

Biology of infection and innate immunity

Signaling pathways involved in the survival of *Mycobacterium tuberculosis* within host cells

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Pathogenic mycobacteria such as *Mycobacterium tuberculosis*, the causative agent of tuberculosis is probably the most successful bacterial pathogen known today. It is responsible for more deaths world wide than any other bacterial pathogen. While mycobacteria are efficiently internalized into macrophages, rather than being destroyed like any other phagocytosed cargo, pathogenic mycobacteria survive for prolonged times.

My laboratory is interested in the mechanisms that are utilized by *Mycobacterium tuberculosis* to avoid innate and adaptive immune defenses. One of the strategies relies on avoiding transfer to lysosomes, and we are characterizing host as well as mycobacterial factors that are involved in blocking intracellular degradation. It appears that mycobacteria utilize both molecular mimicry as well as the hijacking of host signal transduction mechanisms to allow them to survive inside macrophages. Strikingly, one of the signaling pathways utilized by *M. tuberculosis* to survive appears also to play a crucial role in the generation of adaptive immune responses. The implications of these findings in the light of the challenges faced in the control of tuberculosis will be discussed.

Pathways and consequences of macrophage cell death in TB

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Mycobacterium tuberculosis is a facultative intracellular pathogen that invades lung-resident alveolar macrophages to establish productive infection in the host. By inhibiting phagosome maturation in macrophages the bacillus creates a protected niche for replication. One countermeasure against intracellular parasitism is the elimination of infected macrophages by apoptosis, which may be triggered by autocrine/paracrine tumor necrosis factor-alpha (TNF) death signaling. Caspase-dependent apoptosis not only eliminates the intracellular niche but also reduces bacillary viability and packages mycobacterial antigens for efficient T cell priming by dendritic cells. The host may activate several apoptotic pathways in TB defense, including TNF receptor and Fas signaling as well as granzyme-mediated apoptosis. Virulent *M. tuberculosis* strains are capable of suppressing TNF-mediated apoptosis in order to defend their preferred growth environment. After a period of optimal intracellular replication to a critical threshold bacillary load these same virulent strains can induce an alternative death mode with initial features of apoptosis but rapid progression to necrosis. This cell death mechanism is independent of TNF, caspases or Bax and Bak and it releases viable bacilli. An emerging concept of macrophage cell

death in TB pathogenesis holds that apoptosis favors host defense while necrosis facilitates spreading infection and creates conditions supporting extracellular bacillary replication that is necessary for transmission to the next host. An increasingly complex model of pathogenesis suggests that multiple pro- and anti-apoptotic pathways are utilized by the host and the pathogen for opposing purposes in the evolution of TB disease.

***Mycobacterium tuberculosis* conA affinity glycoproteomics based on lectin affinity capture of mannosylated glycoproteins**

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Protein glycosylation, a key post-translation modification, plays a range of biological functions in bacteria such of interaction with mammalian cells contributing to bacterial infection. At date only a few glycoproteins had been identified in the human pathogen *M. tuberculosis*. In order to find the expression profile of *M. tuberculosis* mannosylated proteins, *M. tuberculosis* Culture Filtrate was enriched in mannose containing proteins by ConA affinity chromatography and the alfa-methyl mannoside eluted proteins were analyzed by two-dimensional electrophoresis. Several spots were detected, but only spots bound to ConA in a ligand 2D-gel assay were consider to be identified via peptide mass fingerprinting by a matrix assisted laser desorption/ionization time-of-flight mass spectrometry. About 32 spots were identified and the majority of them corresponded to lipoproteins with glycosylation sites predicted with NetOGlyc 3.1 software. Furthermore, it was confirmed that LprG (Rv1411c) a lipoprotein that increases the susceptibility of mice to *M. tuberculosis* infection is also a glycoprotein; this molecule was identified in a *M. tuberculosis* Culture Filtrate ammonium sulphate fraction with a rabbit polyclonal antibody. The purified ConA protein was then compared with the recombinant protein expressed in *E. coli*. It is worth of note that the enrichment of Culture Filtrate glycoproteins with ConA allowed us to identify new unreported proteins in *M. tuberculosis* proteome databases. The construction of ConA affinity glycoprotein database of *M. tuberculosis* would contribute to the subsequent research of mycobacteria glycoproteomics, in order to resolve the significance of glycoprotein and find out the biological functional importance of these molecules.

A TLR-induced vitamin D-dependent antimicrobial mechanism in human infection

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The innate immune system provides a rapid mechanism for host defense against microbial pathogens. Activation of the human innate immune response is mediated in part by Toll-like receptors (TLRs), specifically, TLR2 and TLR1

heterodimers mediate the response to bacterial lipopeptides, including those present in mycobacteria. Activation of TLRs results in a direct antimicrobial response against *M. tuberculosis* in monocytes and macrophages *in vitro*. In mice, this activity is mediated principally through the generation of nitric oxide. However, TLR2/1-induced antimicrobial activity in human macrophages against intracellular *M. tuberculosis* is vitamin D receptor (VDR)-dependent. Activation of the TLR2/1 heterodimer on human monocytes upregulates gene expression of both the VDR and the vitamin D1-hydroxylase, CYP27b1, leading to the VDR-dependent induction of two antimicrobial peptides, cathelicidin and DEFb4. Induction of these peptides requires the conversion of the vitamin D3 prohormone (25D3) into the active form (1,25D3) by CYP27b1. These data support a link between vitamin D and innate antimicrobial immunity in human infectious disease.

Insight into *M. tuberculosis* adaptation to macrophages in the human respiratory system

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During infection, *M. tuberculosis* (*M.tb*) bacilli traverse the lung airways and settle in the alveolar spaces where they encounter alveolar macrophages (AM ϕ). The alveolus is a highly immune-regulated microenvironment and AM ϕ contribute to this by displaying an anti-inflammatory phenotype also known as an "alternative activation state", characterized by increased expression of a subset of pattern recognition receptors (PRRs) such as the mannose receptor and scavenger receptors, and a reduced oxidative response. This biological state allows AM ϕ to effectively clear microbes and particles within the alveolus while minimizing collateral inflammatory damage, but on the other hand may be exploited by the host-adapted *M.tb*. Our ongoing studies highlight the importance of *M.tb* surface mannosylation and host cell C-type lectin PRRs in macrophage recognition and response. *M.tb* clinical isolates vary in their degree of surface mannosylation impacting the use of these pathways and potentially disease outcome. We are also exploring the effects of the lung surfactant collectins SP-A and SP-D in shaping AM ϕ phenotype and function which impact the phagocytosis and intracellular fate of *M.tb*. For example, we have found that SP-A regulates the expression, activity, and intracellular trafficking of Toll-like receptor (TLR) PRRs in human macrophages. SP-A dampens the TLR2- and 4-mediated pro-inflammatory response by inhibiting the phosphorylation of key TLR signaling molecules that lead to NF κ B activation and TNF α secretion in response to their ligands. Thus, bacterial surface mannosylation and surfactant components that "shape" AM ϕ biology alter the battlefield in favor of *M.tb* providing examples of *M.tb* adaptation to its environment.