

ABSTRACT

Harmful algal blooms are on the rise globally and pose serious health concerns due to the release of cyanotoxins, which are harmful to humans and the environment. Microcystin-LR (MC-LR) is one of the most frequently produced cyanotoxins and has recently been detected in aerosols generated by the normal motions of affected bodies of water. However, the human pulmonary health effects of MC-LR aerosols remain largely unknown. It has been previously observed that MC-LR exposure has a pro-inflammatory influence on the airways of mice, however this has yet to be observed in the context of aerosol exposure. Additionally, it is unknown whether this is a concern for human airways as the potential inflammation and associated downstream effects have yet to be thoroughly characterized. Therefore, we set out to address these knowledge gaps.

Herein we describe MC-LR mediated pro-inflammatory signaling in a 3D model of primary human airway epithelium. We further characterize this inflammation utilizing inherent biological variation in a strain and sex comparison of a murine model of MC-LR aerosol inhalation. In which, we found an upregulation of type 1 and type 17 immunity characterized by neutrophilic inflammation. Next, we addressed the risk for patients with the pre-existing airway inflammatory disease, neutrophilic asthma. In both an in vitro and in vivo model, asthma-related pro-inflammatory signaling was further upregulated after MC-LR exposure. Finally, we propose a novel mechanistic explanation for the pro-inflammatory responses which involves the amplification of NF- κ B activity through the inhibition of protein phosphatase 1 (PP1) and 2A (PP2A).



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DISSERTATION
PRESENTATION

by

**Joshua D.
Breidenbach**

February 16th, 2023

**Harmful Algal Bloom
Toxin Aerosol Exposure
and Airway Inflammation**

**Ph.D. in Biomedical
Sciences**

AWARDS/ LEADERSHIP

- 2022 Recipient of the “Best Student Presentation” at the Fourth International Scientific Conference of Al-Kafeel University
- 2022 Recipient of the MMI Graduate Student Research Scholar Award
- 2021 Recipient of a trainee award at the Central Society for Clinical and Translational Research—Midwest Clinical and Translational Research Meeting
- 2020 Recipient of a trainee award at the Central Society for Clinical and Translational Research—Midwest Clinical and Translational Research Meeting
- 2022 Second place for Poster presentation at the Graduate Research Forum (GRF) 2022, University of Toledo, College of Medicine and Life Sciences
- 2021 Breidenbach, J. 2021. UT research investigates how inhaling cyanotoxins affect our health. Toledo Blade.
- 2021 First place for oral presentation at the Graduate Research Forum (GRF) 2021, University of Toledo, College of Medicine and Life Sciences
- 2019 Vice President for the Council of Biomedical Graduate Students (CBGS) for the year 2018-2019, at the University of Toledo.

ACADEMIC RESEARCH SUPPORT

Title: Inhalation Exposure to Aerosolized Cyanotoxins Worsens Pre-Existing Asthma via Activation of Granulocytic Inflammation

Agency: NIH – NHBLI - PA-21-051 - Ruth L. Kirschstein National Research Service Award (NRSA) Individual Predoctoral Fellowship (Parent F31)

Period: 7/01/2021 - 6/30/2024

Direct Costs: \$227,600 (total)

PI: **Breidenbach JD** (Haller ST Sponsor/Kennedy DJ Co-sponsor)

Role: The overall goal of this project is to test the hypothesis that inhalation exposure to the harmful algal bloom toxin microcystin-LR worsens the symptoms of neutrophil-driven asthma by promoting granulocytic inflammation and stimulating effector immune mediators of airway inflammation

PUBLICATIONS

- Breidenbach JD**, French BW, et al. (2022) Microcystin-LR Aerosol Induces Inflammatory Responses in Healthy Human Primary Airway Epithelium. *Environment International*, 107531.
- Dube P, Khalaf FK, DeRiso A, Mohammed CJ, Connolly JA, Battepati D, Lad A, **Breidenbach JD**, et al. (2022) Cardio-protective Role for Paraoxonase-1 in Chronic Kidney Disease. *Biomedicines*, 10(9), 2301.
- Lad A, Hunyadi J, Connolly J, **Breidenbach JD**, et al. (2022) Antioxidant Therapy Significantly Attenuates Hepatotoxicity following Low Dose Exposure to Microcystin-LR in a Murine Model of Diet-Induced Non-Alcoholic Fatty Liver Disease. *Antioxidants (Basel, Switzerland)*, 11(8), 1625.
- Breidenbach JD**, Begue III EF, et al. (2022) GeneToList: A Web Application to Assist with Gene Identifiers for the Non-Bioinformatics-Savvy Scientist. *Biology*, 11(8):1113.
- Khalaf FK, Mohammed CJ, Dube P, Connolly JA, Lad A, Ashraf UM, **Breidenbach JD**, et al. (2022) Paraoxonase-1 Regulation of Renal Inflammation and Fibrosis in Chronic Kidney Disease. *Antioxidants*, 11(5):900.

