Understanding the roles of complement regulators in thromboinflammation

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Diseases that manifest with vascular inflammation, including cardiovascular, inflammatory lung and bowel disease, and diabetes, are all associated with increased levels of circulating platelet/granulocyte aggregates (PGAs) in the bloodstream. Formation of PGAs naturally occur in response to platelet activation with subsequent interaction with, and activation of, granulocytes. PGAs help promote hemostasis not only by enhancing platelet activation, but also limiting thrombus formation by phagocytosing platelets, degrading the platelet agonist ADP, and limiting the interaction of fibrinogen with a key platelet receptor. While PGA formation occurs naturally, increased PGA levels associated with multiple human diseases suggests a pathologic role, a hypothesis supported by animal vascular injury models that show benefits to inhibiting PGA formation in vivo. Platelets can directly stimulate granulocytes to secrete proinflammatory cytokines and chemokines, produce reactive oxygen species, upregulate adhesion molecules, and express tissue factor to promote fibrin deposition. Clinical and experimental evidence thus suggests the existence of a threshold at which point PGA formation becomes pathologic rather than homeostatic. Given the array of diseases presenting with increased circulating PGA levels, understanding mechanisms that regulate the extent of PGA formation could provide researchers with key therapeutic targets to limit thromboinflammation in diverse clinical settings.

The complement system has emerged as a key mediator of platelet/granulocyte interactions in human whole blood. The complement system is a group of soluble and membrane-bound proteins that are a key part of the innate immune system. It activates in a cascade-like manner leading to the production of several different effector molecules that promote biological functions. Three distinct pathways initiate complement activity: (1) the classical pathway (CP), (2) the lectin pathway (LP), and (3) the alternative pathway (AP). While the CP and LP require recognition of specific molecular patterns to activate, the AP is a safeguard system that activates spontaneously at a continuous low rate in the fluid phase of blood. The AP amplifies its own activity as well as preexisting complement activity initiated by the CP and LP, thus it is a powerful amplification loop for all complement activity. Tight regulation is needed to prevent excessive AP activation and subsequent effects. AP activation is intrinsically short-lived, however the fluid-phase positive regulator, properdin, can increase AP activity 5-10 fold. Contrarily, Factor H (FH) is a soluble glycoprotein that is the primary negative regulator of the AP in the fluid phase. FH can also be recruited to host cell surfaces to help limit AP activation.
While there are known links between complement activity and PGA formation, the mechanisms that account for the effects of complement and the role of the AP regulators, properdin and FH, were previously unknown.

Our laboratory recently published a paper in the Journal of Immunology that defined complement-mediated mechanisms and the roles of properdin and FH in PGA formation in human whole blood stimulated with thrombin receptor-activating peptide (TRAP), a peptide that directly stimulates thrombin receptors. Utilizing an ex vivo flow cytometric assay we demonstrated that physiological properdin oligomers increase PGA formation and complement activity when added exogenously to whole blood stimulated with TRAP. Properdin is unique among complement proteins in that it is primarily produced by stimulated leukocytes, including neutrophils, thus its local concentration at inflammatory sites can be significantly higher than its concentration in plasma. Adding properdin exogenously to whole blood simulates such conditions that could lead to high local concentrations of properdin, and exemplifies the powerful potential effect of locally released properdin on thromboinflammation. Furthermore, inhibitory anti-properdin monoclonal antibodies significantly inhibited TRAP-mediated PGA formation in whole blood to approximately the same level as inhibition of all complement activity, indicating a key role for endogenous properdin (circulating in the plasma or produced locally by leukocytes) in promoting PGA formation in our system.

Investigation into the mechanisms for the effects of properdin, revealed a role for the CP in initiating complement activity, and C5a was the primary complement effector molecule that accounted for properdin-mediated effects on PGA formation. Inhibition of the C5a-C5a receptor axis prevented increases in PGA formation mediated by addition of exogenous properdin and was also equally effective as inhibition of properdin in limiting TRAP-mediated PGA formation. C5a directly enhanced TRAP-mediated PGA formation via upregulation of complement receptor 3 (CD11b/CD18) expression on the granulocyte surface, a receptor known to mediate stable binding of platelets to granulocytes. Collectively, these results point toward a model by which properdin aids in the amplification of complement activity initiated by the CP, leading to the production of C5a that promotes granulocyte activation and stable binding to platelets. Finally, we showed that inhibiting FH function specifically on the cell surface (rather than in the fluid phase), significantly increased PGA formation in a properdin-dependent manner. This indicates that FH is key for controlling properdin-mediated enhancement of PGA formation in TRAP-stimulated whole blood.

