibitory complexes. We will also delineate the pathways controlling the differentiation of ABCs into CD11c+ PCs. Specifically, we will: a) Determine whether IRF5-expressing ABCs contribute to the development of lupus in DKO mice, b) Delineate the mechanisms leading to the downregulation of MAF in DKO ABCs, and c) Characterize the pathways controlling the differentiation of ABCs. Since peripheral expansion of ABC-like cells in SLE correlates with SLEDAI and specific clinical manifestations, in particular lupus nephritis and since ABCs from

SLE patients are poised to differentiate into PCs and are the major producers of autoAbs, understanding the molecular mechanisms responsible for their regulation and differentiation will provide critical information into SLE pathogenesis and help uncover novel therapeutic targets.

Felipe Andrade, MD, PhD
Johns Hopkins University

Rediscovering Ro52 in Systemic Lupus Erythematosus
Innovative Research Award

It is widely accepted that the current standard of care for patients with systemic lupus erythematosus (SLE) is inadequate, in part because of the absence of lupus specific therapies and also because of the lack of appropriate biomarkers to monitor the disease. In SLE, antibodies to self-antigens are considered to play a crucial role in disease pathogenesis. Understanding the mechanisms that initiate immune responses to autoantigens may therefore provide opportunities for the discovery of novel biomarkers and disease mechanisms, which can lead to new tools for diagnosis and target-specific therapies. To better understanding the source and mechanisms of autoantigen production in SLE, we focused on neutrophils and interferons (IFNs), two important players in SLE pathogenesis. Using biochemical and proteomic approaches to identify neutrophil-specific autoantigens linked to IFN activation in SLE, we found that neutrophils express at least four splicing variants of Ro52, which differ in structure, E3 ubiquitin ligase activity and immunogenicity. Interestingly, the expression of the distinct forms of Ro52 is strongly linked to interferogenic activation in patients with SLE, suggesting that these are IFN-induced autoantigens. Moreover, we identified a new set of autoantibodies in SLE patients, which target a unique sequence found in two novel Ro52 isoforms described for the first time in this proposal.

Building on these results, we hypothesize that neutrophils are a major source of immunogenic isoforms of Ro52, which are linked to IFN-induced activation in SLE. In addition, we propose that Ro52 isoforms have unique regulatory functions on the production of type I IFNs (IFN-I), via ubiquitination-induced degradation of IFN regulatory factors (IRFs), which may be relevant for SLE pathogenesis. We will examine these hypotheses directly in the human model in two specific aims. In Aim 1, we will use in vitro and cellular assays to define the regulatory role of the Ro52 isoforms in the ubiquitination of IRFs and the activation of IFN-I genes. In cGAMP+ and cGAMP- patients. Because we have identified oxidized mitochondrial DNA (ox-mtDNA) as a potent trigger of IFN-b through the STING pathway, in Aim 1B we will determine whether ox-mtDNA is the likely trigger of cGAS-STING by developing new ELISAs to detect ox-mtDNA and will correlate levels with the optimal markers of activation of cGAS-STING determined in Aim 1A. cGAMP+ and cGAMP- patients.

Because we have identified oxidized mitochondrial DNA (ox-mtDNA) as a potent trigger of IFN-b through the STING pathway, in Aim 1B we will determine whether ox-mtDNA is the likely trigger of cGAS-STING by developing new ELISAs to detect ox-mtDNA and will correlate levels with the optimal markers of activation of cGAS-STING determined in Aim 1A. In the third Aim (1C), we move from ex vivo to in vivo studies in humans. In this Aim, we will expose photosensitive cGAMP+ and cGAMP- patients to UVB, a known inducer of oxDNA (although the source is not clear). We will seek to determine whether the oxDNA is derived from neutrophil NETS, whether ox-DNA generation is concordant with activation of cGAS-STING and IFN-I.

These studies should establish the frequency and significance of cGAS-STING activation in SLE. They may lead to new approaches to therapy targeting ox-DNA, cGAS or STING.

Henri Tiedge, PhD
SUNY Downstate Medical Center

An RNA Mechanism in Systemic Lupus Erythematosus

Innovative Research Award

Neuronal regulatory Brain Cytoplasmic (BC) RNAs are translational regulators that operate in synapto-dendritic neuronal domains. Lack of rodent BC1 RNA in BC1 knock-out (BC1 KO) animals cause cognitive impairment and epileptogenesis. BC RNAs are delivered to synapto-dendritic subdomains by heterogeneous nuclear ribonucleoprotein A2 (hnRNP A2), a trans-acting RNA transport factor. hnRNP A2 recognizes a noncanonical GA motif in the BC RNA dendritic targeting element (DTE) in interactions that are required for the dendritic delivery of these RNAs.

