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Drug discovery strategies to outer membrane targets in Gram-negative pathogens

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ABSTRACT

This review will cover selected recent examples of drug discovery strategies which target the outer membrane (OM) of Gram-negative bacteria either by disruption of outer membrane function or by inhibition of essential gene products necessary for outer membrane assembly. Significant advances in pathway elucidation, structural biology and molecular inhibitor designs have created new opportunities for drug discovery within this target-class space.

1. Introduction

The outer membrane (OM) of Gram-negative bacteria poses a significant barrier to unwanted molecules from entering the cell and thus accumulating to toxic levels inside of the pathogen.[1] Since the OM serves as a protective barrier, disruption or interference with the biogenesis of the OM presents an attractive strategy for antibacterial drug discovery. These strategies can be viewed as leading to both direct antibacterial agents which shut-down essential mechanisms, as well as agents that are not intrinsically active by themselves, but work by increasing the potency of other antibacterial compounds through weakening of the cell wall structure. This review will only address those mechanisms which have known molecular inhibitors, thus the reader is directed to a more thorough reviews of the OM biology for other pathways of interest of which are largely unexploited in drug discovery settings.[1–4]

The specific pathways covered in this review are depicted in Figure 1. These pathways are divided into three major categories which are the following; (a) lipopolysaccharides (LPS), (b) outer membrane proteins (OMP), and (c) lipoproteins. A fourth major class of membrane architecture involves the biogenesis and transport of phospholipids, but little is known about this area relative to these other mechanisms.[3] Finally, the synthesis of peptidoglycan may represent a fifth major class and is inhibited by well-known and traditional classes of antibiotics such as β-lactams which inhibit penicillin-binding proteins,[5] and glycopeptides such as vancomycin which bind to *N*-acyl-D-ala-D-ala peptidoglycan precursors.[6] Peptidoglycan is found in the periplasmic space in Gram-negative pathogens and is presumed to provide rigidity to the overall cell wall membrane. An excellent review of this area has recently been published by Silver and will not be covered in this text.[7]

A distinguishing feature of Gram-negative pathogens is not only the double-membrane architecture, but the presence of LPS extruding from the outer-membrane. LPS is highly variant among Gram-negative pathogens, but is made of the basic elements of Lipid A, core polysaccharides and O-antigen repeats (**4**, Fig. 2).[8,9] LPS is highly functionalized with anionic charges, and subsequently cross-linked to create a large defensive web which can block the entry of molecules as a first line of defense for the bacteria. Furthermore, LPS itself is antigenic and strongly stimulates the innate immune system, thus serving as not only a defensive mechanism, but an offensive mechanism for bacterial survival.[10]

The synthesis of LPS involves nine conserved enzymes within the cytosolic inner membrane (LpxA-M and KdtA),[11] which result in the formation of Lipid A, an essential component of LPS (Fig. 2). Transport of LPS across the inner membrane is facilitated by MsbA, a lipid-activated ATP-ase which is proposed to function as a flippase leading to proper orientation of budding LPS towards the periplasmic and OM transport system.[12,13] The transport from the inner membrane to the OM is accomplished with the lipopolysaccharide transport system (Lpt). The LptBCFG complex is an ATP-driven mechanism which results in delivery of LPS to

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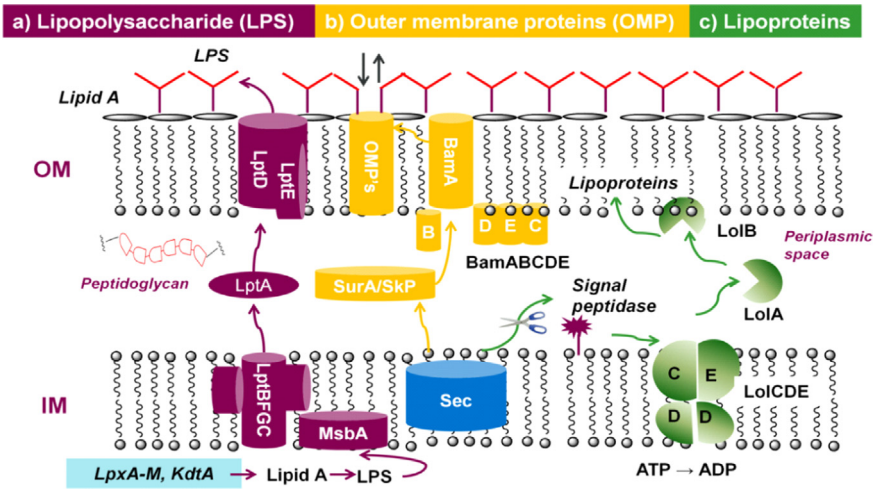