Lad A, **Breidenbach JD**, Su RC, et al. (2022) As We Drink and Breathe: Adverse Health Effects of Microcystins and Other Harmful Algal Bloom Toxins in the Liver, Gut, Lungs and Beyond. *Life*, 12(3):418.

Breidenbach JD*, Su RC*, Alganem K, Khalaf FK, French BW, et al. (2021) Microcystin-LR (MC-LR) Triggers Inflammatory Responses in Macrophages. *Int J Mol Sci*, 22(18):9939.

Zhang S, **Breidenbach JD**, Russell B. H., George J, Haller ST. (2020) CD40/CD40L Signaling as a Promising Therapeutic Target for the Treatment of Renal Disease. *J Clin Med*, 9(11):3653.

FUTURE PLANS

Josh has accepted a Postdoctoral Research Associate appointment at Los Alamos National Laboratory.

PUBLICATIONS, CONT.

- Bekbolsynov D, Mierzejewska B, Borucka J, Liwski RS, Greenshields AL, **Breidenbach J**, et al. (2020) Low Hydrophobic Mismatch Scores Calculated for HLA -A/B/DR/DQ Loci Improve Kidney Allograft Survival. *Front Immunol*, 11:580752.
- Breidenbach JD**, Dube P, Ghosh S, Abdullah BN, et al. (2020) Impact of Comorbidities on SARS-CoV-2 Viral Entry-Related Genes. *J Pers Med*, 10(4):146.
- Su RC, Meyers CM, Warner EA, Garcia JA, Refsnider JM, Lad A, **Breidenbach JD**, et al. (2020) Harmful Algal Bloom Toxicity in *Lithobates catesbeiana* Tadpoles. *Toxins*, 12(6):378.
- Su RC, Warner EA, **Breidenbach JD**, et al. (2020) CD40 Receptor Knockout Protects against Microcystin-LR Prolongation and Exacerbation of Dextran Sulfate Sodium (DSS)-Induced Colitis. *Biomedicines*, 8(6):149.
- Su RC, Lad A, **Breidenbach JD**, et al. (2020) Assessment of Diagnostic Biomarkers of Liver Injury in the Setting of Microcystin-LR (MC-LR) Hepatotoxicity. *Chemosphere*, 257:127111.
- Zhang S, **Breidenbach JD**, et al. (2020) Renal Fibrosis Is Significantly Attenuated Following Targeted Disruption of *Cd40* in Experimental Renal Ischemia. *J Am Heart Assoc*, 9(7):e014072.
- Su RC, Lad A, **Breidenbach JD**, et al. (2019) Hyperglycemia Induces Key Genetic and Phenotypic Changes in Human Liver Epithelial HepG2 Cells Which Parallel the Leprdb/J Mouse Model of Non-Alcoholic Fatty Liver Disease (NAFLD). *PLoS One*, 14(12):e0225604.
- Khattar M, Baum CE, Schroder P, **Breidenbach JD**, Haller ST, Chen W, Stepkowski S. (2019) Interleukin 21 (IL-21) Regulates Chronic Allograft Vasculopathy (CAV) in Murine Heart Allograft Rejection. *PLoS One*, 14(11):e0225624.
- Lad A, Su RC, **Breidenbach JD**, et al. (2019) Chronic Low Dose Oral Exposure to Microcystin-LR Exacerbates Hepatic Injury in a Murine Model of Non-Alcoholic Fatty Liver Disease. *Toxins*, 11(9):486.
- Su R, Blomquist T, Kleinhenz A, Khalaf F, Dube P, Lad A, **Breidenbach J**, et al. (2019) Exposure to the Harmful Algal Bloom Toxin Microcystin-LR Prolongs and Increases Severity of Dextran Sulfate Sodium (DSS)-Induced Colitis. *Toxins*, 11(6):371.