

ABSTRACT

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.

DISSERTATION COMMITTEE

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DISSERTATION PRESENTATION

Adam Blatt

June 6, 2016

Role of complement
regulatory proteins
properdin and Factor
H in platelet/
granulocyte aggregate
formation

Ph.D. in
Biomedical Sciences

PUBLICATIONS

A.Z. Blatt, G. Saggi, K.V. Kulkarni, C. Cortes, J.M. Thurman, D. Ricklin, J.D. Lambris, J.G. Valenzuela, V.P. Ferreira. Properdin-mediated C5a production enhances stable binding of platelets to granulocytes in human whole blood. Published online 25 April 2016. *J Immunol.* doi:10.4049/jimmunol.1600040.

Blatt, Adam, "Cardiovascular disease: immune system's battle with blood vessels." Toledo Blade. Mar. 2, 2015.

A.Z. Blatt, S. Pathan, V.P. Ferreira. Properdin: a tightly regulated critical inflammatory modulator. *Immunol. Rev.* (Under review).

A.Z. Blatt, G. Saggi, C. Cortes, A.P. Herbert, D. Kavanagh, D. Ricklin, J.D. Lambris, J.G. Valenzuela, V.P. Ferreira. Factor H C-terminal domains are critical for regulation of human platelet/granulocyte aggregate formation. (In preparation).

FUTURE PLANS

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

PRESENTED ABSTRACTS PUBLISHED IN JOURNALS

A.Z. Blatt, G. Saggi, C. Cortes, A.P. Herbert, D. Kavanagh, D. Ricklin, J.D. Lambris, J.G. Valenzuela, V.P. Ferreira. Role of factor H and effects of C-terminal mutations on control of human platelet/granulocyte aggregate formation. To be presented at the 16th International Congress of Immunology, Aug. 2016, Melbourne, Australia. To be published in *Immunobiology*.

A.Z. Blatt, G. Saggi, J.M. Thurman, C. Cortes, V.P. Ferreira. Critical role of the alternative pathway complement regulatory proteins properdin and factor H in platelet/leukocyte aggregate formation. 25th International Complement Workshop, Sep. 2014, Rio de Janeiro, Brazil. *Molecular Immunology*. 61(2): 247.

A.Z. Blatt, G. Saggi, C. Cortes, V.P. Ferreira. Essential regulatory role for factor H and properdin in the formation of platelet/granulocyte aggregates in human whole blood. 101st annual meeting of the American Association of Immunologists (AAI), May 2014, Pittsburgh, PA. *Journal of Immunology* 192 (1 supplement): 52.1.

AWARDS

National Institutes of Health Ruth L. Kirschstein 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

OTHER PRESENTED ABSTRACTS

A.Z. Blatt, G. Saggi, J.M. Thurman, C. Cortes, V.P. Ferreira. Critical role of alternative pathway complement regulatory proteins properdin and factor H in platelet/leukocyte aggregate formation. Infection, Immunity, and Transplantation/Medical Microbiology and Immunology Research Forum, Oct. 2015, Toledo, OH.

A.Z. Blatt, G. Saggi, C. Cortes, D. Ricklin, J.D. Lambris, J.M. Thurman, V.P. Ferreira. Differential roles for properdin and complement activity in the formation of platelet/granulocyte and platelet/monocyte aggregates. 8th International Conference on Complement Therapeutics, Sep. 2015, Chania, Greece.

A.Z. Blatt, H.N. Emch, K.V. Kulkarni, S.K. Pathan, M.M. Bickford, S.A. Moledina, C.M. DeAngelis, C. Cortes, J.M. Thurman, V.P. Ferreira. Characterization of anti-properdin monoclonal antibodies in their ability to recognize physiological forms of human properdin and inhibit their function. 8th International Conference on Complement Therapeutics, Sep. 2015, Chania, Greece.

A.Z. Blatt, G. Saggi, 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.

A.Z. Blatt, G. Saggi, C. Cortes, V.P. Ferreira. Essential regulatory role for factor H and properdin in the formation of platelet/granulocyte aggregates in human whole blood. Graduate Research Forum and Midwest Graduate Research Symposium, Mar. 2014, University of Toledo, OH.