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CHRB Grant Title: ***Interaction of Host Chemokines with Pathogenic Bacteria: A Novel Antimicrobial Strategy***

CHRB Project Summary:

Chemokines are small proteins that are produced in response to a variety of infections and are involved in the host inflammatory response. We have found that three related chemokines called MIG, IP-10, and ITAC, exhibit antimicrobial effects on the spores and vegetative cells of the bacterium, *Bacillus anthracis*. Thus, these naturally occurring immune mediators may function as host antimicrobial agents in addition to their known function of recruiting white blood cells and other inflammatory cells to the site of infection to fight an invading pathogen. This would represent a novel mechanism by which the host combats pathogenic bacteria. By understanding the mechanisms by which chemokines inhibit *B. anthracis*, and given the increasing incidence of antibiotic-resistance amongst bacteria globally with the relative scarcity of new classes of antibiotics to counter the emergence of resistance, this project may open up new therapeutic strategies for use against a broad range of pathogens.

CHRB Project Outcome:

Chemokines are small proteins produced in response to pulmonary infections such as pneumonia and other infections, and they play a key role in the host inflammatory response against microbes. We found that three related chemokines (a family of three "CXC" chemokines known as CSCL9, CLCL10, and CSCL11 that are induced by interferons) show antimicrobial effects against the microbe, *Bacillus anthracis*, which has two distinct forms: a spore form and a bacillus form.

In our original CHRB proposal, we proposed to determine the mechanism by which these chemokines affected growth of this microbe, and we proposed two specific objectives: 1) Define the mechanism(s) underlying the *in vitro* effects of the interferon-inducible CXC chemokines on growth of *B. anthracis* spores and bacilli; and 2) Characterize the *in vivo* effects of the interferon-inducible CXC chemokines on outcome of *B. anthracis* infection in a mouse model of anthrax.

As a direct result of CHRB funding, we have found in Objective 1 that these chemokines have direct killing effects against both the *B. anthracis* spores and bacilli (which has never been found with any known antibiotics), and we have identified what molecule in the bacteria the chemokines are targeting as their mechanism of action. Importantly, the chemokines target a critically important ABC transporter protein acts as a permease that imports nutrients into the microbe. Since the ABC transporter is found in many different microbes, especially those that cause clinically important infections, this finding is especially exciting since it now opens up an entirely new avenue for developing novel therapies, based on the chemokines as new antibiotics.

Our studies using a mouse model of infection in Objective 2 have led to the understanding that the chemokines have a direct killing effect against the microbe during actual infection in the lungs; this animal model may now serve as a pre-clinical model for testing of chemokines or smaller versions of them, as antibiotics during infection.

The findings generated by this project are very exciting for both their novelty in finding a new class of antibiotic and their potential for the development of these chemokines as novel antibiotics. We plan to continue this research project and further study the mechanism by which the chemokines kill the spore and vegetative form of *Bacillus anthracis*, continue our translational studies using a pre-clinical mouse model of infection, and expand the research to include studies of antimicrobial effects of the chemokines against other bacterial pathogens such as Gram-negative pathogens. The ultimate goal of these studies is to develop a new class of antimicrobial agents to help treat infections caused by multi-drug resistant bacterial pathogens, which are resistant or may become resistant to the currently available antibiotics.

Comments regarding CHRB Grant Funding

The support I have received from the CHRB is greatly appreciated. It made all the difference in terms of being able to gather data to then successfully compete for NIH grant funds. Thank you to the CHRB!

Leveraged Funding as a result of CHRB Grant Award: $1,536,300

Awarded:

Project title: *Bacillus anthracis Targets Involved in Chemokine-Mediated Antimicrobial Activity*
Funding agency: NIH/NIAID
Awarded: April 5, 2013 to March 31, 2017
Amount awarded $1,536,300

Publications

Antimicrobial Effects of Interferon-Inducible CSC Chemokines against Bacillus anthracis Spores and Bacilli. Matthew A. Crawford, Yinghua Zhu, Candace S. Green, Marie D. Burdick, Patrick Sanz, Farhang Alem, Alison D. O'Brien, Borna Mehrad, Robert M. Streiter, and **Molly A. Hughes.** Infection and Immunity, Apr. 2009, p. 1664-1678

Interferon-inducible CXC Chemokines Directly Contribute to Host Defense Against Inhalational Anthrax in a Murine Model of Infection
Matthew A. Crawford, Marie D. Burdick, Anne E. Boyer, John R. Barr, Borna Mehrad, Robert M. Strieter, and **Molly A. Hughes.** *PLoS Pathogens* 2010, 6:e1001199.

Identification of the Bacterial Protein FtsX as a Unique Target of Chemokine-mediated Antimicrobial Activity Against Bacillus anthracis.
Matthew A. Crawford, David E. Lowe, Debra J. Fisher, Scott Stibitz., Jason Zemansky, John W. Beaber, Borna Mehrad, Ian J. Glomski, Robert M. Strieter, and **Molly A. Hughes.**
Proceedings of the National Academy of Science USA, 2011,
www.pnas.org/cgi/doi/10.1073/pnas.1108495108.

The Antimicrobial Activity of CXCL10 Against Bacillus anthracis is Mediated Through Both FtsX-dependent and FtsX-independent Mechanisms.
Katie R. Margulieux, Jay W. Fox, Robert K. Nakamoto, and **Molly A. Hughes.**
Manuscript submitted, 2015.