 Obada Shamaa  
PhD Candidate  

“**Intracellular and extracellular regulation of the inflammatory protease caspase-1**”  

April 14, 2014  
Davis Heart and Lung Research Institute  
Room 165  
9:00 AM
VITA

October 29, 1986 . . . . . . . . . . . . . . . . . . . . . . . . . Born – Elgin, IL

May 2008 . . . . . . . . . . . . . . . . . . . . . . . . . . B.S. Pharmacology & Toxicology, University at Buffalo, Buffalo, NY

June 2008-Present . . . . . . . . . . . . MD/PhD Biomedical Sciences Graduate Program, Ohio State University, Columbus, OH

COMMITTEE MEMBERS

Mark Wewers, MD

Elliott Crouser, MD

Peter Mohler, PhD

Larry Schlesinger, MD

AWARDS AND HONORS

2013 Award for Excellence in Research – Student Category, 1st Annual Internal Medicine Physician Scientist Research Day, Ohio State University Wexner Medical Center

2011 Excellence in Volunteer Community Service, Ohio State University

2011-2013 Susan L. Huntington’s Dean’s Distinguished University Fellowship, Ohio State University

2010 MD/PhD Leadership and Academic Achievement Scholarship, Ohio State University College of Medicine

FUTURE PLANS

I will be returning to medical school to finish my clinical training.


**ABSTRACT**

Caspase-1 is an inflammatory cysteine protease which cleaves the pro-inflammatory cytokines, interleukin-1β (IL-1β) and IL-18, into their mature, bioactive forms. In addition, caspase-1 plays a role in an inflammatory form of programmed cell death termed pyroptosis. Caspase-1 is constitutively expressed in monocytes as a zymogen which requires the assembly of a multi-protein complex, termed the inflammasome. Assembly of the inflammasome occurs in response to pathogen or danger associated molecular patterns (PAMP/DAMPs) that are sensed by pattern recognition receptors (PRR) which facilitates caspase-1 dimerization and cleavage into the mature enzyme. Where and how caspase-1 activation occurs is poorly understood. To address this question we compared the activation of caspase-1 in a cell-extract model, in which concentrated monocyte lysates endogenously activate caspase-1, to an *in vitro* model in which monocytes release mature caspase-1 from the cell upon inflammasome activation. We also assessed the role of mitochondria which have been identified as playing a role in the localization and assembly of the inflammasome.

In the cell-extract model, concentrated monocyte lysates incubated at 37°C spontaneously activate caspase-1 which cleaves endogenous proIL-18, but rapidly lose function, with a t1/2≈10 min. Addition of mitochondria to the monocyte lysates induced mature caspase-1. We found that the inflammasome adaptor protein, ASC, was enriched on the mitochondria and this was critical for caspase-1 activation. High concentrations of Ca^{2+}, which are tightly regulated by the mitochondria, induced the formation of a stable caspase-1 intermediate. At more physiologic concentrations, Ca^{2+} enhanced the activity of caspase-1 in the cell-extract.

Intact monocytyc cells release mature caspase-1 in response to inflammatory stimuli. We used the classic inflammasome activators, endotoxin (LPS) and adenosine triphosphate (ATP) and found that in contrast to the cell-extract activated caspase-1 the released caspase-1 has stable activity over 12h and is inhibited by
the tetra-peptide caspase-1 specific inhibitor, YVAD-cmk. This released caspase-1 activity existed in a high molecular weight complex distinct in size from inactive lower molecular weight forms. The active caspase-1 was not immunodepleteable nor able to cleave exogenous proIL-1β.

These results suggest that in the intracellular environment, caspase-1 is rapidly activated and that Ca^{2+} and the mitochondria play a role in this process. However, in this cytosolic environment caspase-1 rapidly loses its activity. Monocytes release mature caspase-1 extracellularly. Active caspase-1 exists in a unique complex that stabilizes its ability to cleave low molecular weight substrates but is sequestered in its interaction with target cytokines after release. An inactive fraction of mature caspase-1 is also released, and this is readily immunodepleteable, and exists in a low molecular weight fraction by gel chromatography. Understanding the significance of the unique release of caspase-1, especially in the context of inflammation and disease, will yield new insights into the function of this protease and the efficacy of therapeutic inhibition of caspase-1.

**RECENT ABSTRACTS AND PRESENTATION**


**Shamaa, OR**, (2013) Mitochondrial enrichment of ASC enhances caspase-1 activity. *Inaugural Internal Medicine Physician Scientist Research Day*. Oral Presentation. Department of Internal Medicine, Wexner Medical Center, Ohio State University, Columbus, OH

