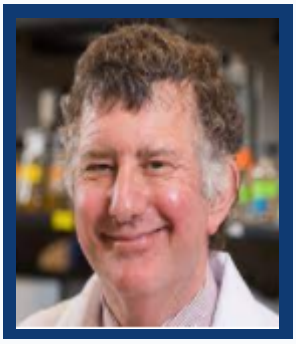
RHEUMATOLOGY RESEARCH FOUNDATION

SUMMER RESEARCH SERIES

NOVEL INSIGHTS IN CONNECTIVE TISSUE DISEASE

FRIDAY, AUGUST 6TH
2:00 - 3:45 PM EDT

David Markovitz, MD
University of Michigan



Centromeres and Scleroderma

Innovative Research Award

Scleroderma is an often-devastating disease that affects not only the skin but multiple other organs and causes considerable morbidity and mortality. Scleroderma is often broken down into two categories based on skin involvement: limited scleroderma and diffuse scleroderma. While scleroderma has generally been considered a disease of autoimmunity, there have been indications in the literature over the years that there is an accompanying chromosomal dysfunction, and indeed patients with scleroderma have a higher incidence of cancer. A vital part of the chromosome is the centromere, which is key to accurate chromosomal partition during mitosis. Interestingly, most of the epigenetic marks involved in centromere biology were discovered because about half of the patients with limited scleroderma have antibodies to these proteins. Nevertheless, the concept that centromere dysfunction might play a role in scleroderma pathogenesis has not been much examined at either the epigenetic or genetic levels. The genetic study of centromeres, like the study of centromeres in other diseases, has previously been greatly hindered by the lack of informative genomic information about these structures, whose tremendously repetitive DNA sequences make genomic annotation extremely difficult. However, recently we have developed PCR-based methods for assessing the status of centromeric DNA in a rapid fashion in almost all of the specific chromosomes. Using this methodology, we discovered that in diffuse scleroderma, but not in limited scleroderma, there is a profound loss of centromeric DNA. Further, multiple chromosomal abnormalities are seen, the latter consistent with previous literature. While patients with limited scleroderma do not exhibit defects in their centromeric DNA, in half of them, specifically those who have antibodies to centromeric proteins, we find kinetochore defects with a migration of the centromere/kinetochore out of the nucleus to a place in the cytoplasm. In view of our recent observations, we now propose to more fully characterize the epigenetic defects seen in the centromeres of patients with scleroderma and to correlate these changes with defects in chromosomes. We will also examine the interplay between the epigenetic abnormalities of the centromere and the pro-fibrotic scleroderma phenotype.

Further, we will ascertain whether the editing of centromere DNA can drive normal fibroblasts into the pro-fibrotic scleroderma phenotype. Taken together, the proposed studies hold the potential to define a previously unexplored mechanism of scleroderma pathogenesis: centromeric dysfunction. This work can therefore lead to new therapeutic approaches and aid in clinical management decisions for patients with scleroderma.

Jason Knight, MD, PhD
University of Michigan

Antiphospholipid antibodies in COVID-19

Innovative Research Award

The outbreak of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread to hundreds of countries and has been declared a global pandemic. Severe COVID-19 results in death due to progressive hypoxemia and multi-organ failure. COVID-19 patients are also at high risk for thrombosis in macro- and microvascular beds. At the same time, abnormal coagulation parameters such as elevations in D-dimer correlate with COVID-19 severity. Antiphospholipid syndrome (APS) is an acquired autoimmune thrombophilia in which patients form autoantibodies to phospholipids and phospholipid-binding proteins such as prothrombin and beta-2-glycoprotein I (β2GPI). These antiphospholipid antibodies (aPL) then engage clotting factors and cell surfaces, where they activate coagulation cascades, endothelial cells, platelets, and neutrophils—thereby tipping the balance toward thrombosis. A defining feature of APS is its ability to promote thrombosis in vascular beds of all sizes, including both arterial and venous circuits. The catastrophic variant of APS (CAPS) is often fatal, and bears many similarities to the coagulopathy seen in patients with COVID-19. Reports of aPL in COVID-19 and their possible relationship to thrombosis have begun to emerge in small case reports and series. While viral infections are known triggers of transient aPL, the extent to which these antibodies may be pathogenic has not been well defined.

