Studying the mechanism of secretion, macrophage substrate characterization, and therapeutic targeting of mycobacterial Protein Kinase G

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Abstract

Mycobacterium tuberculosis (M.tb), the causative agent of tuberculosis (TB), remains one of the most formidable global health threats, largely due to its remarkable ability to manipulate host immune responses and establish long-term persistence within macrophages. This success is attributed in part to its repertoire of secreted virulence factors that not only modulate host cell signalling but also enable evasion of bactericidal mechanisms such as phagosomal maturation and apoptosis. Among these factors, Protein kinase G (PknG), a mycobacterial Ser/Thr kinase, has emerged as a central player in pathogenesis, mediating both intracellular survival and immune modulation. However, the mechanism underlying its secretion and functional switching remained unclear. Here, we uncover a novel, infection-triggered secretion mechanism of PknG driven by host immune signals. We demonstrate that the early Th1-skewed immune response to M.tb infection induces the generation of reactive nitrogen intermediates (RNI), which subsequently mediates S-nitrosylation of PknG at cysteine 109. S-nitrosylated PknG's thereafter leaves behind its intramycobacterial metabolic role and interacts with the chaperone SatS to get secreted across the mycobacterial membrane via the SecA2 pathway. Subsequent to the release of PknG into the phagosomal lumen and owing to increasing acidification of this lumen with increasing time of infection, a ubiquitously secreted virulence factor MPT63 plays a secondary role in the secretion of PknG. We observe that acidification induced oligomerization and phagsosomal membrane pore formation by MPT63 thereinafter enables the secretion of S-nitrosylated PknG into the macrophage cytosol. Once inside the macrophage cytosol, S-nitrosylated PknG phosphorylates Supressor of Death Domain (SODD) so as to make it irreversibly interact with the death domain of TNF receptor. This prevents the pro-inflammatory trigger driven activation of Caspase8 and thereby prevents the apoptosis of the mycobacteria infected macrophages. Additionally S-nitrosylated PknG was also observed to induce the degradation of ATF2 which then resulted in dampened TNFa production by infected macrophages. An alternative host-directed nanotherapeutic approach was thereafter envisaged to target PknG as well as induce immunomodulation of the M.tb infected macrophages. We synthesized PknG inhibitor AX20017 encapsulated mannose functionalized diVitD3-OEG nanocapsules. This self-assembling amphiphilic molecule were thoroughly characterized and its efficiency in immunomodulating M2 polarized Mtb infected macrophages to M1 polarization and thereby reanabling it to eliminate the parasite through lysosomal transfer. Collectively, this study offers mechanistic insights into how M.tb hijacks host immune signalling to orchestrate the secretion and function of virulence factors like PknG and MPT63. It highlights the critical roles of post-translational modifications, host–pathogen protein interactions, and membrane remodelling in immune evasion and pathogenesis. Furthermore, a promising alternative therapeutic strategy has been proposed that combines targeted immune reprogramming with direct inhibition of bacterial virulence, laying the foundation for improved host-directed therapies against both drug-sensitive and drug-resistant TB.

Key words: Mycobacterium, PknG, S-Nitrosylation, MPT63, immunomodulation, nanocapsule