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Mini-review

Pathogenic and immunosuppressive properties of mycobacterial phenolic glycolipids

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ABSTRACT

Phenolic glycolipids (PGL) are polyketide synthase products that are uniquely produced by a subset of pathogenic mycobacteria and are displayed at the bacterial cell surface, in a strategic position to interfere with host immune cells. Their expression has been associated with enhanced mycobacterial virulence *in vivo*, and suppression of the inflammatory responses of host phagocytes *in vitro*. In this review, we will present our current understanding of the mode of operation of PGL, along with functional evidence that demonstrates the evolutionary advantage conferred by PGL production for host cell invasion, intracellular persistence and evasion of host immune and bactericidal responses.

1. Introduction

Mycobacterium is a genus of *Actinobacteria*, the sole member of the *Mycobactericeae* family in the order *Actinomycetales* [1]. Mycobacteria can be subdivided into fast- and slow-growing species, a subdivision that is recapitulated by 16S rRNA sequence homology analysis across the genus [2,3] (Fig. 1). Most mycobacterial species are innocuous saprophytes living in the soil, water, plants and in the air, however the slow-growing species subfamily contains major pathogens for humans. *Mycobacterium tuberculosis* and the genetically-related members of the *M. tuberculosis* complex (*M. canetti, M. africanum, M. microti* and *M. bovis*) cause tuberculosis in humans and other animals [4]. *M. tuberculosis* is considered to be one of the most successful pathogens on the planet, with about one third of the human population infected worldwide [5]. *M. leprae* causes leprosy, the second most common mycobacterial disease in humans. Of note, the anti-tuberculous vaccine *M. bovis* Bacille Calmette Guérin (BCG) was generated empirically by Calmette and Guérin by serial passages of a virulent strain of *M. bovis* in bile salts [6–8]. Aside from its use as a live vaccine, and a treatment for superficial bladder cancer, BCG represents a useful vector for the delivery of heterologous antigens *in vivo*. It also provides researchers with a genetically engineerable reference strain for the study of species-specific products, such as phenolic glycolipids (PGL) [9].

The mycobacterial cell wall is unique among bacteria due to its lipid-rich, exceptionally impermeable structure [10–12]. From interior to exterior, this cell wall consists of a plasma membrane and an outer, Gram-negative-like membrane (called mycomembrane) containing mycolic acids (MA). Besides MA, the mycomembrane also contains non-covalently bound lipids such as phthiocerol dimycocerosates (DIM), polyacyl trehaloses (PAT), diacyltrehaloses (DAT), sulfoglycolipids (SGL), lipomannan (LM) and lipoarabinomannan (LAM). In addition, the mycomembrane is coated by a detergent-labile capsule containing arabinomannan, alpha-glucan and oligomannosyl-capped glycolipids and proteins, which together with the cell wall forms the mycobacterial cell envelope. While the architecture and composition of this envelope is largely conserved within the Mycobacterium genus, individual lipid constituents can differ across strains or clinical isolates. This is well exemplified by PGL, a family of polyketide synthase-derived, cell surface-displayed lipids that are only found in a limited subset of pathogenic species (Fig. 1) [13,14]. Structurally, mycobacterial PGL all contain a phenol ring that is linked to a DIM-related lipid backbone on the one hand, and an oligosaccharide moiety on the other hand (Fig. 2). While the lipid core is highly conserved across PGL-producing species, the oligosaccharidic domain that is attached to the phenol ring varies [15,16]. PGL-bovis, PGL-mar and

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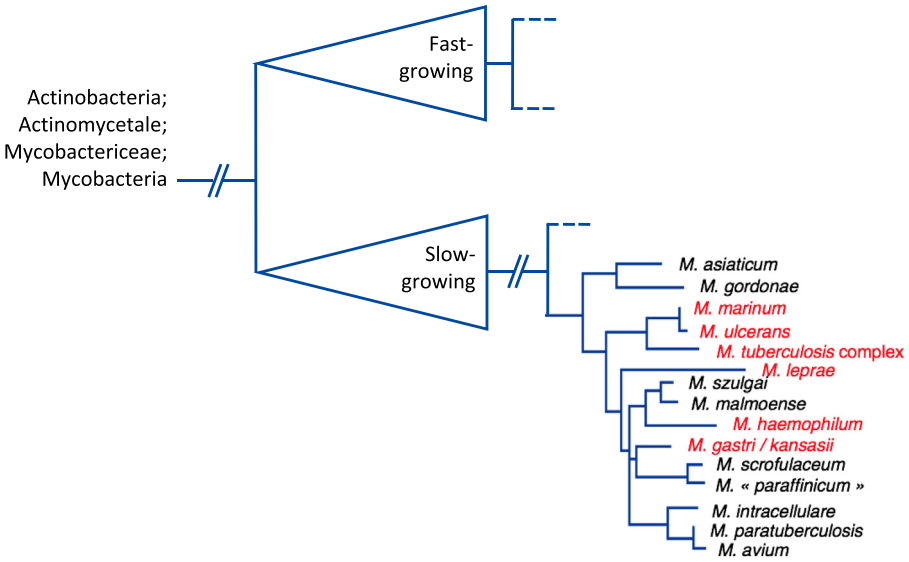

Fig. 1. Phylogenetic position of the PGL-producing species within the genus Mycobacterium. PGL-producing species are shown in red, in the context of their phylogenetic branch, in the subfamily of slow-growing mycobacteria. Adapted from Springer et al., 1996 [2,3].