Our work has defined key roles for the AP regulators properdin and FH in controlling PGA formation induced by the thrombin-like agonist, TRAP. The diverse array of diseases associated with increased levels of circulating PGAs, suggests therapeutics that inhibit properdin function, that promote FH activity, or that inhibit C5a-C5a receptor interactions, used alone or in combination, may find utility in the clinic to limit thromboinflammation resulting from PGA formation. Our findings also expand on the pathophysiological mechanisms of the complement system in the human host. The role of complement in the human host goes beyond its ability to promote the elimination of pathogens. It is a ubiquitous system that exerts powerful effects on multiple biological processes. Understanding its mechanisms of activation and regulation could provide scientists with key tools to combat a plethora of diseases. Our paper is just one example of a broader base of work that validates the potential for complement-directed therapeutics in the clinic. In the future, the Ferreira lab is focused on better understanding the molecular mechanisms that mediate the interactions of properdin and FH at the platelet/granulocyte interface, and finding novel ways to inhibit properdin function, and/or promote FH activity, in a thromboinflammatory setting.

**New Division**

Division of Endocrinology, Diabetes and Metabolism

Juan C. Jaume, MD.
Professor/Chief Endocrinology
Director, Center for Diabetes and Endocrine Research

The mission of the new division, under Dr. Jaume’s direction is to maintain excellence in service and education, and promote research in the field of Endocrinology, Diabetes and Metabolism. The full mission statement is available on the Division webpage. Administratively, the Division’s research program is conducted at the Center for Diabetes and Endocrine Research (CeDER). The group conducts research at the Block Health Science Building site (basic and translational) and at outpatient clinics and inpatient service sites within the University of Toledo Health Campus (UT Medical Center, Ruppert Health Center, Dana Cancer Center) and the ProMedica Health Campus (The Toledo Hospital and the Falzone Diabetes Center). Scientifically, the Division’s research encompasses clinical, translational and basic areas as described below.

Current Clinical/Translational projects include:

1. Latent Adult Autoimmune Diabetes Database Study
2. Anti-Programed Cell Death-1 Pathway Blockade and Type 1 Diabetes Development
3. Calcitonin Enhanced Localization of Parathyroid Adenomas
4. Thyroid Nodule Database Study
5. Insulin Antibodies and Insulin Resistance

Current Basic/Translational projects include:

1. Characterization of a New Humanized Transgenic Model of Type 1 Diabetes
2. Experimental Immunotherapeutic Approaches for Treatment of Type 1 Diabetes
3. Thyroid Cancer Biomarkers
4. Tumor Immunity Studies

More specifically, our group's research has focused on trying to better understand mechanism of disease in endocrine autoimmunity (diabetes, autoimmune thyroid disease), with the ultimate goal of preventing its development or altering its course.

At the University of California-San Francisco, while studying human, MHC-class II restricted, beta-cell autoantigen presentation to autoreactive T-cells in vitro, my lab discovered a novel way of antigen specific T-cell inactivation. Human antigen-specific B cells and the antibodies they secrete, appear to modulate the autoimmune T-cell repertoire by down-regulating T-cell epitope presentation located in immunodominant areas. The B-cells studied were from human patients with diabetes. The antigen studied was a beta-cell human autoantigen clinically relevant in diabetes (Glutamic Acid Decarboxylase, GAD, 65 isoform). To study and confirm the observed phenomena in-vivo, an animal model, in which human beta-cell autoantigens are presented to effector T-cells in the context of human diabetes-susceptibility genes, needed to be developed.

Now in Ohio, our group has finally established such an animal model of antigen specific insulitis and diabetes. The group has developed for the first time, an animal model of antigen-specific insulitis and diabetes in double-transgenic mice carrying the HLA-DQ8 diabetes-susceptibility haplotype instead of mouse MHC-class II and expressing the human beta-cell autoantigen GAD65 in pancreatic beta cells. Having shown that inactivation of antigen-specific T-cells is feasible in vitro, we are continuing now with an animal model to study the same phenomenon in-vivo. The rational is that by restricting antigen presentation of immunodominant regions, we can inactivate antigen-specific T-cells and abrogate insulitis and diabetes in this model.