Figure 1. Schematic of the outer membrane (OM) processes discussed within this review.[8–16] The lipopolysaccharide (LPS) pathway involves synthesis of Lipid A by the LpxA-M and KdtA enzymes. Lpt = lipopolysaccharide transport system which culminates in the outer membrane delivery of LPS. The secretory system (Sec) is involved in both the secretion of proteins such as outer membrane proteins (OMP) as well as lipoproteins. The β-barrel assembly machinery (BamA-D) is responsible for the folding and insertion of β-barrel proteins which regulate transport of molecules across the OM. Lipoproteins are directed by protein signals which are cleaved by signal peptidases, and then passed from the inner membrane (IM) to the OM via the localization of lipoprotein pathway (LolA-E).

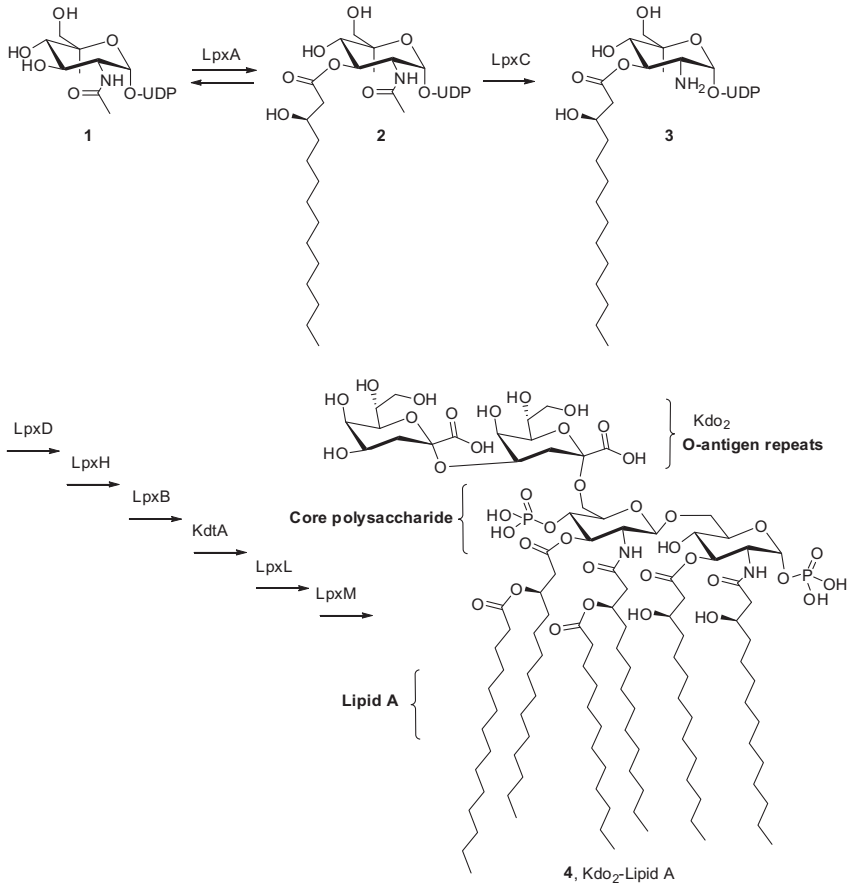

Figure 2. Synthesis of LPS.[12]

the periplasmic space, where LptA then serves as a chaperone to the LptD/E complex.[14] LptD is a β-barrel protein which delivers LPS to is final port-of-call, the OM.[15] Once LPS is on the outer membrane, significant cross-linking by divalent cations of Mg^{2+} to the negatively charged phosphate groups finalize the interlocked defensive barrier.[16,17]

The second major pathway outlined in Figure 1 is the Sec/Bam pathway which is responsible for outer membrane protein (OMP) β-barrel assembly (Bam) and trafficking.[18] These β-barrel OMPs are important for passage of selected molecules across the OM as well as for the structure and architecture of the OM.[19,20] This pathway utilizes the Sec transport mechanism for introduction into the