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Sensing Gram-negative bacteria: a phylogenetic perspective

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Gram-negative bacteria represent a major group of pathogens that infect all eukaryotes from plants to mammals. Gram-negative microbe-associated molecular patterns include lipopolysaccharides and peptidoglycans, major immunostimulatory determinants across phyla. Recent advances have furthered our understanding of Gram-negative detection beyond the well-defined pattern recognition receptors such as TLR4. A B-type lectin receptor for LPS and Lysine-motif containing receptors for peptidoglycans were recently added to the plant arsenal. Caspases join the ranks of mammalian cytosolic immune detectors by binding LPS, and make TLR4 redundant for septic shock. Fascinating bacterial evasion mechanisms lure the host into tolerance or promote inter-bacterial competition. Our review aims to cover recent advances on bacterial messages and host decoding systems across phyla, and highlight evolutionarily recurrent strategies.

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Introduction

Gram-negative bacteria form a major group of pathogens that affect a broad range of hosts, from plants to vertebrates. They differ from Gram-positive bacteria by an additional outer lipid membrane that makes them resistant to the action of lysozymes. Because of this additional layer of defence, they represent a special challenge to the host immune system. Here we review the mechanisms used to recognize Gram-negative bacteria as microbial agents and to distinguish them from other microbes.

Bacterial mixed messages — immune triggers and evasion

Gram-negative bacteria protect themselves against environmental and host attacks by a double layer of inner peptidoglycans (PGN) and outer lipopolysaccharides (LPS) (Figure 1). Break-down products of this cell wall are microbe associated molecular patterns (MAMPs). Sensing of MAMPs by host pattern recognition receptors (PRRs) elicits immune responses. To scramble the MAMP message, bacteria and especially virulent species have evolved ingenuous evasion mechanisms to hide from their hosts.