Our ultimate goal is to develop tools that precisely sequester the fuel (autoantigenic peptides) that sustains beta cell destruction (antigen/epitope-specific autoreactive T-cells) while preserving an immune system capable of defending the individual from foreign (germs) and self (tumors) insults. If feasible in humanized mice, the next goal would be to apply this approach to human diabetes. Furthermore, this approach would also provide proof of principle for therapeutic interventions in other autoimmune diseases.

In the thyroid field, our group has achieved progress in understanding pathogenesis of both Graves disease and Hashimoto thyroiditis. We have shown, for the first time, the direct interaction of human thyrotropin (TSH) receptor autoantibodies with the TSH receptor and determined their pathogenic potential in Graves disease. Also, we have dissected a unique inheritance pattern of autoimmunity to thyroid peroxidase (TPO) in Hashimoto thyroiditis. Furthermore, the group has identified a crucial mechanism of immune modulation were by B-cells (the antibody-making immune cells), working as antigen presenting cell, has the capacity of modifying the T-cell (the effector immune cell) response against TPO in Hashimoto thyroiditis. More recently, we became involved in the study of thyroid cancer. Using a comprehensive database analysis we have uncovered an unexpectedly high frequency of cytological Hashimoto's thyroiditis in otherwise normal individuals. Later on, the discovery that higher serum TSH (the earliest manifestation of Hashimoto’s) was associated with greater risks of thyroid cancer was the first hint that a link between the two conditions exists. Our group’s most recent work has now confirmed that euthyroid Hashimoto thyroiditis as opposed to hypothyroid Hashimoto’s, is a major risk factor for thyroid cancer. Moreover, the immune microenvironment accompanying thyroid cancer is now being defined.

A full publication list will be located on the Division webpage.
New Research Consortium

Hypertension Microbiome Consortium (HyMiCo):
A University of Toledo initiative to understand the impact of gut microbiota on high blood pressure.

The University of Toledo Center for Hypertension and Personalized Medicine announces the creation of the Hypertension Microbiome Consortium (HyMiCo), a research and academic multi-disciplinary collaborative effort that draws on strengths from investigators in various colleges within our institution. It is the main goal of HyMiCo to advance current knowledge on the functions of gut microbiota in hypertension and hypertension-related vascular and metabolic disorders through an agenda that includes basic/translational research and an educational program. For additional information and related links, visit the HyMiCo website.

New Funding Awards

Resp18 a novel genetic determinant of blood pressure regulation and renal function

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Member, Center for Hypertension and Personalized Medicine

Hypertension, also known as high blood pressure (BP), is a complex polygenic trait influenced by multiple genetic elements, of which the number, magnitude of effect and identities are largely unknown. Hypertension is a highly prevalent and significant risk factor for heart attack, stroke, and chronic kidney disease. Chronic kidney disease (CKD) is a leading cause of death in the United States with an estimated 31 million people (10% of the U.S. adult population) being affected. According to kidney statistical reports, 1 in 3 American adults are currently at risk for developing kidney disease. Marked ethnic variation in the familial aggregation of kidney disease exists, with high rates seen in African American, Native American, and Hispanic American families. This tells us that the likelihood of an individual developing CKD is determined by the interaction between complex genes/genetic components and environment.

Natural genetic heterogeneity among individuals impedes the identification of genes conferring a predisposition to progressive renal disease in humans. Investigation of animal models, through comparison of strains that are prone to develop CKD with those that are not, may aide identification of specific gene contributions. The most extensively studied experimental model of salt-sensitive hypertension is the Dahl salt sensitive (SS) rat. Dahl rats were developed by Dr. Lewis K. Dahl, and inbred by Dr. John Rapp to study the genetics of hypertension at our Institution and are currently studied extensively in our lab, directed by Dr. Bina Joe, Chair of the Department of Physiology and Pharmacology and Director of UT’s Hypertension and Personalized Medicine Center. SS rats are not only a model for hypertension studies but also serve as a model to study salt-induced CKD. The SS rat exhibits tubular dysfunction, which contributes to enhanced medullary vascular sensitivity and may predispose these animals to the development of hypertension. Studies in humans and animals have shown that a high salt intake can significantly increase BP and induce severe renal damage. However, the molecular basis of increased risk for developing high BP and renal damage remains largely unknown. Uncovering genetic factors that predispose individuals to progressive kidney diseases would lead to an improved understanding of the molecular basis of hypertension-induced renal pathology.

