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Partners in Crime: Phenolic Glycolipids and Macrophages

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Abstract

Two recent articles advance our understanding of mycobacterial pathogenesis, revealing key roles for bacterial-derived phenolic glycolipids (PGL). In leprosy, *Mycobacterium leprae*'s PGL-1 uniquely subverts local macrophages to produce neurotoxic nitric oxide, leading to nerve demyelination. In a related model, *M. marinum* PGL stimulates the recruitment of growth-conductive monocytes to sites of initial infection as an early immune evasion strategy.

Hiding in Plain Sight

Intracellular pathogens gain access to the cell interior and must rapidly gain control of numerous innate cellular mechanisms that have evolved to thwart and destroy them. In the well-chronicled cases of *Salmonella* and *Legionella*, this subversion is accomplished via secretion of hundreds of interfering proteins, remodeling the cell into a replication vessel for the pathogen [1,2]. With pathogenic mycobacteria, analogous proteins that can subvert host cell machinery have been sought. However, the list of secreted bacterial-derived proteins that gain access to the cytosol and alter cell function, remains a short one.

Pathogenic mycobacteria are well-known for producing complex lipids, comprised of up to 90 carbons in length. However, to date, such lipids have not been extensively investigated as virulence effector molecules within macrophages. Two recent studies by Ramakrishnan's laboratory address the function of **phenolic glycolipids (PGL, see Glossary)** in mycobacterial pathogenesis, demonstrating that, indeed, such mycobacterial lipids—not proteins—play pivotal intracellular roles in the pathogenic programs of both *M. leprae* and *M. marinum* [3,4]. Indeed, key virulence effectors in pathogenic mycobacteria might have been “hiding in plain sight”, overlooked in the hunt for protein mediators of pathogenesis.

What Are Phenolic Glycolipids?

PGLs are polyketide-derived surface-exposed bioactive glycolipids produced by mycobacteria. The precise PGL structures are species-specific, though the significance of these differences has not been fully elucidated. *M. leprae* PGL-1 was first identified as an antigen and virulence factor of leprosy in 1981 [5] and was characterized chemically in 1987

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[6]. Production of a structurally distinct PGL by *M. tuberculosis* has also been associated with tuberculosis virulence in mouse models[7]. *M. tuberculosis* PGL expression results in repression of pro-inflammatory cytokines IL-1 β and TNF- α , while PGL-deficient strains lead to increased pro-inflammation in infected macrophages [8].

PGL-1 -Intoxicated Macrophages Can Mediate Demyelination and Neuronal Damage in Leprosy

A recent report published in *Cell* describes a novel mechanism of neuronal damage in leprosy, a disease caused by the pathogen *M. leprae* which remains uncultivable *in vitro* [3]. Specifically, the authors capitalized on the *M. marinum*-zebrafish model in which host-pathogen interactions may be visualized in real time, owing to the transparency of zebrafish larvae[3]. Special experimental tools were generated, including a transgenic zebrafish strain expressing a fusion protein characterized by myelin basic protein (mbp)-green fluorescence protein (GFP), which enabled direct visualization of demyelinated glial cells; red-fluorescent protein (RFP)-expressing *M. marinum*; and remarkably --despite the difficulty of working with an uncultivable pathogen-- fluorescently labeled *M. leprae* itself (derived by growing the pathogen in athymic mouse footpads in the presence of fluorescent markers) [3]. Lastly, six key *M. leprae* genes were cloned into *M. marinum*, generating a recombinant strain (Mm:PGL-1) expressing bona fide triglycosylated *M. leprae* PGL-1, in contrast to the PGL molecule of *M. marinum*, which is monoglycosylated [6].

The authors showed that *M. leprae* and Mm:PGL-1, but not *M. marinum* alone, elicited myelin protrusions along myelinated axons in the developing larval spinal cord, indicating that *M. leprae*-derived triglycosylated PGL-1, uniquely elicited myelin damage[3]. The bacilli localized to macrophages which patrolled intact myelin sheaths. Control larvae lacking macrophages (either by treatment with **clodronate** or by use of a **pu.1 morphant**) did not develop myelin protrusions [3]. Using transmission electron microscopy, the authors assessed the effects of a series of nitric oxide synthase (NOS) inhibitors and nitric oxide (NO) donors in the zebrafish embryo model, demonstrating that myelin damage, axonal swelling and axonal mitochondrial toxicity resulted from increased NOS expression as a consequence of bacterial-derived PGL-1 [3] (Fig. 1A).

This work establishes a new paradigm in the pathogenesis of leprosy-associated neuropathy because earlier models proposed that glial cell toxicity was the result direct *M. leprae* binding to glial cell laminin [9]. In contrast, the work of Madigan et al. now shows that *M. leprae*-infected macrophages are the source of neuronal damage, and derived PGL-1 intoxication of these perineural macrophages prompts a pathologic reactive nitrogen species (RNS) response that can lead to demyelination, at least in these animal models [3]. Furthermore, the work is clinically relevant because it suggests that novel NOS inhibitors such as **ronopterin (VAS-203)** -- currently in development as candidate neuroprotective agents to treat brain injury [10], might potentially be efficacious in treating leprosy. However, this remains to be tested.

