**[PS2.6]**

# Kinome Response of Human Macrophage Proteins to Mycobacterial Infection

VP Poirier*, HB Bach, YAG Av-Gay
*University of British Columbia, Canada*

Introduction Since the 1990s, tuberculosis infection has reemerged causing 2 million deaths annually[1]. Tuberculosis is a growing health concern and understanding the host-pathogen interaction is a crucial requirement in achieving control of this deadly disease.

Macrophages engulf invading pathogens in phagosomes which fuse with lysosomes for degradation. *Mycobacterium tuberculosis* (Mtb), the etiological agent of Tuberculosis, blocks phagosome maturation by stopping the phagosome-lysosome fusion. This allows survival of pathogens inside the host. We have recently shown that Mtb pathogenicity is dependent on PtpA, a secreted tyrosine phosphatase, which blocks phagosome maturation by dephosphorylating VPS33B, a host protein involved in membrane fusion[2].

Hypothesis We hypothesize that upon mycobacterial infection of macrophages, Mtb signaling molecules interfere with macrophages' signaling pathways resulting in observable changes in the phosphorylation status of host proteins.

Methods THP-1 cells were infected with Mtb (wild-type, PtpA and protein kinase G mutants) and the phosphorylation profiles of host proteins were determined by 2D electrophoresis before and after infection. Also, an array of mammalian phospho-site-specific antibodies was used to track changes in the phosphorylation status of host proteins. For validation of PtpA and PknG dependent protein responses, downstream effects of the reported phosphorylation events on modulated host proteins were analyzed using biochemical assays.

Results The effect of PtpA and PknG on macrophage signal transduction was examined and we discovered changes in host signaling pathways upon infection. PtpA causes phagosome maturation arrest and PknG alters the phosphorylation profile of several signaling molecules.

Discussion The interaction between Mtb and the macrophage needs to be elucidated since it leads to the interference of the macrophage's killing machinery and promotion of the pathogen survival. With these proteomic approaches, we hope to show the extent of post-translational modifications occurring on macrophage proteins upon Mtb infections and to have a better understanding of how Mtb eludes the attention of the immune system.

[1] Butler, D. (2000). New Fronts in an Old War, Nature (406): 670-672.

[2] Bach *et al.* (2008). *Mycobacterium tuberculosis* Virulence is Mediated by PtpA Dephosphorylation of Human Vacuolar Protein Sorting 33B, Cell Host and Microbe, 316-322.

Keywords: Kinase/Phosphatase, Signal Transduction, Mycobacterium tuberculosis, Alveolar Macrophage