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Structural Determination and Toll-like Receptor 2-dependent Proinflammatory Activity of Dimycolyl-diarabion-glycerol from *Mycobacterium marinum*

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Abstract:
Although it was identified in the cell wall of several pathogenic mycobacteria, the biological properties of dimycolyl-diarabino-glycerol have not been documented yet. In this study an apolar glycolipid, presumably corresponding to dimycolyl-diarabino-glycerol, was purified from *Mycobacterium marinum* and subsequently identified as a 5-*O*-mycolyl-β-Ara*f*-(1→2)-5-*O*-mycolyl-α-Ara*f*-(1→1')-glycerol (designated Mma_DMAG) using a combination of nuclear magnetic resonance spectroscopy and mass spectrometry analyses. Lipid composition analysis revealed that mycolic acids were dominated by oxygenated mycolates over α-mycolates and devoid of *trans*-cyclopropane functions. Highly purified Mma_DMAG was used to demonstrate its immunomodulatory activity. Mma_DMAG was found to induce the secretion of proinflammatory cytokines (TNF-α, IL-8, IL-1β) in human macrophage THP-1 cells and to trigger the expression of ICAM-1 and CD40 cell surface antigens. This activation mechanism was dependent on TLR2, but not on TLR4, as demonstrated by (i) the use of neutralizing anti-TLR2 and -TLR4 antibodies and by (ii) the detection of secreted alkaline phosphatase in HEK293 cells co-transfected with the human *TLR2* and secreted embryonic alkaline phosphatase reporter genes. In addition, transcriptomic analyses indicated that various genes encoding proinflammatory factors were up-regulated after exposure of THP-1 cells to Mma_DMAG. Importantly, a wealth of other regulated genes related to immune and inflammatory responses, including chemokines/cytokines and their respective receptors, adhesion molecules, and metalloproteinases, were found to be modulated by Mma_DMAG. Overall, this study suggests that DMAG may be an active cell wall glycoconjugate driving host-pathogen interactions and participating in the immunopathogenesis of mycobacterial infections.

Full text: http://www.jbc.org/content/287/41/34432.abstract