Time to Expand the Picture of Mycobacterial Lipids: Spotlight on Nontuberculous Mycobacteria

One of the key characteristics that distinguish mycobacteria from other bacteria is the lipid richness of their cell wall, which can comprise up to 60% of the cell mass, compared with 20% in gram-negative bacteria (1). The vast majority of mycobacteria are primarily found in the environment, and the lipid-rich cell wall of these nontuberculous mycobacteria (NTM) allows them to survive in rough environments, ranging from desert soil to municipal water supply systems. In *Mycobacterium tuberculosis* (MTB), which has adapted to the human species as a primary host so well that it is not an environmental organism anymore, lipid constituents of the cell wall were recognized early on as crucial factors in its virulence. A prominent example is trehalose dimycolate (TDM), better known as the cord factor, which has been recognized as a key virulence factor with a plethora of immunological effects, including granuloma formation and induction of a vast number of cytokines (2). Subsequently, a diverse array of lipid molecules were identified, resulting in a complex picture of various lipid molecules and their interaction with host immune cells. Although we have learned much about MTB, we are just beginning to understand the role of different cell wall compounds in NTM pathogenesis. One might say that in contrast to the large canvas painting of MTB, the NTM counterparts still fit on the pages in a sketchbook, and we have just started to add them together to form a cohesive picture (2).

Cathelicidin LL-37, an antimicrobial peptide, is an important effector molecule of innate immunity. It is produced by phagocytes and epithelial cells, and plays an important role in the control of MTB infection. Its antimicrobial activity has been associated with its capacity to bind to the MTB cell wall and form pores, leading to mycobacteria lysis (3). This ancient antimicrobial mechanism has evolved over a long time, and, interestingly, only a few bacterial species have developed strategies to evade this mechanism. In MTB, for example, a *lysX* gene–encoded mechanism whereby lysine residues are added to surface phospholipids, leading to a change in their net charge and thus repelling antimicrobial peptides, including cathelicidin, has been well described (4). Indeed, the amount of LysX protein produced by MTB is an important modulator of MTB virulence (5).

Although LL-37 has potent antimicrobial activity against MTB, clinically relevant species of NTM are resistant to the effect of LL-37. Moreover, some pathogenic isolates are able to inactivate this cathelicidin. NTM-specific cell envelope components are suspected to be the cause of this resistance. Among the leading suspects were the glycopeptidolipids (GPLs), which are lipid molecules that are unique to the cell wall of several NTM species and are not found in MTB. These components have been associated with colony morphology and biofilm formation; however, somewhat against expectation, it was found that they are not responsible for NTM resistance to LL-37 (6).

In this issue of the *Journal*, Honda and colleagues (pp. 354–363) provide new insight into this issue (7). In the first set of experiments in their study, they demonstrated that virulent NTM isolates of different species were capable of neutralizing the antibacterial activity of LL-37. Furthermore, they found a correlation between NTM's capacity to neutralize LL-37 and its ability to survive in human immune cells. Most of these experiments were conducted in a THP-1 macrophage system. The authors then validated their findings in a subset of experiments using human monocyte-derived and alveolar macrophages. Remarkably, they found that these cathelicidin-neutralizing factors are soluble, as supernatants from LL-37–treated NTM cultures were unable to kill LL-37–susceptible *Escherichia coli* strains despite the considerable abundance of LL-37. Given that NTM pulmonary infections are often polymicrobial in nature, the NTM-mediated inactivation of LL-37 could benefit other bacteria that are susceptible to its antimicrobial activity. Clinical reports are inconclusive in this regard: although patients with pulmonary NTM infections are often coinfected with other bacteria, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* (8), observations from large registries suggest that NTM infection is negatively correlated with coisolation of other bacteria (9). Because we are just beginning to understand the complex role of the pulmonary microbiota in mycobacterial disease (10), the authors' observation adds to our evolving understanding of host defense mechanisms in NTM.

In further experiments, using TLC of total lipid extracts (*M. chimera*) and various lipid fractions (*M. intracellulare* and *M. abscessus*) combined with IB for LL-37, the authors demonstrated that LL-37 binds specifically to the polar fraction of NTM lipids, which are part of all mycobacterial cell walls, including phosphatidylinositol (PI). Furthermore, mass spectrometry of BAL fluid sampled from a patient with *M. intracellulare* infection demonstrated that PI was the most abundant fraction. Mass spectrometry of *M. abscessus*–conditioned medium using biotinylated LL-37 confirmed the specific binding to phospholipids. To identify individual candidates, the authors used synthetic soy lysophosphatidylinositol as well as synthetic cardiolipin (CL), phosphoethanolamine, PI mannosides (PIM), and phosphocholine as surrogate molecules in parallel with mycobacterial lipid extracts. Interestingly, both CL (which is one of the most abundant phospholipids in mycobacteria) and PIM (which is the basic subunit of lipoarabinomannan) showed a significant interaction with LL-37, with CL having the strongest interaction. This was confirmed in an *E. coli*

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bioassay, in which CL effectively inactivated LL-37. These data make a strong case for these candidate phospholipids—just short of proof obtained by using purified individual lipids from NTM cultures instead of the synthetic lipids used by the authors.

These findings are surprising, given that the identified candidate lipids are a ubiquitous part of *all* mycobacterial cell walls (in contrast to the aforementioned GPLs, which are found only in NTM), including in MTB, whereas the neutralizing function is restricted to certain NTM species. The authors hypothesize that the relative abundance of these phospholipids may vary among species, leading to differences in their neutralizing capability. Future studies should allow the construction of a more nuanced picture of the role of species-dependent differences in lipid composition.

Clinically significant NTM disease is a growing concern, as its incidence is increasing and antimicrobial treatment options are limited (11). Most of the species designated as NTM are usually not pathogenic to normal hosts, and significant attention has been directed to host factors such as underlying structural lung disease, impaired mucociliary clearance, and immune function (12). To complement this picture, we need to gain a better understanding of pathogen-related factors, and the current study provides valuable information toward that end. Strain-specific markers that can help identify a strain's potential for virulence could be used to select patients early on who would benefit from antimicrobial therapy. In addition, along with any newly discovered pathogenic mechanism comes the hope that it may represent a novel therapeutic target. Aside from its role as a host defense peptide, LL-37 has been implicated in autoimmune diseases such as psoriasis and inflammatory bowel disease, as well as in cancer (13). Although much remains to be learned about its function in these disease entities, it is known to act as an immune modulator, and has been found to be upregulated in ovarian, breast, and lung cancer tissue, where it has been associated with angiogenesis and cell proliferation (14). It is intriguing that CL and PIM are able to alter its activity, and one may speculate that this could have therapeutic potential for those conditions as well.

Overall, although the current findings are intriguing, it is too early to firmly conclude that the cell wall composition of bacteria determines their propensity to cause invasive disease. We agree with the authors that future studies are needed to correlate markers of *in vitro* infectivity with clinical and radiographic disease severity. We hope that by the time these clinical correlations are established, we will be able to bring the sketchbook drawings of NTM lipidology onto the canvas to paint a meaningful picture, using lipid-containing oil paint (15). ■

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