MCW
GRADUATE SCHOOL

Doctoral Dissertation Defense Announcement

"Metabolism supports CD8 T cell differentiation in chronic inflammation"

Ashley K. Brown

Candidate for Doctor of Philosophy

Microbiology & Immunology

School of Graduate Studies

Medical College of Wisconsin

Committee in Charge:

Weiguo Cui, PhD (Mentor)

Yi-Guang Chen, PhD

Michelle Riehle, PhD

John Corbett, PhD

Joseph Barbieri, PhD

Date: Monday, April 29, 2024

Time: 1:30 PM (CST)

Defense Location: VBRI Seminar Room

Zoom: contact asbrown2@mcw.edu for zoom link

Graduate Studies:

Reading and Research

Doctoral Dissertation

Ethics & Integrity in Science

Research Ethics Discussion Series

Techniques in Molecular & Cell Biology

Foundations in Biomedical Science III

Microbiology & Immunology Seminar Course

Immunological Tolerance

Dissertation

“Metabolism supports CD8 T cell differentiation in chronic inflammation”

CD8 T cells are a fundamental component of the immune system providing protection against intracellular pathogens, transformed cells, and self-antigens. In response to chronic inflammatory conditions, CD8 T cells experience persistent antigen stimulation and differentiate into exhausted cells that are generally characterized by reduced cytolytic capacity and loss of cytokine production. However, recent studies have shown that exhausted CD8 T cells are functionally heterogenous, consisting of a self-renewing progenitor population that gives rise to potent cytolytic effector and terminally exhausted cells under the control of distinct transcriptional and epigenetic programming. Emerging evidence also shows that this differentiation process is further supported by metabolic reprogramming to meet the bioenergetic demands of the distinct subsets of exhausted CD8 T cells. However, the mechanisms by which metabolism is regulated in exhausted cell differentiation is not well understood. Specifically, questions remain regarding how metabolism may support the differentiation of the cytolytic effector subset for control of infection and cancer and persistence of autoimmunity.

We first characterized the metabolic heterogeneity of the progenitor, effector, and exhausted CD8 T cell subsets in chronic infection by utilizing the Compass algorithm, which provides metabolic state predictions based on single-cell RNA sequencing (scRNA-seq) data and flux-based analysis. We applied this algorithm to an integrated scRNA-Seq dataset of virus-specific CD8 T cells in late chronic viral infection. Our Compass analysis, in combination with gene set enrichment analysis (GSEA), revealed metabolic programs distinct to each exhausted subset. Specifically, oxidative phosphorylation, glycolysis, and glutamine metabolism were more active in effector cells. Recent work in chronic viral infection shows that the differentiation of progenitor to effector CD8 T cells depends on interleukin (IL)-21- producing CD4 T cells. Therefore, we hypothesized that a pathway downstream of IL-21 signaling may support the observed metabolic programing of effector CD8 T cell differentiation in chronic viral infection. One candidate is the PIM kinase family, specifically PIM1 kinase, which functions downstream of JAK-STAT and IL-21 signaling, displays high gene expression in the effector CD8 T cell subset, and is a known regulator of cellular energy metabolism. Using the LCMV Clone 13 model of chronic viral infection, we showed that CD8 T cell specific deletion of PIM1 kinase impairs the differentiation and cytolytic function of late effector CD8 T cells. Furthermore, deficiency in PIM1 kinase reduced oxidative and glycolytic metabolism, potentially contributing to the diminished effector differentiation and function. Overall, these data revealed not only the metabolic heterogeneity of exhausted CD8 T cells, but also how metabolic regulation through the IL-21-PIM1 axis impacts CD8 T cell differentiation.