PGL Helps *M. marinum* “Buy Time” during Early Phases of Mycobacterial Infection

Work published in *Immunity* by Cambier et al. revealed a second subversion mechanism--mediated in this case, by *M. marinum* PGL [4]. In this study, mycobacteria used PGL to mediate escape from hostile tissue-resident macrophages during the early stages of intracellular infection. Using zebrafish larvae and *M. marinum*-expressing PGL, the authors monitored the dynamics and kinetics of initial recruitment of myeloid cells and identified a novel sequence of first-and second-responder cells to the site of mycobacterial infection. For this analysis, the authors exploited the zebrafish hindbrain ventricle (HBV), which is protected by the blood brain barrier, to chronicle the succession of cells that were recruited to this compartment and could phagocytose virulent mycobacteria. In contrast to the conventional dogma--namely, that mycobacteria initially reside in naïve macrophages until 3–4 weeks when cell-mediated immunity leads to the recruitment of bactericidal macrophages--this study showed that resident macrophages (RM) which were already present in the HBV were the first to phagocytose mycobacteria and harbored bactericidal capability [4]. The authors demonstrated that mycobacterial PGL expression within these RMs prompted RMs to secrete a **chemokine (C-C motif) ligand 2 (CCL2)** signal that recruited naïve monocytes to the infection site in the HBV. These exogenous, naïve second-responder monocytes could take up the infecting mycobacteria, but unlike RMs, the monocytes were non-bactericidal and therefore, unable to prevent mycobacterial proliferation (Fig 1B) [4].

This report introduces a novel pathogen escape mechanism. While bactericidal RMs are the first cells to respond, and could normally eliminate the infection, mycobacteria use PGL as a virulence factor to trigger a detrimental CCL2-mediated recruitment signal[4]. Rather than being destroyed by RMs, bacilli “buy time” by leading RMs to recruit non-bactericidal monocytes to the infection site [4]. These non-bactericidal monocytes can then act as a temporary “safe harbor” where otherwise doomed mycobacteria, subsequently escape killing and proliferate [4].

Take Home Messages

PGL-driven macrophage subversion is a key thread in both recent studies by Ramakrishnan’s group. Each article shows that pathogenic mycobacteria—like other intracellular pathogens--subvert macrophage homeostasis by secreting bacterial-derived molecules. But in contrast to *Salmonella*, *Listeria*, and *Legionella*, mycobacteria can utilize lipids rather than proteins, to execute intracellular subversion. Consequently, these studies strongly suggest structure-specific interactions of microbial lipids (e.g. PGL and PGL-1) with intra-macrophage targets. Indeed, Madigan et al. beautifully demonstrated that triglycosylated PGL-1 (made uniquely by *M. leprae*), but not monoglycosylated PGL (made by *M. marinum*), specifically elicited a neurotoxic RNS response in perineural macrophages. The fact that specific foreign lipids delivered from intracellular bacteria are capable of modulating macrophage responses thus raises the possibility that other biological lipids may serve as critical mediators in other processes of macrophage function. Elucidating the host cell molecules that recognize PGL and PGL-1 promises to enlighten our, not only of bacterial pathogenesis, but also of macrophage cell biology at large.

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Glossary

Phenolic glycolipid (PGL)

Subset of a complex branched PGL linked to increased virulence, in a set of clinical isolates of *M. tuberculosis* (e.g. members of W-Beijing family of strains). *M. marinum* PGL is monoglycosylated while *M. leprae* PGL-1 is triglycosylated.

CCL2 (Chemokine (C-C motif) ligand 2

Monocyte chemoattractant protein 1 (MCP1) produced in response to infection or tissue injury. It recruits monocytes, dendritic cells and T cells at the site of inflammation caused by infection.

Demyelination

Damage to the protective covering (myelin sheath) that surrounds nerve fibers. Debilitating morbidity associated with leprosy is caused by axon demyelination in neurons.

Ronopterin (VAS-203)

Nitric oxide synthase inhibitor (4-amino-tetrahydrobiopterin) currently under development as a neuroprotective agent for the treatment of traumatic brain injury

Clodronate

A bisphosphonate anti-osteoporotic drug approved for prevention of osteoporosis. Selectively depletes macrophages at high doses in animal models.

Pu.1 morphant

Zebrafish treated with a morpholino antisense oligonucleotide targeting the *pu.1* genetic locus, depleting myeloid progenitor cells in the fish.

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stable signal. RMs are bactericidal and in the absence of PGL, they will eliminate these bacilli. However, in a PGL-dependent process, infected RMs release a chemokine C-C motif ligand 2 (CCL2) signal which binds its ligand C-C chemokine receptor type 2 (CCR2), recruiting permissive, non-bactericidal, second-responder monocytes to the HBV. The subversive PGL signal leads to bacilli transfer from bactericidal RMs to these newly recruited growth-permissive monocytes, thus allowing *M. marinum* to escape killing and proliferate. By contrast, PGL-deficient *M. marinum* bacilli are eventually cleared by first-responder RMs.

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