Doctoral Dissertation Defense Announcement

Melissa L. Whyte

“Alterations to the surface glycoproteome of T cells during HHV-6A infection”

Candidate for Doctor of Philosophy in Microbiology & Immunology
Graduate School of Biomedical Sciences
Medical College of Wisconsin

Committee in Charge
Amy W. Hudson, PhD (Advisor)
Scott Terhune, PhD
Ravit Boger, MD
Subramaniam Malarkannan, PhD
Carol Williams, PhD

Thursday, July 7th, 2022 at 1 pm (CST)

Hybrid defense
Room: Alumni Center
Live Public Viewing: https://mcw-edu.zoom.us/j/94865677710?pwd=bkJOWIJK0pPcVJORWIQVrLzBmZz09
Graduate Studies:

Cellular Microbiology

Cellular & Molecular Immunology

Graduate Student Seminar

Advanced Protein Chemistry

Ethics & Integrity in Science

Research Ethics Discussion Series

Mechanisms of Cellular Signaling

Classical & Molecular Genetics

Biochemistry

Techniques in Molecular & Cellular Biology

Molecular & Cellular Biology
Abstract

Like all herpesviruses, the roseoloviruses (HHV6A, -6B, and -7) establish lifelong infection within their host, requiring these viruses to evade host antiviral responses. One common host-evasion strategy is the downregulation of host-encoded, surface-expressed glycoproteins. Roseoloviruses have been shown to evade the host immune response by downregulating NK-activating ligands, class I MHC, and the TCR/CD3 complex. To more globally identify glycoproteins that are differentially expressed on the surface of roseolovirus-infected cells, we performed cell surface capture of N-linked glycoproteins present on the surface of T cells infected with HHV6A, and compared these to proteins present on the surface of uninfected T cells.

We found that the protein tyrosine phosphatase CD45 is downregulated in T cells infected with HHV6A. We also demonstrated that CD45 is similarly downregulated in cells infected with HHV7. CD45 is essential for signaling through the T cell receptor and, as such, is necessary for developing a fully functional immune response. Interestingly, the closely related β-herpesviruses human cytomegalovirus (HCMV) and murine cytomegalovirus (MCMV) have also separately evolved unique mechanisms to target CD45. However, while HCMV and MCMV target CD45 signaling and trafficking, HHV6A acts to downregulate CD45 transcripts. Because CD45 is targeted by a unique mechanism in HHV6A-infected cells, we hypothesized that the viral gene product(s) mediating this downregulation, would be unique to the roseoloviruses. To identify the viral gene product(s) responsible for the downregulation of CD45 in HHV6A-infected T cells, we infected T cells with a mutant HHV6A virus lacking open reading frames (ORFs) U21-U24 (ΔU21-U24 HHV6A) and examined CD45 expression. The U21-U24 locus contains a series of ORFs unique to roseoloviruses, some of which have been shown to downregulate surface-expressed proteins. Unlike cells infected with wild-type HHV6A (wtHHV6A), ΔU21-U24 HHV6A-infected cells displayed CD45 expression close to that of non-infected control T cells, suggesting an ORF encoded within the U21-U24 locus is required for the downregulation of CD45 during HHV6A infection. To determine which individual ORF is required for HHV6A to downregulate CD45, we created a series of mutant viruses, each with a stop-mutation introduced in place of the initiating methionine for a single ORF encoded within the U21-U24 locus. We infected cells with these stop-mutant viruses and again examined CD45 expression. We found that cells infected with U24-stop HHV6A had levels of CD45 expression identical to those of ΔU21-U24 HHV6A, implicating U24 as the viral ORF responsible for the downregulation of CD45 during HHV6A infection.

Our surface glycoproteome screen also revealed several perturbations to the purinergic signaling pathway during HHV6A infection. In purinergic signaling, ATP is released into the extracellular space (eATP), often during cell death, where it acts as a signaling molecule by binding to surface-expressed P2 receptors. Purinergic signaling is an important mediator of the inflammatory response, with eATP acting as a ‘danger signal’ drawing immune cells to the site of dead or dying cells. CD39 is a surface-expressed ectonucleotidase that converts eATP into ADP and AMP, and as such, expression of CD39 acts to decrease the inflammatory response by decreasing the local concentration of eATP. We found that during HHV6A infection, there is a decrease in surface-expressed CD39 and a corresponding increase in the concentration of eATP. However, CD39 is not downregulated in cells infected with ΔU21-U24 HHV6A, suggesting the downregulation of CD39 is caused the virus, and not a cellular response to viral infection. We also found that the P2X4 receptor is upregulated on the surface of HHV6A-infected cells. P2X4 binds eATP resulting in an influx of calcium. Calcium is often utilized by viruses to promote infection and as such, these alterations to the purinergic signaling pathway may be a means to increase intracellular calcium for the benefit of the HHV6A virus. We have also shown that ectopically-expressed CD39 is not downregulated during wtHHV6A infection. Surprisingly, wtHHV6A-infected CD39-overexpressing T cells have increased rates of cell death, suggesting that the downregulation of CD39 during HHV6A infection may aid in prolonged survival of host cells.
MELISSA L. WHYTE
Curriculum Vitae, July 2022

Education
2016-present  Medical College of Wisconsin, Microbiology & Immunology. Milwaukee, WI. PhD in Biomedical Sciences. Anticipated July 2022.
2015-2016  University of Wisconsin – Milwaukee, Biological Sciences. Milwaukee, WI. M.S. in Biological Sciences
2010-2014  University of Wisconsin – Milwaukee, Microbiology. Milwaukee, WI. B.S. in Microbiology

Research Experience
2017-present  Dr. Amy Hudson, Medical College of Wisconsin (MCW)
            Alterations to the surface glycoproteome of T cells during human herpesvirus-6A infection.
            Human herpesvirus-6A infection of human salivary gland-derived epithelial cells.
2014-2016  Dr. Charles Wimpee, University of Wisconsin – Milwaukee
            A highly divergent bioluminescent bacterium suggests a new species in the genus Aliivibrio.
            Lateral replacement of the lux operon in a Vibrio isolated from the intestine of a coral reef fish.