In contrast to chronic infection, autoimmune conditions such as type 1 diabetes (T1D) result from the destruction of tissues by T cells. Therefore, treatments aim to attenuate CD8 T cell differentiation and cytolytic function. Similar to chronic viral infection, the non-obese diabetic (NOD) mouse model requires IL-21 production from CD4 T cells to support the sustained CD8 T cell effector functions for pancreatic β-cell destruction and T1D development. Therefore, we hypothesized that the IL-21-PIM kinase axis could be targeted to decrease effector metabolism and cytolytic function to prevent diabetes onset. Analogous to chronic viral infection, autoreactive CD8 T cells are heterogenous and can be characterized by a self-renewing progenitor TCF-1^{+} population that gives rise to a

$CXCR6^+$ terminal effector population. Analysis of scRNA-sequencing data of CD8 T cells reactive to the islet autoantigen islet-specific glucose-6-phosphatase catalytic subunit-related protein $(IGRP)_{206\text{-}214}$ showed metabolic heterogeneity between progenitor and effector cells, with effector cells displaying high *Pim1* expression. Pharmacological inhibition of PIM kinase with AZD1208, a pan-PIM kinase inhibitor, significantly delayed T1D incidence in the NOD mouse model. Therefore, these data suggest that the IL-21-PIM kinase axis supports CD8 T cells effector function in T1D pathogenesis.

Overall, the work presented in this dissertation characterizes the metabolic heterogeneity of CD8 T cells in both chronic viral infection and T1D systems. In addition, PIM1 kinase may function in both systems to support effector CD8 T cell differentiation, with this function resulting in viral control in chronic infection or pancreatic β-cell destruction in T1D. Taken together, these findings may provide new pathways to target in the design of novel immunotherapies for chronic infections or T1D.

Ashley K. Brown
Curriculum Vitae
asbrown2@mcw.edu

EDUCATION

Medical College of Wisconsin
MD, expected May 2026
PhD, Immunology, expected May 2024

Boston College
Morrissey College of Arts and Sciences Biology Graduate Program
Biology
Master of Science, May 2016

Boston College
Morrissey College of Arts and Sciences Honors Program
Biology and Classics
Bachelor of Science, Cum Laude, May 2014

RESERCH EXPERINCE

PIM kinases are essential for CD8 T cell effector function and metabolism in chronic viral infection and type 1 diabetes
Medical College of Wisconsin
PhD Candidate
Mentor: Weiguo Cui, PhD
February 2021 – present

Developing a standardized battery of clinically relevant resilience challenges and outcome measures to assess healthy aging
Mayo Clinic**,** Robert & Arlene Kogod Center on Aging
Senior Research Technician
Mentor: Nathan LeBrasseur, PhD
June 2016 – 2018

Investigated the effects of energy substrates and LPS-activation on the in-vitro energy metabolism of tumorigenic and non-tumorigenic cells
Boston College**,** Biology Department
Master's Student
Mentor: Thomas Seyfried, PhD
June 2014 – May 2016

Etomoxir induced triglyceride accumulation and reduced energy production in murine glioblastoma cells
Boston College, Biology Department
Undergraduate Student
Mentor: Thomas Seyfried, PhD
August 2012 – May 2014

Protein and lipid oxidative damage in breast cancer
The Hormel Institute, Nutrition and Metabolism
Summer Undergraduate Research Experience Intern
Mentor: Margot Cleary, PhD
June 2012 – May 2012

ADDITIONAL PROFESSIONAL EXPERIENCE

Assessment of a novel glutaminolysis inhibitor and restricted ketogenic diet in a pre-clinical metastatic cancer model
Collaboration between Agios Pharmaceuticals and Dr. Thomas Seyfried Laboratory
Boston College
August 2014 – August 2015

GRANTS

F30-DK132807-01A1 (April 2023-present)
"Mechanisms by which PIM kinase modulates the effector function of autoreactive CD8 T cells in type 1 diabetes"

PROFESSIONAL MEMBERSHIP

American Association of Immunology (AAI) Trainee (2022-present)

