Lipid A Is More than Acyl Chains

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The acyl chain length, number, and distribution have been considered the major factors contributing to this biological activity of lipid A. The charged head groups on the dihexosamine backbone have also been implicated in contributing to this biology. In *Neisseria*, it has now been shown that loss of the 4' phosphoethanolamine has an impact on virulence in an animal model and on the organism's susceptibility to cationic antimicrobial peptides. Such studies offer potential insight into targets for novel antimicrobial agents.

In this issue of *Infection and Immunity*, Packiam and coworkers (1) have demonstrated that phosphoethanolamine decoration of gonococcal lipid A both protects the organism from innate immune factors and is involved in the induction of immunostimulatory factors. These investigators have shown that purified lipooligosaccharide (LOS) from a phosphoethanolamine transferase (*lptA*) mutant and an *lptA* LOS mutant strain induced significantly lower levels of NF- κ B in tissue murine embryonic fibroblasts and in a female mouse model of lower genital tract infection than LOS from the wild type. In addition, the mutant strain was more sensitive to human and murine cathelicidins. Previous studies had shown that the *lptA* mutant was less fit than the wild-type strain in an experimental model of competitive gonococcal infection in men (2).

The LOSs of *N. gonorrhoeae* has been shown to play a role in the pathogenesis of human infections (3–5). The LOS is composed of three major components: the oligosaccharide chain extensions, the core region, and lipid A (6–8). The oligosaccharide extensions of the LOS contain determinants that resemble human glycosphingolipid antigens, which play a role in molecular mimicry (9–11). *Neisseria gonorrhoeae* lipid A has a dihexosamine backbone and a hexa-acyl structure (composed of two lauric, two hydroxymyristic, and two myristic acid residues) similar to that of enterobacterial lipopolysaccharide (LPS). The 4' position of the dihexosamine backbone is replaced with a phosphoethanolamine. This head group structure has been shown by a number of groups to impart a number of important biological features on the gonococcus. It has been shown to be important in the resistance of the organism to cationic antimicrobial peptides, in killing by normal human serum, and in stimulating an inflammatory response (12–17).

The importance of the charged LPS head groups on membrane integrity has been known for many years. Leive showed that treatment with EDTA resulted in a loss of approximately 50% of the LPS from the *Escherichia coli* outer membrane and a resultant increase in the permeability of the cell (18). This resulted from a loss of the divalent cations involved in linking the LPS structures to each other and to membrane proteins. Using synthetic lipid A structures, Kotani and collaborators showed that diphosphoryl structures (e.g., compound 506) were a significantly more potent mitogen and pyrogen than a structure with a monophosphoryl structure (e.g., compound 504) or a nonphosphorylated structure (e.g., compound 505) (19, 20). In 1985, Peterson and coworkers showed that polycationic antibiotics and polyamines bound to head groups, resulting in disruption of the integrity of the outer membrane of *Pseudomonas aeruginosa* (21). Removal of the head

group phosphorylation has been shown to reduce inflammation in *Neisseria* commensals (14). Recently, Kong and coworkers have shown that the phosphate groups of the lipid A of *Salmonella enterica* serovar Typhimurium impact both innate immunity and virulence in mice (22).

The role of acyl chains and head groups on the inflammatory response initiated by lipid A has been elucidated by Seydel and coworkers, who have determined its conformation and supramolecular structure (23–26). Using biophysical approaches, Seydel and his group have written extensively about the relationship between the conformation of lipid A and its ability to act as an agonist and antagonist (23). They have shown that lipid A modifications in the acyl chain number, distribution of the chains (symmetrical or asymmetrical), and head group substitution were accompanied by a change in the tilt angle of the backbone with respect to the acyl chains, which impact the conformation of the lipid A structure and, potentially, the ability to engage Toll-like receptor 4 (TLR4). They proposed that only those lipid A molecules that assume a conical shape were biologically highly active and that structures assuming a cylindrical shape were antagonistic (23). The loss of head groups causing changes in membrane integrity and the reduction in the ability of modified lipid A to interact with TLR4 most probably explain the loss of the inflammatory response and the enhanced activity of the cationic antimicrobial peptide on the organism.

The paper by Packiam et al. has implications for the development of novel approaches to therapy for gonococcal infections. The recent evolution of antimicrobial resistance to all of the drugs introduced for the treatment of gonorrhea as well as organisms resistant to multiple antibiotics has made the need for new agents imperative (27–29). Studies such as this paper and a previous manuscript by Hobbs and coworkers (2), which combine basic experimentation with *in vivo* analysis of outcome, are crucial in logical antibiotic design. Such approaches will give us new targets for antimicrobial development and, hopefully, new para-

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digms for treatment (multidrug therapies) to control the increasing problem of antimicrobial resistance in gonococcal infections.

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