



Tailor made: New insights into lipoarabinomannan structure may improve TB diagnosis

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Detecting the mycobacterial glycolipid lipoarabinomannan (LAM) in urine by anti-LAM antibodies fills a gap in the diagnostic armamentarium of much needed simple rapid tests for tuberculosis, but lacks high sensitivity in all patient groups. A better understanding of LAM structure from clinically relevant strains may allow improvements in diagnostic performance. De *et al.* have recently determined the structures of LAM from three epidemiologically important lineages of *Mycobacterium tuberculosis* and probed their interaction with an anti-LAM monoclonal antibody. Their results not only identify a series of tailoring modifications that impact antibody binding but also provide a roadmap for improving U-LAM-based diagnostics.

A host of mycobacterial species are found in nature, ranging from environmental organisms that are nonpathogenic in most humans to those that cause serious disease (1). The disease tuberculosis (TB), an age-old scourge, arises from infection by the most notorious of all mycobacteria, *Mycobacterium tuberculosis* (*Mtb*). Around a quarter of the world is estimated to have latent *Mtb* infection, and 5 to 10% develop disease over their lifetime, causing over 10 million new cases with 1.5 million associated deaths annually (2). A characteristic feature of mycobacteria is their unusual cell wall, which is composed primarily of polysaccharides and glycolipids with unique and atypical structures (3). These molecules protect the organism from its environment, modulate the host immune response during infection, and play important roles in the pathogenesis of *Mtb* infection (3).

A major component of the mycobacterial cell wall is the glycolipid lipoarabinomannan (LAM), which constitutes 15% of the *Mtb* mass and is a potent immunomodulator and inducer of antibodies (4). Moreover, detection of LAM in urine (U-LAM) by anti-LAM antibodies is the basis of a simple TB point-of-care diagnostic (5). Historically, the utility of this diagnostic has been limited to individuals with suppressed immunity, for example, HIV⁺ individuals, because they have higher *Mtb* burden and thus a higher amount of

antigen shed into the urine, although recent improvements in U-LAM detection have resulted from the generation and use of high-affinity anti-LAM mAbs (6), leading to increased sensitivity in HIV⁺ and HIV⁻ individuals (5, 7). The attractiveness of a U-LAM-based TB diagnostic has catalyzed interest in generating new anti-LAM antibodies to improve performance even further (8). Knowledge of the immunogenic epitopes in LAM and those most commonly displayed in U-LAM is essential to achieve this goal. However, detailed structural work on *Mtb* LAM has been carried out only for the H37Rv laboratory *Mtb* strain. Scientists in the field have thus wondered if unknown differences between LAM produced by H37Rv and clinical strains are hindering the development of improved anti-LAM mAbs for U-LAM detection and other applications. A recent JBC paper by De *et al.* (9) addresses this knowledge gap. Through the combined use of nuclear magnetic resonance spectroscopy, mass spectrometry, and enzymatic digestion, the authors have completed the arduous task of characterizing LAM from three clinical *Mtb* strains using H37Rv LAM as a reference. The clinical strains selected are representatives from three of the seven global disease-causing lineages (L1–L7), which are strongly correlated with geography. Those studied include one strain from a lineage geographically associated with East Asia (L2) and two strains from lineages (L1 and L3) present in areas surrounding the Indian Ocean.

Lipoarabinomannan has a tripartite structure (3, 5), with a lipidated mannan core composed of D-mannopyranose (Man_p) residues, a D-arabinofuranose-containing arabinan, and capping motifs decorating the termini of the arabinan (Fig. 1). The identity of the capping motifs, and the degree of capping, varies from species to species. In *Mtb*, the predominant capping motifs are Man_p oligosaccharides, some of which are further modified by a single 5-methylthioxylofuranose residue. The study highlighted here focused on the structure of the arabinan termini and capping motifs, which are the epitopes recognized by the majority of the anti-LAM mAbs characterized to date (6). A major, and perhaps unexpected, conclusion of this study is that the glycan structures of the LAM from the three clinical strains are similar to that produced by H37Rv. The core mannan and arabinan domains are essentially the same, although the degree of Man_p-capping is lower in the

