Biochimica et Biophysica Acta 1848 (2015) 3026–3031

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Review

Antimicrobial peptide resistance in *Neisseria meningitidis*☆

Yih-Ling Tzeng [a], David S. Stephens [a,b,*]

[a] Department of Medicine, Emory University School of Medicine, Atlanta, GA 30322, USA
[b] Laboratories of Microbial Pathogenesis, Medical Research Service, Veterans Affairs Medical Center, Decatur, GA 30033, USA

ARTICLE INFO

Article history:
Received 20 November 2014
Received in revised form 6 May 2015
Accepted 12 May 2015
Available online 19 May 2015

Keywords:
Neisseria meningitidis
Antimicrobial resistance
Efflux pump
Phosphoethanolamine modification of lipid A

ABSTRACT

Antimicrobial peptides (AMPs) play an important role as a host defense against microbial pathogens and are key components of the human innate immune response. *Neisseria meningitidis* frequently colonizes the human nasopharynx as a commensal but also is a worldwide cause of epidemic meningitis and rapidly fatal sepsis. In the human respiratory tract, the only known reservoir of *N. meningitidis*, meningococci are exposed to human endogenous AMPs. Thus, it is not surprising that meningococci have evolved effective mechanisms to confer intrinsic and high levels of resistance to the action of AMPs. This article reviews the current knowledge about AMP resistance mechanisms employed by *N. meningitidis*. Two major resistance mechanisms employed by meningococci are the constitutive modification of the lipid A head groups of lipooligosaccharides by phosphoethanolamine and the active efflux pump mediated excretion of AMPs. Other factors influencing AMP resistance, such as the major porin PorB, the pilin biogenesis apparatus, and capsular polysaccharides, have also been identified. Even with an inherently high intrinsic resistance, several AMP resistance determinants can be further induced upon exposure to AMPs. Many well-characterized AMP resistance mechanisms in other Gram-negative bacteria are not found in meningococci. Thus, *N. meningitidis* utilizes a limited but highly effective set of molecular mechanisms to mediate antimicrobial peptide resistance. This article is part of a Special Issue entitled: Bacterial Resistance to Antimicrobial Peptides.

Contents

1. Introduction

Neisseria meningitidis, the meningococcus, is a Gram negative aerobic encapsulated diplococcal β-proteobacterium. Meningococci are carried asymptomatically by 5 to 10% of the overall population in non-epidemic periods and are transmitted from a carrier by aerosol droplets or respiratory secretions. *N. meningitidis* is unique among the major bacterial agents of meningitis in that it causes epidemic as well

☆ This article is part of a Special Issue entitled: Bacterial Resistance to Antimicrobial Peptides.

* Corresponding author at: Woodruff Health Sciences Center, 1440 Clifton Road, NE, Atlanta, GA, 30322, USA. Tel.: +1 404 727 8357.
E-mail address: dstep01@emory.edu (D.S. Stephens).

http://dx.doi.org/10.1016/j.bbamem.2015.05.006
0005-2736/

as endemic (sporadic) disease. Approximately 500,000 cases of invasive meningococcal disease have occurred annually worldwide, with at least 50,000 deaths and as many survivors suffering neurological sequelae [1]. The meningococcus causes a range of disease: rapid onset meningitis and severe sepsis (meningococcemia), septic arthritis, pneumonia, purulent pericarditis, conjunctivitis, otitis, sinusitis, and urethritis. Meningococci are classified by serologic typing based on the biochemical composition of the capsular polysaccharides (serogroup), major outer membrane porin proteins (serotype), other outer membrane proteins (serosubtype), and lipooligosaccharide (immunotype). Of the 12 serogroups identified, almost all of invasive cases are caused by meningococci that express one of six capsular polysaccharides (serogroups A, B, C, X, Y, and W) and most epidemic and endemic cases of meningococcal disease are caused by a limited number of clonal groups defined genetically using multilocus sequence typing (MLST). The US licensed vaccines against *N. meningitidis* are based on capsular polysaccharides (CPS) with the more recent development of CPS-protein conjugate vaccines for different combinations of serogroups A, C, Y and W [2–5]. New serogroup B vaccines using sub-capsular surface antigens are now approved in the US, Europe, Australia and Canada.

2. Antimicrobial peptides

Antimicrobial peptides play an important role in host defense against microbial infection. In addition to being major components of the innate immune response, AMPs also have many potential roles in inflammatory responses by inducing the secretion of chemokines and cytokines [6]. Antimicrobial peptides are peptides of 12–50 amino acids with excess of basic amino acids (arginine, lysine and histidine), thus resulting in a net positive charge (cationic). AMPs also generally have significant portion of hydrophobic amino acids residues and are amphipathic to facilitate interaction with bacterial membranes. Based on their structural characteristics, AMPs are classified into different categories [7]. The most common classes are β-sheet peptides stabilized by disulfide bonds such as β-defensins [8,9], and amphipathic α-helices formed upon contact with membranes such as α-defensins, cathelicidin and LL-37 [10–12]. Less common are extended peptides with a predominance of one or two amino acids (e.g. proline, tryptophan or histidine) and peptides with loop structures formed by either a single disulfide bond such as bactenecins. A cyclic lipopeptide, polymyxin B (PMB), has long been used as a model compound to define the mechanisms by which AMPs kill bacteria and how bacteria develop resistance to antimicrobial actions of AMPs.