PGL-ulc from *M. bovis*, *M. marinum* and *M. ulcerans*, respectively, display glycosidic domains that are restricted to a single monosaccharide. Most strains of *M. tuberculosis*, including the laboratory strain H37Rv, do not produce PGL due to a frameshift mutation in the polyketide synthase gene *pks15/1* [17]. However, strains of the W-Beijing *M. tuberculosis* family have an intact *pks15/1*, and are known producers of PGL (hereafter named PGL-tb) [18,19]. As shown in Fig. 2, PGL-tb and PGL-1 of *M. leprae* contain extended, structurally distinct trisaccharidic domains.

2. PGL and pathogenesis of mycobacterial infections

The first indication that PGL production may confer additional virulence to mycobacteria came in 2004 with the observation that clinical isolates of *M. tuberculosis* belonging to the W-Beijing family (which express PGL-tb) show a highly lethal phenotype in animal models of infection [18]. While hyper-pathogenicity was lost upon disruption of PGL production in these isolates, it was not observed with a H37Rv strain engineered to produce PGL-tb [20]. This suggested that PGL production is required but not sufficient for *M. tuberculosis* hyper-pathogenicity. Moreover, it implied that the mycobacterial genetic background interacts with PGL to increase virulence. Notably, ectopic expression of PGL-tb by *M. tuberculosis* efficiently downregulated the production of inflammatory cytokines in infected monocytes or macrophages, irrespective of the strain used [18,20]. Studies in zebrafish showed that PGL expression by *M. marinum* favors the recruitment of macrophages to the site of infection, through a host chemokine receptor 2 (CCR2)-mediated pathway [21]. Yet, DIM and PGL-mar both contributed to *M. marinum* virulence in infected zebrafish [22]. By applying signature-tag mutagenesis to *M. bovis* and infection of guinea pigs, Collins *et al.* identified attenuated mutants with mutated *pks1*, a gene that is essential for PGL biosynthesis [23]. PGL-1, made by *M. leprae*, is detected in all clinical isolates and considered a key player in leprosy pathogenesis [24,25]. PGL-1 was indeed shown to mediate the selective tropism of *M. leprae* to Schwann cells, through selective binding of its trisaccharidic moiety to Laminin-2 expressed at the basal lamina [26]. More recently, PGL-1 was shown to interact with complement receptor (CR3), either purified and immobilized or displayed at the surface of phagocytes [9,27]. Altogether, these studies thus suggest that PGL production contributes to mycobacterial pathogenicity, by directly interfering with host cell invasion and/or by suppressing the generation of innate immune responses by the host. The next section reviews the state of the art with regard to PGL recognition by the innate immune system, and mode of operation.

3. Molecular mechanisms governing PGL recognition and biological activity

3.1. PGL and CR3 signaling

CR3, also known as Macrophage-1 antigen (MAC-1) is a heterodimeric complex composed of an alpha (CD11b) and beta subunit (CD18). CR3 belongs to the β2-integrin family, which also comprises CR4 (CD11c/CD18) and the lymphocyte function-associated antigen 1 (CD11a/CD18) [28]. CR3 is a widely expressed receptor found on monocytes, tissue resident macrophages, dendritic cells, neutrophils, NK cells, basophils, eosinophils and platelets. It is also detected on activated CD8 cytotoxic T cells during acute viral infection [29,30] and CD4 T cells [31]. Its major functions are cellular adhesion, intracellular signaling and phagocytosis. Notably, CR3 has three conformational states of low-, intermediate- and high-affinity for ligands, co-existing in dynamic equilibrium that can be altered by the activity of other cellular receptors through a process called inside-out signaling. For instance, TLR2 and TLR5 ligation on leukocytes signal to augment β2-integrin affinity, resulting in increased cellular adhesion to immobilized ICAM-1 and fibronectin [32]. CR3 is also able to induce phagocytosis of opsonized and non-opsonized particles, with its I-domain (which binds ICAM-1, fibrinogen and complement component iC3b) and a carbohydrate-binding lectin domain [33,34].

Phagocytosis of mycobacteria is known to occur in both opsonic and non-opsonic conditions [35], with CR3 being responsible for 40–50% of non-opsonic binding and 50–60% opsonic binding of *M. tuberculosis* to macrophages infected *in vitro* [36]. CR3 also mediates the phagocytosis of *M. leprae* by monocyte-derived macrophages in both conditions [37]. Also, it was shown that *M. bovis* BCG promotes its own phagocytic uptake by macrophages, through an inside-out activation of CR3 involving TLR2 [38]. While

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