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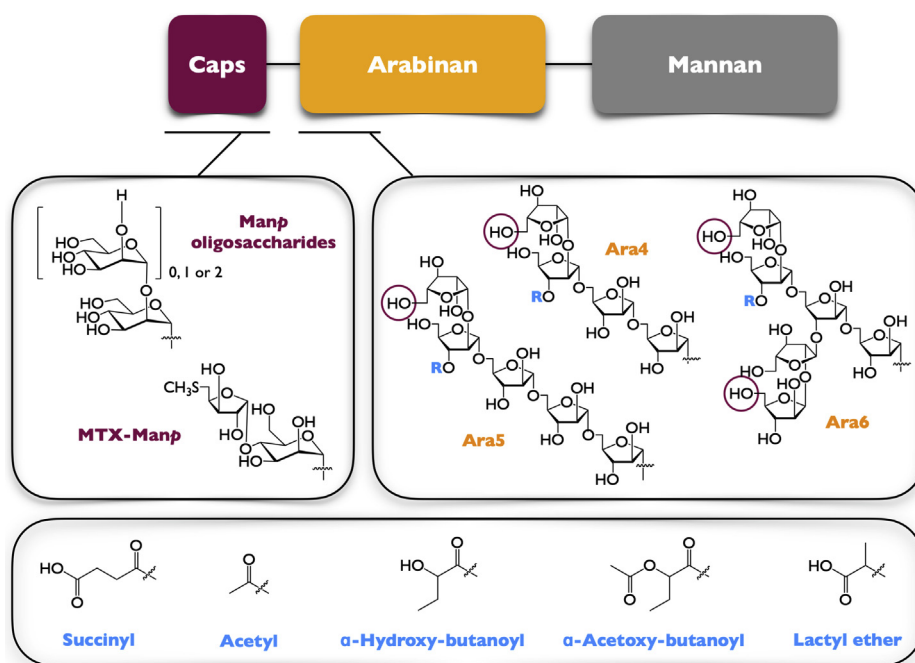


Figure 1. Top, schematic drawing of mycobacterial LAM showing tripartite structure consisting of the mannan domain, arabinan domain, and capping motifs. Middle left, capping motifs found in *M. tuberculosis* LAM, Manp oligosaccharides, and the MTX-Manp motif. Middle right, termini in arabinan domains including Ara4, Ara5, and Ara6; maroon circles indicate attachment sites of the capping motifs, and location of tailoring substituents are indicated by R groups in aqua. Bottom, structures of tailoring modifications identified in highlighted paper. The stereochemistry of the stereogenic center in the α -hydroxy-butanoyl, α -acetoxy-butanoyl, and lactyl ether substituents is undetermined. LAM, lipoarabinomannan; MTX, 5-methylthioxylofuranose.

clinical strains (49.5–63.0%) compared to H37Rv (72.8%). On the other hand, the presence and degree of LAM ‘tailoring’ modifications differ considerably. Prominent among these modifications is acylation, with the most significant being the addition of a succinate residue, although acetate and α -hydroxy/ α -acetoxy-butyrate modifications are also present, as are ether-linked lactyl groups. All of these tailoring functionalities are linked to the O-3 position of D-arabinofuranose-residues in one of the three glycan sequences located at the nonreducing termini (Ara4, Ara5, and Ara6) (Fig. 1). The degree of substitution of these modifications ranges from 0.1% to 90%, with succinylation being found, on average, on ~45% of these motifs in LAM from the clinical strains, but only ~30% in H37Rv. Although succinylation has previously been found in LAM, its presence was predominantly thought to be limited to the internal regions of the arabinan, not the termini (3).

The impact of the tailoring modifications was evaluated through the binding of the four LAMs to the high-affinity anti-LAM mAb CS-35, generated from *Mycobacterium leprae*-immunized mice, and the only mAb for which there is published X-ray crystallographic support detailing epitope-binding (10). Computational docking of Ara6 and the corresponding succinylated derivative in the binding site of the CS-35 crystal structure revealed that the latter binds in an inverted orientation, presumably because of succinylation-induced steric hindrance. In full-length LAM, which contains a much larger glycan, binding in this orientation may not be possible because of steric clashes between other parts of the ligand and the mAb. Chemical deacylation of the LAMs led to enhanced binding by CS-35, further supporting the deleterious effect that acylation has on binding.

These results suggest two approaches for improving the performance of U-LAM-based TB diagnostics. One is sample deacylation before testing, and the second is developing new mAbs that specifically recognize these acylated glycan motifs. With regard to the latter approach, although some of these motifs are present in small amounts, they may nevertheless lead to the generation of useful mAbs. In this respect, it is important to note that the recent improvements in U-LAM detection (7) involve a ‘capture mAb’ that specifically recognizes the 5-methylthioxylofuranose motif, present only at a ratio of 1 to 2 per LAM molecule.

With a better structural understanding of LAM from these three clinical strains, many future studies can be anticipated. In addition to generating and possibly tailoring new mAbs for U-LAM-based diagnostics, and potentially improving current assays by sample deacylation, the biological importance of these motifs in *Mtb* infection and their abundance in U-LAM during TB should be studied, as should the pathways by which they are added to the polysaccharide. Whether these results could impact the development of arabinomannan/LAM-based TB vaccines, an area of increasing interest (4), through providing new haptens (small molecules that induce antibodies when bound to larger particles), warrants investigation. It also would be of interest to characterize the LAM structures from other clinical strains, in particular from the L4 to L7 lineages, which were not the focus of the present study.

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Abbreviations—The abbreviations used are: LAM, lipoarabinomannan; *Mtb*, *Mycobacterium tuberculosis*; TB, tuberculosis.

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