The initial electrostatic interaction of the positively charged AMPs with the negatively charged lipopolysaccharides of the outer leaflet of the outer membrane is believed to initiate the self-promoted uptake of AMPs in Gram-negative bacteria [13]. Subsequently, both electrostatic and hydrophobic interactions between AMPs and the inner membrane phospholipids are critical for AMP's antimicrobial activity that disrupts membrane integrity. As the membrane–peptide complexes are insoluble and non-crystalline, solid-state NMR studies of AMPs [14] have been used to obtain structure, dynamics, orientation, and oligomeric states of AMPs in a membrane environment [15,16] as well in lipopolysaccharide micelles [17–20]. These biochemical studies provide important information about the mechanism of action of AMPs at molecular level. Further, recent studies suggested that AMPs are also able to act on intracellular targets following their translocation across the inner membrane either as a main mode of action or as additive effects combining with membrane disruption [13,21]. Expression of AMPs is widespread in many cell types. AMPs are constitutively produced by phagocytic cells such as macrophages and neutrophils [6]. For example, defensins have been shown to be the most abundant protein species in neutrophils [22]. Mucosal epithelial cells also constitutively expressed AMPs and AMP production can be further induced following exposure to bacterial determinants [23]. AMPs can also be formed by proteolytic digestion of larger cationic proteins such as lactoferricin, a proteolytic cleaved product from the N-terminus of lactoferrin [24].

3. AMP resistance mechanisms in *N. meningitidis*

Following acquisition through close contact with a carrier, meningococci overcome clearance and other local specific and nonspecific mucosal host defenses in order to colonize the upper respiratory mucosal surfaces (e.g., the nasopharynx). Colonization of *N. meningitidis* may also result in invasion of epithelial surfaces, access to the bloodstream and the production of systemic and focal infections. As the only natural reservoir of *N. meningitidis* is the human nasopharynx, meningococci constantly encounter endogenous antimicrobial defense including antimicrobial peptides during colonization and infection. Thus, it is not surprising that meningococci have developed mechanisms for conferring intrinsic and/or inducible resistance to the action of AMPs. AMP resistance mechanisms have been well-characterized in various Gram negative bacteria to include (i) efflux pumps that export AMP from the periplasmic and intracellular compartments [25]; (ii) structural modifications of lipopolysaccharide (LPS) and lipooligosaccharide (LOS) to reduce interaction with AMPs; (iii) modulation of outer membrane permeability to limit entry and/or enhance excretion of AMPs; and (iv) proteases that degrade AMPs [26,27]. Here we summarize the current knowledge of AMP resistance mechanisms as well as other characteristics that influence AMP resistance in *N. meningitidis* (Fig. 1) in the order of importance. The inducible effects of AMPs on some of these resistance determinants will also be discussed.

3.1. Lipooligosaccharide (LOS)

Lipopolysaccharide (LPS) is the major component of the outer leaflet of the outer membrane of Gram-negative bacteria. Meningococcal LPS is a lipooligosaccharide (LOS) that is structurally similar to LPS, but does not have repeating O-antigens. Both LOS and LPS have a conserved inner core region composed of heptose and 3-deoxy-D-manno-2-octulosonic acid (KDO) attached to a lipid A moiety. The meningococcal lipid A structure has a symmetrical distribution of acyl chain (C12 and C14) attachments to the di-galactosamine backbone (Fig. 1) and thus differs from that described for *E. coli* with an asymmetrical distribution of C14 and C16 acyl chains [28]. One of the best-characterized mechanisms of AMP resistance is remodeling of LPS [29]. It is believed that AMPs interacts with phosphorylated head groups of lipid A, and modification of the lipid A head groups correlates with increased PMB resistance. Such structural modifications that have been shown to affect CAMP resistance include i) removal of the phosphate head groups of the lipid A disaccharides [30,31]; (ii) modifications of lipid A head groups by the addition of positively charged moieties, such as aminoarabinose [32–35], glucosamine [36], galactosamine [37] or phosphoethanolamine (PEA) [38,39]; and (iii) alteration in the degree of lipid A acylation such as the formation of hepta-acylated lipid A [40,41].

Among the different structural modifications of the lipid A head group identified in Gram negative bacteria, only the PEA modification has been demonstrated in *N. meningitidis*. This is consistent with the fact that meningococci only encodes the PEA transferase LptA [42] and the gene cassette encoding the aminoarabinose modification machinery is absent in the meningococcal genomes. In contrast to the lipid A of *E. coli* and *Salmonella enterica*, which may be modified by PEA after induction by certain environmental conditions [38], meningococcal lipid A is constitutively substituted with PEA [43,44], which is a key factor that defines the intrinsic high level resistance of meningococci to PMB [39]. An *lptA* mutation caused ~250 fold reduction in PMB resistance to reach levels similar to those of *E. coli*. In comparison, an *mtr* mutation that inactivates major efflux pumps in meningococci resulted in a 16-fold reduction [39], demonstrating the LptA-mediated PEA decorations of lipid A is vital to meningococcal resistance. Recently, a poly-T_8 tract