PUBLICATIONS

1. **Brown AK**, Mazula DL, Roberts L, Roos C, Zhang B, Pearsall VM, Schafer MJ, White TA, Huang R, Kumar N, Miller JD, Miller RA, LeBrasseur NK. Physical Resilience as a Predictor of Lifespan and Late-Life Health in Genetically Heterogeneous Mice. J Gerontol A Biol Sci Med Sci. 2024 Jan 1;79(1):glad207. doi: 10.1093/gerona/glad207. PMID: 37701988; PMCID: PMC10733175.
2. Kasmani MY, Topchyan P, **Brown AK**, Brown RJ, Wu X, Chen Y, Khatun A, Alson D, Wu Y, Burns R, Lin CW, Kudek MR, Sun J, Cui W. A spatial sequencing atlas of age-induced changes in the lung during influenza infection. Nat Commun. 2023 Oct 18;14(1):6597. doi: 10.1038/s41467-023-42021-y. PMID: 37852965; PMCID: PMC10584893.
3. Topchyan P, Zander R, Kasmani MY, Nguyen C, **Brown A**, Lin S, Burns R, Cui W. Spatial transcriptomics demonstrates the role of CD4 T cells in effector CD8 T cell differentiation during chronic viral infection. Cell Rep. 2022 Nov 29;41(9):111736. doi: 10.1016/j.celrep.2022.111736. PMID: 36450262; PMCID: PMC9792173.
4. Kasmani MY, Ciecko AE, **Brown AK**, Petrova G, Gorski J, Chen YG, Cui W. Autoreactive CD8 T cells in NOD mice exhibit phenotypic heterogeneity but restricted TCR gene usage. Life Sci Alliance. 2022 Jun 6;5(10):e202201503. doi: 10.26508/lsa.202201503. PMID: 35667687; PMCID: PMC9170949.
5. Volberding PJ, Xin G, Kasmani MY, Khatun A, **Brown AK**, Nguyen C, Stancill JS, Martinez E, Corbett JA, Cui W. Suppressive neutrophils require PIM1 for metabolic fitness and survival during chronic viral infection. Cell Rep. 2021 May 25;35(8):109160. doi: 10.1016/j.celrep.2021.109160. PMID: 34038722; PMCID: PMC8182757.
6. Schafer MJ, Zhang X, Kumar A, Atkinson EJ, Zhu Y, Jachim S, Mazula DL, **Brown AK**, Berning M, Aversa Z, Kotajarvi B, Bruce CJ, Greason KL, Suri RM, Tracy RP, Cummings SR, White TA, LeBrasseur NK. The senescence-associated secretome as an indicator of age and medical risk. JCI Insight. 2020 Jun 18;5(12):e133668. doi: 10.1172/jci.insight.133668. PMID: 32554926; PMCID: PMC7406245.
7. Schafer MJ, Mazula DL, **Brown AK**, White TA, Atkinson E, Pearsall VM, Aversa Z, Verzosa GC, Smith LA, Matveyenko A, Miller JD, LeBrasseur NK. Late-life time-restricted feeding and exercise differentially alter healthspan in obesity. Aging Cell. 2019 Aug;18(4):e12966. doi: 10.1111/acel.12966. Epub 2019 May 21. PMID: 31111669; PMCID: PMC6612646.
8. Flores RE, **Brown AK**, Taus L, Khoury J, Glover F, Kami K, Sarangarajan R, Walshe TE, Narain NR, Kiebish MA, Shelton LM, Chinopoulos C, Seyfried TN. Mycoplasma infection and hypoxia initiate succinate accumulation and release in the VM-M3 cancer cells. Biochim Biophys Acta Bioenerg. 2018 Sep;1859(9):975-983. doi: 10.1016/j.bbabio.2018.03.012. Epub 2018 Mar 23. PMID: 29580805.

AWARDS AND HONORS

2023	Karen Evangelista Student Humanitarian Award
2023	MCW Travel Award
2022	Medical College of Wisconsin (MCW) Center for Immunology Growth and Research in Immunology Training (GRIT) Award
2022	MCW Center for Immunology Travel Award
2018	Best Poster Presentation Mayo Clinic Young Investigators Research Symposium
2015	Outstanding Poster Presentation Award, SACNAS East Coast Regional Meeting
2013 – 2014	Boston College Biology Honors Program
2013	Undergraduate Research Fellowship Recipient
2013	ACC-IAC Thesis Research Advanced Study Grant Recipient
2010 – 2014	Boston College Morrissey College of Arts and Sciences Honors Program

POSTER PRESENTATIONS

Brown AK, Shen J, Volberding PJ, Cui W. PIM kinases are essential for CD8 T cell effector function and metabolism during chronic viral infection. Autumn Immunology Conference. Chicago, IL. November 17 – 20, 2023.

