RUTGERS
School of Graduate Studies

Office of Student Affairs and Admissions
SGS at New Jersey Medical School
Rutgers, The State University of New Jersey
185 South Orange Avenue, MSB C-696
Newark, NJ 07103

http://njms.rutgers.edu/gsbs/
p. 973-972-4511
f. 973-972-7148

YOU ARE INVITED TO ATTEND THE

DEFENSE OF THE DOCTORAL

DISSERTATION

"Not So Fas: The Diverse Mechanisms of Leukotoxin-Mediated Cell Death"

by

Brian Vega
Infection, Immunity, and Inflammation Program

B.S. 2013, University of Notre Dame, Notre Dame, IN

Thesis Advisor, Scott Kachlany, Ph.D.
Associate Professor
Department of Oral Biology

Wednesday, September 12th, 2018
10:00 A.M.
Dental School, B721

ABSTRACT

Leukotoxin (LtxA) is a protein secreted from the oral bacterium *Aggregatibacter actinomycetemcomitans*. LtxA helps the bacterium evade the host immune response by triggering cell death via a specific interaction with the active conformation of lymphocyte function-associated antigen-1 (LFA-1). LFA-1 is a β_2 integrin composed of the proteins CD11a and CD18, which are exclusively expressed on white blood cells. LtxA interaction with LFA-1 triggers different cell death pathways in lymphocytes and monocytes. In lymphocytes, LtxA kills cells via a caspase-8 dependent signaling pathway that, in addition to LFA-1, may involve the death receptor Fas. In monocytes, LtxA binds to LFA-1 and triggers a lysosomal cell death pathway. In this study, the diverse roles of CD18, CD11a and Fas were evaluated. We generated knockouts of CD18, CD11a and Fas in Jurkat T cells and/or THP-1 monocytes using CRISPR/Cas9 gene editing. Knockouts were validated with DNA sequencing and protein analyses. These are the first known human white blood cells lines with knockout of CD18, CD11a or Fas protein. Using these knockouts, we evaluated the roles of each protein in LtxA-mediated cell death. In lymphocytes, deletion of CD18 or CD11a rendered cells completely resistant to the effects of LtxA. LtxA was not able to overcome the loss of either protein. Additionally, LtxA was unable to activate caspases in the absence of CD18 or CD11a. Deletion of Fas also rendered Jurkat cells highly resistant to low doses of LtxA; however, at high concentrations, LtxA was able to overcome the loss of Fas and intoxicate cells, but to a lesser extent than wild-type cells. Low levels of LtxA were unable to trigger caspase activation or mitochondrial membrane permeabilization in the absence of Fas. These studies clearly identify a requirement for Fas in LtxA-mediated cell death in T cells. Deletion of Fas had no impact on the ability of LtxA to kill malignant B cells. Thus, we demonstrate that the mechanisms of LtxA-

mediated cell death differ in B- and T- lymphocytes. In monocytes, CD18 knockouts were highly resistant to the effects of LtxA. LtxA was unable to overcome the loss of CD18 and could not be internalized and rupture the lysosomal membrane. THP-1 CD11a knockout cells were efficiently killed by LtxA via the same lysosomal cell death pathway. THP-1 CD11a knockout cells were found to express CD11b/Mac-1. Studies with THP-1 knockout cells have clearly identified Mac-1 as a receptor for LtxA and that CD18 is the functional receptor for LtxA.