ABSTRACT

Chagas disease is a chronic disabling disease caused by the protozoan *Trypanosoma cruzi*. There is no standardized treatment or preventative vaccine against Chagas disease. The complement system is an integral part of the immune system that comprises the classical, lectin and alternative pathways. The infective trypomastigote form of *T. cruzi* is highly resistant to killing by complement. Factor H (FH), an important negative regulator of the alternative pathway (AP) on cell surfaces and in blood, contains 20 short consensus repeat domains and binds to complement activation products (i.e., C3b/C3d) and select glycan markers on host cells, including sialic acid. The four N-terminal domains of FH inactivate the AP, while the other domains interact with C3b/d and glycan markers on cell surfaces. Various pathogens bind FH to inactivate the AP and evade complement. *T. cruzi* uses its trans-sialidase enzyme to transfer host sialic acids to its own surface, which could enable it to bind FH. Previous studies have shown that FH binds to complement-opsonized *T. cruzi* and that desialylation of the parasite surface increases complement-mediated lysis of *T. cruzi* trypomastigotes. However, the molecular basis and functional consequences of FH binding to *T. cruzi* are not known. Only trypomastigotes, but not epimastigotes (non-infective, complement susceptible) bound FH directly (i.e., independent of C3 deposition) in a dose-dependent manner. Although domain 20, the only known sialic acid-binding region in FH, was believed to be important for FH binding to *T. cruzi*, domain mapping experiments using 3-5 domain fragments of FH showed that domains 5-8 competitively inhibited FH binding to the trypomastigotes by ~30%, but did not decrease trypomastigote survival in complement. FH-Fc fusion proteins (3-4 contiguous FH domains fused to the IgG Fc) also did not kill trypomastigotes. FH-related protein-5 (FHR-5), whose domains bear significant sequence identity to all known polyanion-binding FH domains (6-7, 10-14, 19-20), fully inhibited FH binding to trypomastigotes. Concomitantly, FHR-5 reduced trypomastigote survival to < 24% in the presence of serum. In conclusion, we have elucidated the role of FH in complement resistance of trypomastigotes. These data provide the foundation for developing novel therapeutics and vaccine candidates against *T. cruzi*.

DISSERTATION COMMITTEE

Viviana P. Ferreira, D.V.M., Ph.D., Major Advisor

Ritu Chakravarti, Ph.D.

Z. Kevin Pan, M.D., Ph.D.

Stanislaw M Stepkowski, D.V.M., Ph.D., D.Sc.

Guillermo Vazquez, Ph.D., FAHA

Jyl Matson, Ph.D.

Graduate School Representative

R. Travis Taylor, Ph.D.

Substitute Committee Member

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

DISSERTATION PRESENTATION

Smrithi S. Menon

January 27, 2023

Mechanisms by which Factor H protects *Trypanosoma cruzi* from the alternative pathway of complement

Ph.D. in Biomedical Sciences

PUBLISHED ABSTRACTS

Menon SS, Ramirez-Toloza G, Wycoff KL, Shaughnessy J, Ram S, Ferreira VP. "Mechanisms by which Factor H protects *Trypanosoma cruzi* from the alternative pathway of complement. *The Journal of Immunology*. 2022, 208 (1 Supplement) 170.20. Published abstract (accepted for Immunology 2022 organized by American Association of Immunologists) in the special online supplement to *The Journal of Immunology*.

Menon SS, Galwankar N, Moore SR, Ferreira VP. Assessment of function of neutrophil-derived properdin in a novel functional assay. *The Journal of Immunology*. 2020, 204 (1 Supplement) 152.19. Published abstract (accepted for Immunology 2020 organized by American Association of Immunologists) in the special online supplement to *The Journal of Immunology*.

PRESENTED ABSTRACTS

Menon SS, Galwankar N, Moore SR, Khuder AS and Ferreira VP. Functional evaluation of neutrophil-derived properdin in a novel functional assay. Poster presentation for 2021 Graduate Research Forum, The University of Toledo.

Menon SS, Ehinger S, Galwankar NS, Ferreira VP. Assessment of function of neutrophil-derived properdin: identification of a novel form of complement regulation? Poster presentation for 2019 Graduate Research Forum, The University of Toledo.

Galwankar NS, **Menon SS**, Ehinger S, Emch HN, Ferreira VP. Novel assay measuring properdin function gives insights into functional differences between sera- and neutrophil-derived properdin. Poster presentation for the 27th International Complement workshop, 2018, Santa Fe, New Mexico.

PUBLICATIONS

Menon SS, Ramirez-Toloza G, Wycoff KL., Ehinger S. Shaughnessy J, Ram S., Ferreira VP "Mechanisms by which Factor H protects *Trypanosoma cruzi* from the alternative pathway of complement." (Submitted to *Frontiers in Immunol*ogy).

Moore SR*, **Menon SS***, Galwankar N, Khuder SA, Pangburn MK, Ferreira VP. ***Both authors contributed equally**. "A Novel Assay that Characterizes Properdin Function Shows Neutrophil-Derived Properdin has a Distinct Oligomeric Distribution." *Frontiers in Immunol*ogy. 2023; 13:918856.

Ramirez-Toloza G, Aguilar-Guzman L, Valck C, **Menon SS**, Ferreira VP, Ferreira A. "Is It Possible to Intervene in the Capacity of *Trypanosoma cruzi* to Elicit and Evade the Complement System? *Frontiers in Immunol*ogy. 2021; 12:789145.

Menon SS, "UT research plot how to keep our immune system from turning on itself." Toledo Blade. September 6, 2021.

Moore SR*, **Menon SS***, Cortes C, Ferreira VP. ***Both authors contributed equally**. "Hijacking Factor H for Complement Immune Evasion." *Frontiers in Immunol*ogy. 2021; 12:602277.

Chen JY, Galwankar NS, Emch HN, **Menon SS**, Cortes C, Thurman JM, Merrill SA, Brodsky RA, Ferreira VP. "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*. 2020; 11:1460.

AWARDS

American Association of Immunologists Careers in Immunology Fellowship, 2019.

Robert N. Whiteford Memorial Scholarship award, 2020.

Second place poster presentation at UT Graduate Research Forum, 2021.

Travel award for poster presentation at the American Association of Immunologists annual meeting, Immunology 2022, as a Careers in Immunology Fellowship trainee recipient, 2022.

FUTURE PLANS

Smrithi has accepted a post-doctoral position at St. Jude's Children's Research Hospital.