Our preliminary data testing 172 patients hospitalized with COVID-19 reveal that half of patients have positive testing for at least one type of aPL. The presence of aPL correlates with neutrophil activation and disease severity. Furthermore, our preliminary data have revealed that purified IgG from COVID-19 patients markedly potentiate thrombosis in mice. Our hypothesis is that aPL are targetable amplifiers of COVID-19 severity. If correct, the hypothesis has significant implications for treating patients with acute disease (anticoagulation, plasmapheresis), as well as disease that has resolved (antibody persistence, convalescent plasma). Aim 1 will expand aPL testing to 1000 individuals hospitalized with COVID-19 in order to understand clinical correlations and determine long-term outcomes. The hypothesis is that aPL will associate with higher rates of macrovascular thrombosis (stroke, venous thromboembolism) and microvascular thrombosis (respiratory failure, kidney injury) in COVID-19. Aim 2 will characterize COVID-19-derived IgG/IgM fractions and affinity-purified aPL in vitro. The hypothesis is that COVID-19-derived aPL will have in vitro activities similar to aPL

isolated from patients with established APS. Aim 3 will determine the extent to which COVID-19-derived aPL are pathogenic in animal models. The hypothesis is that transfer of COVID-19 antibody fractions into mice will potentiate thrombosis, thereby confirming the pathogenic potential of these antibodies in vivo.

Sarah Gaffen, PhD
University of Pittsburgh

Regulation of Renal IL-17 Signaling in Antibody-Mediated Kidney Diseases
Innovative Research Award

Antibody-mediated glomerulonephritis (AGN) is a clinical manifestation of autoimmune kidney diseases including lupus nephritis and Goodpasture Syndrome. AGN is the third leading cause of kidney dysfunction in US, accounting for 20-30% of total renal failure cases. Response to immunosuppressive drugs is often inadequate and associated with significant side effects. Chronic inflammation in the glomerular and tubular compartments drives tissue damage leading to irreversible loss of renal function. In recent years, proinflammatory cytokine IL-17 has been implicated in the pathogenesis of AGN.

Although the major emphasis in the field has been placed on understanding how Th17 cells are generated, comparatively little is known about regulation of downstream IL-17 signaling in the target cells of the nephritic kidney. Understanding this is important because the actual tissue damage occurs locally, and interventions to limit such damage could be highly valuable clinically to treat the end-organ damage that characterizes AGN. We recently identified a novel inhibitor of IL-17 inflammatory activity, known as Regnase-1. Regnase-1 is an RNA binding protein (RBP) that is a feedback negative regulator of the IL-17 signaling pathway. Mice deficient in Regnase-1 show exaggerated AGN, a phenotype that is reversed in the absence of IL-17 signaling, indicating that activity in AGN is driven by IL-17 more than other inflammatory cytokines.

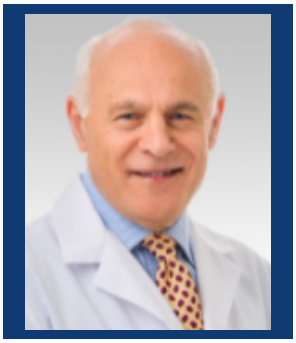
We also discovered that Regnase-1 suppresses the expression of Lipocalin-2 (Lcn2), an IL-17-responsive factor with potent kidney-damaging activities. Our preliminary data show that the ability of Regnase-1 to inhibit IL-17 signaling is blocked by another RBP, Arid5a, which drives Lcn2 production from IL-17 responsive cells. Here, we propose to identify the key IL-17-responding cell types in the nephritic kidney that promote AGN. We will also determine the molecular basis for regulation of IL-17 signaling by Regnase-1 and Arid5a. Our hypothesis is that Regnase-1 limits Lcn2 expression in the nephritic kidney by counteracting Arid5a, which normally prevents AGN development.