Brown AK, Shen J, Volberding PJ, Cui W. PIM kinases are essential for CD8 T cell effector function and metabolism during chronic viral infection. 38th Annual MD/PhD National Student Conference. Copper Mountain, CO. July 7-9, 2023.

Brown AK, Shen J, Volberding PJ, Cui W. PIM kinases are essential for CD8 T cell effector function and metabolism during chronic viral infection. Immunology 2023. Washington, DC. May 11-15, 2023.

Brown AK, Shen J, Volberding PJ, Cui W. PIM kinases are essential for CD8 T cell effector function and metabolism during chronic viral infection. Autumn Immunology Conference. Chicago, IL. November 18 – 21, 2022.

Brown AK, Shen J, Volberding PJ, Cui W. PIM kinases are essential for CD8 T cell effector function and metabolism during chronic viral infection. The Center for Immunology 12th Annual Immunology Scientific Retreat. Milwaukee, WI. June 9, 2022

Brown AK, Mazula DL, Zhang B, Roos CM, White TA, Miller RA, Miller JD,LeBrasseur NK. Physical Resilience as a Determinant of Healthy Aging. 9th Annual Robert and Arlene Kogod Center on Aging Conference. Rochester, MN. October 4-6, 2018

Brown AK, Mazula DL, Zhang B, Roos CM, White TA, Miller RA, Miller JD,LeBrasseur NK. Physical Resilience as a Determinant of Healthy Aging. Experimental Biology. San Diego, CA. April 21-25, 2018

Brown AK, Martínez-Gálvez G, Coffman KE, Matchett WE, and Horazdovsky BF. Twin Talk Series: A novel method to foster peer-to-peer pedagogical training. Experimental Biology. San Diego, CA. April 21-25, 2018

Brown AK, Mazula DL, Zhang B, Roos CM, White TA, Miller RA, Miller JD,LeBrasseur NK. Physical Resilience as a Determinant of Healthy Aging. Mayo Clinic Young Investigators Research Symposium. Rochester, MN. March 23-24, 2018.

Brown AK, Ta L, Flores R, Seyfried TN. Etomoxir Induced Triglyceride Accumulation and Reduced Energy Production in Murine Glioblastoma Cells. SACNAS East Coast Regional Meeting. Chestnut Hill, MA. March 28, 2015.

ORAL PRESENTATIONS

Brown AK, Shen J, Volberding PJ, Cui W. PIM kinases are essential for CD8 T cell effector function and metabolism during chronic viral infection. Autumn Immunology Conference. Chicago, IL. November 17 – 20, 2023.

Brown AK, Shen J, Volberding PJ, Cui W. PIM kinases are essential for CD8 T cell effector function and metabolism during chronic viral infection. Autumn Immunology Conference. Chicago, IL. November 18 – 21, 2022.

INVITED TALKS

51st Annual American Aging Association Meeting
Physical Resilience as a Predictor of Lifespan and Late-Life Health in Genetically Heterogenous Mice
Oklahoma City, OK. June 8-11, 2023

9th Annual Robert and Arlene Kogod Center on Aging Conference
Physical Resilience as a Determinant of Healthy Aging
Rochester, MN. October 4-6, 2018

LEADERSHIP AND COMMUNITY SERVICE

2021 – 2022	MCW Graduate Student Association (GSA) MSTP Representative
2022	MCW 5th Annual GSA Symposium Steering Committee Member
2018 – 2019	Greater Milwaukee Free Clinic (GMFC)
2018 – 2019	MCW Saturday Clinic For the Uninsured
2018 – Present	Mayo Clinic Education Technology Forum Convener
2018 – Present	MCW Memory Arts Program Leader
2018 – Present	MCW American Geriatrics Society (AGS) Treasurer and Co-president
2018 – 2019	Medical Scientist Training Program M1 Student Council Representative
2017 – 2018	Mayo Clinic SACNAS Social Media Chair
2016 – 2018	Mayo Clinic Young Investigators Research Symposium (YIRS) Steering Committee Member and Marketing and Media Chair
2016 – 2018	Mayo Clinic Pedagogy Interest Group Executive Board Member and President
2015 – 2016	Boston College Society for Advancement of Chicanos/Hispanics and Native Americans in Science (SACNAS) Founding Executive Board Member and Treasurer