Platelet binding to leukocytes is a normal response to vascular damage and inflammation, however excessive platelet/leukocyte interactions have significant pathologic potential due to the ability of platelets to directly stimulate leukocytes. Formation of stable platelet/granulocyte aggregates (PGAs) induces neutrophils to release inflammatory cytokine/chemokines and damaging proteases, produce reactive oxygen species, and upregulate adhesion molecule and tissue factor expression. High levels of circulating PGAs are found in patients with diverse inflammatory vascular diseases, and animal models of vascular injury have shown benefits to limiting PGA formation in vivo. The complement system enhances PGA formation in human blood stimulated with thrombin receptor-activating peptide (TRAP), however precise mechanisms for the effects of complement are unknown. Here, we utilized ex vivo and in vitro flow cytometry assays to show that properdin, a positive regulator, and Factor H (FH), a negative regulator, of the alternative complement pathway (AP) have key roles in controlling the extent of TRAP-mediated PGA formation in human whole blood. Physiological properdin oligomers added exogenously to TRAP-stimulated whole blood enhance PGA formation and AP activity, while blocking endogenous properdin function with inhibitory antibodies limits PGA formation. Blocking C5a-C5a receptor interactions limits PGA formation to similar levels seen with inhibition of properdin and abrogates exogenous properdin-mediated increases in PGA formation. C5a directly enhances TRAP-mediated PGA formation and CD11b expression on granulocytes, indicating C5a is the key complement effector molecule for PGA formation. The effects of properdin on PGA formation, are tightly controlled by FH cell-surface protection. FH C-terminal domains 19-20 are key for regulating AP activity on the surface of platelets and neutrophils, and for controlling C5a generation at the platelet/granulocyte interface in TRAP-stimulated blood. Finally, mutations in domains 19-20 associated with atypical hemolytic uremic syndrome (aHUS) have differential effects on the ability of FH to control PGA formation and limit AP activity on platelets and neutrophils, suggesting that increased PGA formation contributes to thrombosis seen in patients with some, but not all, aHUS-related FH mutations. Our work has elucidated critical mechanisms governing PGA formation, and could potentially be used to develop novel therapeutics to limit thromboinflammation in inflammatory diseases.


A.Z. Blatt, G. Saggu, C. Cortes, V.P. Ferreira. Essential regulatory role for factor H and properdin in the formation of platelet/granulocyte aggregates in human whole blood. Published in Immunobiology.


A.Z. Blatt, G. Saggu, J.M. Thurman, C. Cortes, V.P. Ferreira. Role of factor H in controlling the formation of platelet/leukocyte aggregates. Graduate Research Forum and Midwest Graduate Research Symposium, Mar. 2015, University of Toledo, OH.

Adam will be returning to medical school to complete his final two years of the M.D./Ph.D. program.

AWARDS
National Institutes of Health Ruth L. Kirschtein National Research Service Award, 2016-2018
American Heart Association Predoctoral Fellowship – 2015-2016
( Relinquished ) American Association of Immunology Career in Immunology Trainee Fellowship - 2015
Earl H. Freimer, M.D. Endowed Scholarship for M.D./Ph.D. students – University of Toledo, 2015
Travel Award – 8th International Conference on Complement Therapeutics - Chania, Greece, 2015
Selected to Attend NIAID/IDSA Infectious Disease Research Careers Meeting – Bethesda, MD, 2015
Robert R. Buell Memorial Scholarship – University of Toledo, 2015
Travel Award – 25th International Complement Workshop – Rio de Janeiro, Brazil, 2014
First place oral presentation – Midwest Graduate Research Symposium - Toledo, OH, 2014