In Aim 1, we will define the role of IL-17 signaling in podocytes and renal tubular epithelial cells (RTECs) in driving AGN, taking advantage of existing mice with a specific deletion of the IL-17R in these cell types. Aim 2 will determine how Regnase-1 regulates IL-17 signaling in kidney-r

esident RTECs. This will be achieved by (i) assessing susceptibility to AGN in mice with an RTEC-specific deletion of Regnase-1, and (ii) dissecting signaling events involved in regulation of IL-17 signal transduction in mouse and human RTECs. Finally, we will evaluate the preclinical efficacy of treating mice with an Arid5a inhibitor.

These studies will advance our understanding of how pathogenic IL-17 signaling is regulated in the nephritic kidney to promote end-organ damage. Additionally, this work may reveal novel drug targets in the IL-17 signaling pathway that can be exploited for treating IL-17-driven end-organ damage in other rheumatic diseases involving this cytokine.

John Varga, MD
University of Michigan



Dysregulated Ciliogenesis in Scleroderma: Novel Pathogenic Mechanism?

Innovative Research Award

Fibrosis, the defining feature of systemic sclerosis (SSc), is characterized by accumulation of activated myofibroblasts that originate from mesenchymal progenitors through fibroblast-myofibroblast transition (FMT) and endothelial cell-myofibroblast transition(EndoMT). However, there is a gap in understanding how in SScfibrosis develops synchronously in multiple non-contiguous organs, and what are the mechanisms that drive and sustain the process. The objective of his application is to capitalize on our recent discoveries that implicate SPAG17 (sperm-associated antigen 17) and primary cilia in the pathogenesis of SSc. Using unbiased RNA seq technology, we found that expression of a little-known protein called SPAG17 was markedly reduced in SSc patients. We showed that SPAG17 regulates the formation, maintenance and function of primary cilia, which are ubiquitously expressed essential sensory antennae for hedgehog and related profibrotic morphogens (Wnt, TGF-ÃŸ). Mesenchymal cells lacking SPAG17 form stunted cilia and demonstrate constitutive profibrotic activity. Notably, SPAG17 null mice spontaneously develop progressive fibrosis in the skin, lung and muscles, and therefore represent an unprecedented mouse model for multi-organ fibrosis. The premise of this proposal is that SPAG17 controls both ciliogenes is and fibrogenesis, and its reduced expression in SSc will result in aberrant profibrotic ciliary signaling and multiple organ fibrosis. We will test this paradigm-shifting premise in three independent aims: 1) Measure cell type-specific SPAG17 expression, ciliogenesis, profibrotic activity and correlation with fibrosis in SSc biopsies; 2) Define the course and mechanisms of organ fibrosis in mice with conditional SPAG17 deletion; and 3) Determine the cellular basis for myofibroblast differentiation regulated by SPAG17. Aim 1 will use patient biopsies from a well-characterized longitudinal SSc cohort to measure SPAG17, ciliogenesis and morphogen activity and their correlation with clinical variables including disease severity and activity. Aim 2uses a new conditional SPAG17 knockout mouse as a unique model to evaluate spontaneous (age-dependent) and inducible (subcutaneous bleomycin) fibrosis in skin, muscle, heart and lung.

Aim 3 will examine ciliogenesis, morphogen signaling, myofibroblast differentiation and fibrotic phenotypes using mouse and patient-derived fibroblasts and endothelial cells with SPAG17 gain and loss of function. Our proposal is highly innovative, since SPAG17 mechanism and role in fibrosis has never been studied, and the contribution of cilia to disease pathology in SSc is unknown. Significance lies in the potential to define novel roles for SPAG17, ciliogenesis and ciliary signaling as fundamental mechanisms driving pathogenic FMT and EndoMT in SSc. Our investigative research team with deep expertise and unique experimental tools in myofibroblast biology, fibrosis and ciliogenesis is poised for successful accomplishment of these aims. Understanding the contribution of SPAG17 and ciliogenesis in fibrosis will inform the development of entirely new approaches for fibrosis therapy.

We hope you will join us. Registration is required.

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