One of the genes that we previously identified as a candidate gene for the inheritance of hypertension and renal disease is Regulated Endocrine Specific Protein 18 (Resp18). Resp18 is an 18 kDa protein that was reportedly identified by screening a rat neurointermediate pituitary cDNA library for transcripts whose expression was regulated in parallel with the endogenous prohormone, Proopiomelanocortin, in response to dopaminergic agents. Studies with Resp18 suggest that it could be involved in the secretory pathway from lumen to the nucleus and its expression levels are modulated in response to dopaminergic agents. To further validate this gene as a bona fide candidate gene for BP regulation and the regulation of CKD, we utilized a cutting-edge ZFN based targeted gene disruption technology to develop a genetically engineered model with targeted disruption of the Resp18 gene on the genome of the SS rat. Our preliminary experimental evidence obtained through these Resp18 mutant rats which have increased BP, renal fibrosis and decreased mean life time compared to the wild-type SS rat, suggest that Resp18 is a modulator of these phenotypes.
To further understand the mechanistic aspects of Resp18 in salt induced BP and renal function, I was recently awarded a $231,000 3-year Scientist Development Grant from the American Heart Association (AHA). Similar to this project, during my training in Dr. Joe’s laboratory I was primarily involved in validating two other candidate genes A disintegrin-like metalloproteinase with thrombospondin motifs-16 (Adamts16) and Nuclear Receptor Subfamily 2, Group F, Member 2 (Nr2f2) for BP regulation. These results have been published (Proc Nat. Acad Sci, 2012 and Nat Commun, 2015). Successful completion of these studies on Resp18 will define a novel role for Resp18 and potentially delineate a unique mechanism of BP regulation and progression of CKD. Our findings may thus provide an avenue to contemplate Resp18 as a target for better clinical management of BP and renal disease.

Clinical Research Snippets

Anand B. Mutgi, MD
Sadik A. Khuder, PhD

Chronic kidney disease (CKD) affects more than 10% of the population in the United States. With increasing occurrence of Diabetes Mellitus (DM) and Hypertension (HTN), the CKD and consequent progression to dialysis is expected to increase. It will consume a significant portion of the Medicare budget. The few modifiable factors that reduce the incidence and progression of CKD include better control of DM and HTN. This month we review a study that examined the association of midlife fitness and reduced occurrence of chronic kidney disease, suggesting exercise as a modifiable factor in preventing CKD.

The data for this study originated from patient visits to the Cooper Clinic in Dallas, Texas, a preventive medicine practice established in 1970. The study cohort was comprised of 29,013 persons who had an exercise treadmill test between 1971 and 2009 and who could be matched with Medicare administrative claims data between 1999 and 2009. The final cohort involved 17,979 men and women at their midlife examination, in which greater than 90% presented for this examination more than 6 years before Medicare entry.

Higher levels of fitness at midlife were inversely associated with CKD incidence in later life after controlling for other risk factors including HTN, DM, nicotine use, cholesterol, and BMI. Hazard ratio (HR) or risk was reduced by 24% comparing moderate fitness to low level fitness and it was reduced by 34% comparing high fitness level to low a fitness level.

The precise role of fitness or mechanism of exercise, in either the prevention of CKD or on the delay of progression of CKD, is uncertain. In those with underlying CKD, there is evidence that fitness levels are lower, but in this cohort they started with similar renal function at baseline.

Previous animal studies have shown improvement in renal function with regular exercise over a two month period independent of systemic blood pressure. In another clinical observational study, regular physical exercise for more than 150 min per week was associated with the lowest rate of glomerular filtration rate loss. A very small scale (20 Patients) 12 month interventional study, showed no significant improvement but reduced mortality in CKD patients. Presently a somewhat large trial is currently underway.

We feel that this study demonstrates the positive effect of mid-life fitness on reducing the occurrence of CKD and if confirmed in a larger prospective study, it may be one of the simplest and effective measures to reduce the incidence and associated cost of CKD and Dialysis.

New Clinical Trials

A Phase 4 Study Evaluating the Effects of Pitavastatin to Prevent Cardiovascular Events in HIV-1 Infected Individuals (REPRIEVE).
Dr. Duggan - Medicine

A Clinical Study of Patients with Symptomatic Neurogenic Orthostatic Hypotension to Assess Sustained Effects of Droxidopa Therapy.

Dr. Elmer - Neurology

A Double Blind, Randomised, Placebo-Controlled Trial Evaluating Efficacy and Safety of Oral Nintedanib Treatment for at least 52 Weeks in Patients with 'Systemic Sclerosis associated Interstitial Lung Disease' (SSc-ILD).

Dr. Kahaleh - Medicine

IRB Corner

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