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Identification of mycobacterial genes involved in survival of pathogenic mycobacteria

Objectives Pathogenic mycobacteria, in particular *Mycobacterium tuberculosis*, the causative agent of tuberculosis, have the remarkable capacity to avoid destruction within the hostile environment of the macrophage by resisting lysosomal delivery, allowing them to survive intracellularly. The aim of this project is to identify mycobacterial genes involved in intracellular survival that could be used as targets for the development of new drugs to combat mycobacterial diseases.

Conclusions The eukaryotic-like serine/threonine protein kinase G (PknG) expressed by pathogenic mycobacteria was shown to be secreted within macrophage phagosomes. PknG functions by inhibiting phagosome-lysosome fusion, thus mediating intracellular survival of the bacteria. Mycobacteria lacking PknG are viable outside host cells but are immediately transferred to lysosomes and rapidly destroyed within infected macrophages.
Since PknG is secreted within the macrophage cytoplasm, pharmaceutical inactivation of PknG is not hampered by the impermeable barrier created by the mycobacterial cell wall, thus providing a new treatment regimen targeting intracellular residing mycobacteria and allowing the macrophage to carry out its innate bactericidal activity (by shuttling these bacteria to lysosomes), thereby potentially circumventing antibiotic resistance. It is to be noted that specific inhibitors of serine/threonine kinases have been successfully developed for therapeutic usage against a variety of diseases.
To conclude, besides identifying a putative target for the control of mycobacterial infections, these findings suggest that pathogenic mycobacteria have evolved eukaryotic-like signal transduction mechanisms capable of modulating host cell trafficking pathways.

Main results and findings

This NRP 49-funded project allowed the discovery that pathogenic mycobacteria utilize an enzyme that shows a high homology to host enzymes to prevent their destruction within the macrophages. It was found that this enzyme, termed protein kinase G (PknG), which is a member of the eukaryotic serine/threonine kinases, is essential for the survival of pathogenic mycobacteria within host cells. Furthermore, a specific inhibitor for this kinase was found that effectively could kill mycobacteria inside macrophages by allowing the bacilli to be transported to lysosomes.

Analysis of a candidate mycobacterial gene involved in virulence (i.e. involved in the regulation of phagosome-lysosome fusion) Characterisation of the eukaryotic enzyme-like serine/threonine protein kinase G (PknG) revealed that:

- PknG is secreted into the cytosol of infected macrophages and acts by inhibiting phagosome-lysosome fusion and mediating intracellular survival of mycobacteria.
- inactivation of *pknG* by gene disruption or chemical inhibition results in lysosomal localization and mycobacterial cell death in infected macrophages.
- in *M. bovis* BCG, deletion of *pknG* did not affect either glutamine uptake or intracellular glutamine concentrations, and in vitro growth is identical to that of the wild type.

To conclude, PknG may play an important role in defining the intracellular fate of mycobacteria, and these findings suggest that pathogenic mycobacteria have evolved eukaryotic-like signal transduction mechanisms capable of modulating host cell trafficking pathways.

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