Endotoxin neutralization by lactoferricin-derived antimicrobial peptides

J. Andrä, J. Howe, M. Rössle\textsuperscript{2}, and K. Brandenburg

Forschungszentrum Borstel, LG Biophysik, Parkallee 10, 23845 Borstel, Germany
\textsuperscript{2}European Molecular Biology Laboratory, Hamburg Outstation, Notkestr. 85, 22603 Hamburg, Germany

Lipopolysaccharide (LPS), if released from the outer membrane of Gram-negative bacteria during bloodstream infections, is causative for sepsis and septic shock syndrome, and thus is denominated endotoxin. Septic shock is characterized by an massive activation of human immune cells by LPS to produce proinflammatory cytokines leading to an overbording systemic response of the immune system and claims 60,000 death annually in Germany particularly in intensive care units. Mortality is extremely high since antibiotic therapy may even enhance the liberation of LPS and no specific therapy to prevent its immune stimulatory function is available.

LPS of Enterobacteriae like \textit{Salmonella} and \textit{E. coli} consists of a N-acetyldiglucosamine backbone with 6 to 7 acyl chains in ester or amide linkage, referred to as lipid A and which anchors the molecule in the bacterial membrane, linked to a polysaccharide moiety (Fig. 1). The endotoxic, i.e. immune stimulatory activity, of LPS is only modulated by the polysaccharide part of the molecule but is located in the lipid A part, which consequently is referred to as the \textit{endotoxic principle} of LPS.

Interesting candidates for a therapeutic intervention are natural antimicrobial peptides (AMP). These amphipathic and cationic compounds are part of the innate immune system of virtually all species. They may act in a dual way: killing of bacteria and \textit{en passant} neutralization of thereby released bacterial LPS. Based on a 11 amino-acid residue stretch (LF11, [1]) of human lactoferricin, an antibacterial and LPS-neutralizing fragment of lactoferrin, we have designed a number of synthetic derivatives with significantly improved activity. These peptides bind to and prevent the LPS-induced activation of \textit{ex vivo} isolated human immune cells. LPS is an amphiphilic molecule and forms stable aggregates in solution which are the endotoxically active units. Immune cell stimulation involves the LBP-mediated intercalation of LPS-aggregates into the plasma membrane and subsequent activation of a transmembrane receptor complex. Decisive for the cell-activation properties of LPS are negatively charged phosphates at the sugar headgroup combined with a certain threedimensional structure of the LPS-aggregates, which is linked to a distinct shape of the lipid A portion, compiled in the ’conformational concept of endotoxicity’ [2]: Endotoxically active hexa- and heptaacyl lipid A have a conical molecular conformation and form unilamellar / inverted cubic aggregate
structures, whereas inactive tetra- and pentacyl lipid A are of cylindrical conformation and form multilamellar aggregates. To understand the molecular basis of LPS-inactivation by AMP it is of utmost importance to analyze the peptide-induced modulation of the properties of LPS-aggregates. In extension to our previous studies with lipid A and deep rough mutant LPS Re, which both lack a significant oligosaccharide chain (Fig. 1), here we have investigated the influence of LF11-derived peptides on the supramolecular structure of LPS Ra, the physiologically relevant chemotype of LPS (Fig. 1). For the first time we could prove that LPS Ra, like lipid A, forms a unilamellar / cubic supramolecular structure (Fig. 2). Active peptides, in a sense of LPS-neutralization, compensate the negative surface charge of LPS-aggregates and convert them into a multilamellar stack (Fig. 2). LPS in such a molecular organization no longer intercalates into the immune cell membrane and no longer stimulates the cells. Loss of charge-charge interactions and of binding epitopes for interacting proteins, or the enhanced binding energy of the LPS-monomers within the aggregate may account for the effective neutralization of LPS Ra by the peptides.

![Figure 2: Small-angle X-ray scattering (SAXS) patterns of rough type LPS Ra without peptide (lower curve) and in the presence of a lactoferricin-derived peptide at equimolar concentration and indicated temperatures.](image)

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**References**