Systemic lupus erythematous (SLE) is an autoimmune disease that often involves neurological or psychiatric impairments. We detected an autoimmune response to BC RNAs in a subset of SLE patients. SLE autoantibodies against BC RNAs (SLE anti-BC abs) belong to the IgG class of immunoglobulins and bind to the same DTE as dendritic transport factor hnRNP A2. SLE anti-BC abs compete with hnRNP A2 for access to the BC RNA dendritic targeting element, as a result impeding BC RNA synapto-dendritic delivery.

Here it is hypothesized that absence, or reduced presence, of regulatory BC RNAs in synapto-dendritic domains, caused by SLE autoimmune anti-BC ab competition with hnRNP A2, will cause phenotypic manifestations that are similar to those observed in BC1 KO animals and may include cognitive dysfunction and epileptogenesis. In the Innovative Research Award project proposed here, it is planned to investigate three key questions. (i) What is the molecular mechanism underlying the ability of autoimmune SLE anti-BC abs to compete with RNA transport factor hnRNP A2? (ii) Does such competition cause defects in the

synapto-dendritic delivery of regulatory BC RNAs in neurons? (iii) Do autoimmune anti-BC abs give rise to cognitive impairment and epileptogenesis in vivo? Are serum levels of SLE anti-BC abs markers of disease status, i.e. of active disease, remission, and flares? There is confidence that investigating a novel RNA mechanism in SLE will in the long-term help address major gaps in our understanding of this complex disease.

Carla Cuda, PhD
Northwestern University

Microglia-Specific Transcriptional Signatures in Neuropsychiatric Symptoms of SLE

Innovative Research Award

Neuropsychiatric symptoms of systemic lupus erythematosus (NP-SLE), including headaches, cognitive dysfunction and psychiatric disorders, affect over 60% of SLE patients, may be among the earliest signs of SLE and often go undetected. Despite the impact of NP-SLE on health-related quality of life and although numerous mechanisms have been proposed, none can solely account for NP-SLE pathogenesis. Microglia are comprised of at least two subsets: CD11chi disease-associated microglia (DAM), a subset of microglia thought to be instrumental in neurodegenerative diseases, and CD11clo microglia. While previous studies in neurodegenerative disease models suggest that CD11chi DAM are a regulatory population, relatively little is known about microglial subsets in NP-SLE. Thus, we will examine microglia subsets from both a mouse model of SLE as well as microglia-like cells from cerebrospinal fluid of patients with SLE to dissect their role in disease pathogenesis. We will also evaluate circulating monocyte populations in both mice and patients for signatures relating to NP-SLE. We generated the CReCOM (Caspase-8 Removed CD11c-specific Overactive MyD88) mouse, which develops an age-dependent aggressive systemic inflammatory disease reminiscent of SLE. Our Preliminary Data demonstrate that CReCOM mice also develop NP-SLE, including behavioral deficits prior to systemic autoimmunity, reduced brain volumes, decreased vascular integrity and brain-infiltrating leukocytes. Further, our Preliminary Data show that microglia from multiple SLE-prone models express a 'NP-SLE signature' as well as genes associated with DAM. Moreover, expression of 'NP-SLE' and 'DAM' signatures correlate with the severity of behavioral deficits in CReCOM mice. We will profile CD11chi DAM and CD11clo microglia from two models of NP-SLE (CReCOM and B6.Sle1.Sle2.Sle3) to determine penetrance of our newly discovered 'NP-SLE' and 'DAM' signatures as well as differences in transcriptional signatures indicative of their subset-specific roles and potential functional deficiencies in NP-SLE-like disease. We will then compare these transcriptional profiles to microglia-like cells from cerebrospinal fluid of corresponding mice as well as SLE patients with central nervous involvement to determine if 'NP-SLE' and 'DAM' signatures are detectable in human disease. We will also evaluate expression of 'NP-SLE' and 'DAM' signatures in circulating classical and nonclassical monocyte subsets of CReCOM and B6.Sle1.Sle2.Sle3 mice and NP-SLE patients to address the utility of these populations as proxies for intrinsically defective microglia during NP-SLE disease. This information will be

invaluable not only for downstream biomarker use but also targeted therapy development. Despite ongoing investigation into microglia subsets, in particular CD11chi DAM, in neurodegenerative disease models, we will be the first to examine the role of microglia subsets in the pathogenesis of NP-SLE. Further, this study will be the first to determine the predictive value of our newly identified microglia-specific 'NP-SLE' and 'DAM' signatures as a surrogate for NP-SLE clinical outcomes.

We hope you will join us. Registration is required.

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