Peer Reviewed Publications
The Roseoloviruses Downregulate the Protein Tyrosine Phosphatase PTPRC (CD45).

HHV-7 U21 exploits Golgi quality control carriers to reroute class I MHC molecules to lysosomes.

Meetings and Presentations
2021  Medical College of Wisconsin. Salivary gland organoids as a model for roseolovirus infection. Research in Progress Talk.
Meetings and Presentations (continued)


2021 MCW Graduate School Poster Session. Salivary gland organoids as a model for roseolovirus infection. Poster Presentation.

2020 Medical College of Wisconsin. Alterations to the surface glycoproteome of HHV6A-infected T cells. Research in Progress Talk.

2019 International Herpesvirus Workshop (IHW). Knoxville, TN. Downregulation of the protein tyrosine phosphatase CD45 in Roseolovirus-infected T cells. Oral and Poster Presentations.

2019 Medical College of Wisconsin. Downregulation of the protein tyrosine phosphatase CD45 in Roseolovirus-infected T cells. Research in Progress Talk.

2019 MCW Graduate School Poster Session. Downregulation of the protein tyrosine phosphatase CD45 in Roseolovirus-infected T cells. Poster Presentation.

2019 MCW Graduate Student Symposium. Downregulation of the protein tyrosine phosphatase CD45 in Roseolovirus-infected T cells. Poster Presentation.

2018 Blood Research Institute Immunology Scientific Retreat. Downregulation of the protein tyrosine phosphatase CD45 in Roseolovirus-infected T cells. Poster Presentation.

2018 MCW Graduate School Poster Session. Downregulation of the protein tyrosine phosphatase CD45 in Roseolovirus-infected T cells. Poster Presentation.

2018 Medical College of Wisconsin. Downregulation of the protein tyrosine phosphatase CD45 in HHV6A-infected T cells. Research in Progress Talk.

2017 MCW Graduate Student Welcome Poster Session. Identification of cellular proteins involved trafficking class I MHC to lysosomes in cells expressing the viral protein U21. Poster Presentation.


Teaching and Mentorship

Guest Lectures Delivered

2019  Cellular Microbiology
2019  Cellular Microbiology
2018  Cellular & Molecular Immunology
2017  Advanced Protein Chemistry

Teaching Assistant Courses

2016  UW-Milwaukee. Foundations of Biological Sciences II.
2015  UW-Milwaukee. Lab Techniques in Molecular Biology.
2015  UW-Milwaukee. Foundations of Biological Sciences II.

Mentorship

2018  1st year graduate student coach. Bailey Sarka. MCW.
2018  1st year graduate student coach. Bryce Podgorsek. MCW.
2018  Mentor. Anna Bower. MCW. High school student summer volunteer.

Professional development

2019-present  Member. American Heart Association.
2014-present  Member. Milwaukee Microbiology Society.
2022  Attendee. Freedom of Expression and Civility in the Laboratory. Responsible Conduct in Research (RCR) Training.
2021  Attendee. Research and the Role of Mentoring and Character. RCR Training.
2020  Attendee. Conflict of Interest. RCR Training.
2020  Attendee. Responsible authorship and publication. RCR Training.
2013-2019  Member. American Society for Microbiology.
2018  Attendee. Scientific Writing Workshop/Course.
2017  Attendee. Peer Review. RCR Training.
Leadership and Service

Committee service

2021-present  **Member.** Microbiology & Immunology Seminar Committee.
2019  **Microbiology & Immunology Department Representative.**
      Graduate Student Association (GSA).
2019  **Chair.** GSA Community Outreach Committee.
2019  **Member.** GSA Graduate Student Symposium Committee.
2019  **Member.** GSA Social Activities Committee. Member.
2019  **Member.** GSA Food Service Committee. Member.
2017-2018  **Chair.** Committee for Enhancing Scholarly Culture.
2016-2019  **Member.** Committee for Enhancing Scholarly Culture.

Community Service Activities

2020  **Judge.** University School of Milwaukee Regional Science Fair.
2019-2020  **Director.** Wauwatosa STEM (WSTEM) Science Fair.
2018  **Project Mentor.** WSTEM Science Fair.
2017-2018  **Judge.** Milwaukee Public Schools STEM Fair.
2017  **Judge.** WSTEM Science Fair.

Honors and Awards

2021  MCW Paper of the Season Award. Fall
2019  Women in Science Award.
2019  MCW GSA Travel Award.
2017  AAAS Program for Excellence in Science.
2015  American Society for Microbiology Student Travel Grant Award.
2014  Student Success Award.
2014  B.S. awarded *Summa Cum Laude* with honors in major (UW-Milwaukee)
2012-2014  Dean’s list, 4 semesters.
2013  Target MD Scholarship.