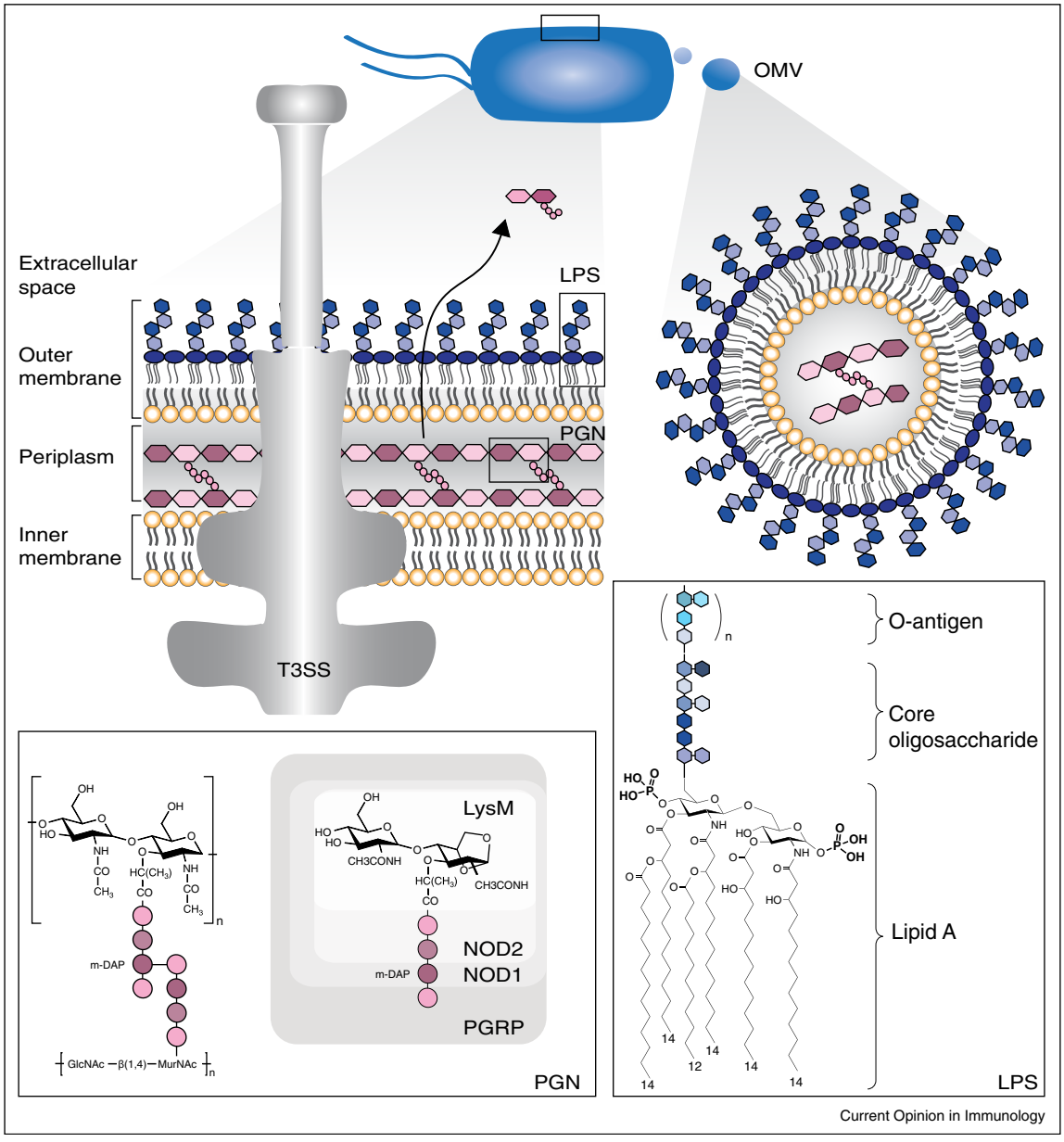
Lipopolysaccharides and peptidoglycans

Central to Gram-negative detection is lipid A, the hydrophobic core of lipopolysaccharide (LPS) (Figure 1). As detailed below, LPS is recurrently targeted by immune receptors throughout evolution to sense Gram-negative bacteria. The second Gram-negative give-away is DAP-type peptidoglycan (PGN), which forms the cell wall of all Gram-negative bacteria and Gram-positive bacilli, while other Gram-positive bacteria contain Lysine-type PGN. In intact bacteria, the outer layer of LPS prevents direct access of immune receptors to PGN. However, bacterial cell wall remodelling releases small peptidoglycan fragments called muropeptides that are not always recycled at full capacity. In addition, type III secretion systems (T3SS) translocate muropeptides along with other virulence factors. Because cell wall remodelling and deployment of secretion systems are hallmarks of actively dividing or invading bacteria, sensing DAP-type muropeptides offers a specific way to detect live as opposed to dead bacteria [1].

Other MAMPs such as bacterial DNA or di-GMP are evolutionarily common in immune detection of bacteria, but as they are not specific for Gram-negative bacteria, they will not be addressed here. Flagellin is a particular case as it occurs in both Gram-positive and Gram-negative bacteria, but reaches the host cytosol more frequently in Gram-negatives as a side effect of type III secretion. Secretion systems, a prominent feature in Gram-negatives, and release of outer membrane vesicles (OMVs), are additional MAMPs or MAMP carriers whose components are sensed by various host receptors. A recent addition to Gram-negative specific MAMPs is the LPS metabolite heptose-1,7-bisphosphate, which triggers immune activation through a novel cytosolic PRR pathway [2••].

Bacteria that infect a broad variety of hosts, including plants, invertebrates and humans, express genes that

Figure 1



Gram-negative bacteria contain a double-layered cell wall consisting of inner and outer membranes surrounding the periplasmic space. Within the periplasmic space, the peptidoglycan layer is cross-linked to inner and outer membranes by proteins. The outer leaflet of the outer membrane contains lipopolysaccharides. Secretion systems such as the Gram-negative specific type III secretion needle serve as translocation pores for virulence factors. Outer membrane vesicles are derived from the outer membrane and can carry cell wall components as well as effectors. *LPS box:* Lipid A is usually hexa-acylated, but acyl chain number and length varies and defines the agonistic or antagonistic properites of LPS [34]. Lipid A is phosphorylated and decorated with hydrophilic carbohydrates (core oligosaccharide) that confer net negative charge. The highly variable external O-antigen defines serotypes for many Gram-negative pathogens. Cationic AMPs attach to the outer layer of bacteria through hydrophilic interactions, creating pores. To prevent lysis, bacteria have evolved mechanisms to hide the negative charge of LPS, either by attaching positively charged carbohydrates or by removing the negatively charged phosphate groups [3]. Modifying the number and length of acyl chains is another escape strategy. *PGN box:* Peptidoglycan is a cross-linked polymer of alternating N-acetylglucosamine and N-acetylmuramic acid sugars, cross-linked by short peptide bridges that confer rigidity and impermeability to the bacterial envelope [61]. Peptidoglycan is assembled de novo from bacterial synthetic enzymes, or recycled from enzymatic breakdown of existing cell wall. A peculiar feature of the released muropeptides is the presence of an anhydro bond on the sugar moiety that results from the action of bacterial transglycosylases. The anhydro GlucNac-MurNac-tetrapeptide entity, historically called tracheal cytotoxin (TCT) because it affects the tracheal epithelium in *Bordetella*-infected hosts, is a particularly efficient immunostimulant that some Gram-negative pathogens release as a virulence factor (e.g. *Bordetella pertussis*). PGN-sensing PRRs vary in their structural requirements: NOD2 detects muramyl dipeptide, which is indistinguishable between Gram-negative and Gram-positive bacteria, while sensing by NOD1 requires the meso-DAP residue in the stem peptide, conferring specificity for Gram-negative bacteria. Sensing by Insect PGRPs involves the recognition of both the N-acetylglucosamine and N-acetylmuramic acid sugars and three amino acids, allowing the discrimination between DAP-type and Lysine-type bacteria. Plant LysM receptors require only the carbohydrate backbone and do not distinguish Gram type.