ABSTRACT

The alternative pathway (AP) of the complement system can be activated excessively in several inflammatory diseases, particularly when there is a defect in regulatory components of the complement system. For instance, deficiency in glycosylphosphatidylinositol-anchored proteins, including complement regulators CD55 and CD59, leads to paroxysmal nocturnal hemoglobinuria (PNH), whereas defects of complement regulatory protein Factor H are associated with atypical hemolytic uremic syndrome (aHUS), both of which cause severe hemolysis and thrombotic pathologies. Despite Soliris being a breakthrough treatment for these two diseases, benefits from Soliris vary considerably among patients. Understanding the molecular mechanisms involved in complement regulation in these diseases is essential for developing new treatments. Properdin, the positive regulator of complement, is essential for complement amplification by stabilizing enzymatic convertases that are formed during complement activation. In this study, the role of properdin in red blood cell (RBC) lysis and endothelial cell opsonization in these human AP-mediated diseases was addressed by developing *in vitro* assays using PNH patient RBCs and human primary endothelial cells, where the effects of inhibition of properdin were compared to other complement inhibitors. In an *in vitro* PNH model using patient samples, inhibition of properdin completely prevented hemolysis on patient PNH type II and III RBCs, impairing the lysis significantly more than inhibition of other complement components, i.e., Factor B, C3, and C5 (>15-fold, >64-fold, or >12-fold lower molar IC_{50} values, respectively, $P<0.0001$-0.05). C5 inhibition by Soliris or OmCI was inefficient as the IC_{50} could not be determined for protection of most PNH patient RBCs from hemolysis (>667nM, >22-fold than the anti-properdin MoAbs IC_{50}). Inhibiting properdin was at least as, or more efficient than the other complement inhibitors when comparing target/inhibitor ratios. In addition, using *in vitro* endothelial cell assays, the data indicate, for the first time, a critical role for properdin in promoting complement activation on human endothelial cells exposed to heme (a hemolysis by-product) and rH19-20 (to inhibit Factor H cell-surface protection), which mimic "aHUS-like" conditions. Inhibiting properdin attenuated complement activation by ~40% on endothelial cells that were exposed to heme and serum, while abolishing complement activation on cells that were exposed to serum and rH19-20. Moreover, inhibition of properdin reduced complement activity by 75% on cells that were exposed to both heme and rH19-20 in the presence of serum. Altogether, the data indicate that properdin plays a key role in promoting RBC lysis and complement activation on human endothelial cells, contributing to the understanding of the pathogenesis of PNH and aHUS. Further studies aimed at determining the therapeutic value of inhibiting properdin in complement-mediated diseases, in particular those that are characterized by AP dysregulation, are warranted.

DISSERTATION COMMITTEE

Viviana P. Ferreira, D.V.M., Ph.D. (Mentor)
Ritu Chakravarti, Ph.D.
Kevin Pan, M.D., Ph.D.
Guillermo Vazquez, Ph.D.
Randall G. Worth, Ph.D.

Nikolai N. Modyanov, Ph.D., D.SC.,
Graduate School Representative

The University of Toledo College of Medicine and Life Sciences

Medical Microbiology and Immunology (MMI) Track

Department of Medical Microbiology & Immunology

THE UNIVERSITY OF TOLEDO
1872

DISERTATION PRESENTATION

Jin Chen

December 4, 2019

Role of Complement Regulatory Protein Properdin in Hemolytic Anemias Caused by Complement Dysregulation

Ph.D. in Biomedical Science

AWARDS

Second place poster presentation - UT Graduate Research Forum, Toledo, OH, 2019

Private Donation ($6000) from Dr. Peter White, UT Professor Emeritus, Toledo, OH, 2019

Travel Award - 27th International Complement Workshop - Santa Fe, NM, 2018

LEADERSHIP

Secretary, Council of Biomedical Graduate Students, UT, 2017-2018

International student representative, College of Medicine and Life Sciences, UT, 2016-2018

Treasurer, Council of Biomedical Graduate Students, UT, 2016-2017

FUTURE PLANS

Jin plans to accept a postdoctoral position at Cleveland Clinic to continue her research career.

PUBLICATIONS

J.Y. Chen, C. Cortes, V.P. Ferreira. 2018. Properdin: A multifaceted molecule involved in inflammation and diseases. *Molecular Immunology*, 2018, 102:58-72.

Jin Chen, "UT researchers aim for cure to genetic cell destruction." *Toledo Blade*. Dec. 3, 2018.

J.Y. Chen, N.S. Galwankar, H.N. Emch, C. Cortes, J.M. Thurman, S.A. Merrill, R.A. Brodsky, V.P. Ferreira. Properdin is a key player in lysis of red blood cells and complement activation on endothelial cells in hemolytic anemias caused by complement dysregulation. *Frontiers in Immunology.* (Under review).

PRESENTED ABSTRACTS PUBLISHED IN JOURNALS

J.Y. Chen, N.S. Galwankar, H.N. Emch, S.A. Merrill, R.A. Brodsky, V.P. Ferreira. "Properdin is a key player in lysis of red blood cells in aHUS and PNH." 27th international complement workshop, Oct. 2018, Santa Fe, NM. *Molecular Immunology*, 102: 139-140.

OTHER PRESENTED ABSTRACTS

J.Y. Chen, N.S. Galwankar, H.N. Emch, S.A. Merrill, R.A. Brodsky, V.P. Ferreira. Properdin inhibition prevents complement-mediated lysis of red blood cells that cannot regulate complement activation. *Graduate Research Forum*, Mar. 2019, Toledo, OH.

J.Y. Chen, N.S. Galwankar, H.N. Emch, S.A. Merrill, R.A. Brodsky, V.P. Ferreira. Properdin is a key player in lysis of red blood cells in aHUS and PNH. *Medical Microbiology and Immunology Research Forum*, Aug. 2018, Toledo, OH.

J.Y. Chen, N.S. Galwankar, S.A. Merrill, R.A. Brodsky, V.P. Ferreira. Antibodies against properdin prevent complement-mediated lysis of red blood cells from paroxysmal nocturnal hemoglobinuria patients. *Graduate Research Forum*, Mar. 2018, Toledo, OH.

J.Y. Chen, V.P. Ferreira. Complement is not involved in collagen- or vWF- mediated thrombi formation in human whole blood subjected to shear stress. *8th Midwest Graduate Research Symposium* and *Graduate Research Forum*, Mar. 2017, Toledo, OH.