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Review

Cationic antimicrobial peptide resistance mechanisms of streptococcal pathogens☆

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ABSTRACT

Cationic antimicrobial peptides (CAMPs) are critical front line contributors to host defense against invasive bacterial infection. These immune factors have direct killing activity toward microbes, but many pathogens are able to resist their effects. Group A *Streptococcus*, group B *Streptococcus* and *Streptococcus pneumoniae* are among the most common pathogens of humans and display a variety of phenotypic adaptations to resist CAMPs. Common themes of CAMP resistance mechanisms among the pathogenic streptococci are repulsion, sequestration, export, and destruction. Each pathogen has a different array of CAMP-resistant mechanisms, with invasive disease potential reflecting the utilization of several mechanisms that may act in synergy. Here we discuss recent progress in identifying the sources of CAMP resistance in the medically important *Streptococcus* genus. Further study of these mechanisms can contribute to our understanding of streptococcal pathogenesis, and may provide new therapeutic targets for therapy and disease prevention. This article is part of a Special Issue entitled: Bacterial Resistance to Antimicrobial Peptides.

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1. Introduction

The genus *Streptococcus* comprises some of the most common, yet potentially deadly, bacterial pathogens of humans. Medically important streptococcal species are typically carried asymptomatically, but have significant pathogenic potential if not restricted to superficial sites. Group A *Streptococcus* (GAS; *Streptococcus pyogenes*) commonly colonizes the mucosal tissues of the nasopharynx or the skin, and is estimated to cause more than 700 million cases of pharyngitis ("strep throat") or superficial skin infections (impetigo) annually worldwide [1]. Less commonly, GAS is associated with severe invasive infections including streptococcal toxic shock syndrome and necrotizing fasciitis, and the pathogen is the trigger of the post-infectious immunologically-mediated syndromes of rheumatic fever and glomerulonephritis [2]. Group B *Streptococcus* (GBS; *Streptococcus agalactiae*) is typically carried

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asymptomatically in the lower gastrointestinal tract or vaginal mucosa. Upon ascending infection of the placental membranes or during passage through the birth canal, GBS can access the newborn infant, where it is a major cause of pneumonia, sepsis and meningitis [3]. GBS is also increasingly associated with invasive infections in adult populations including pregnant women, the elderly and diabetics [4]. The pneumococcus (*Streptococcus pneumoniae*), which colonizes the nasal mucosa, is a major cause of mucosal infections such as otitis media and sinusitis, as well as pneumonia, sepsis in meningitis, especially at the ends of the age spectrum and throughout the developing world [5,6]. *Streptococcus mutans* colonizes the mouth, where it is a major contributor to tooth decay. Additional *Streptococcus* spp., more rarely associated with disease in humans, are pathogenic for other animal species, e.g. *Streptococcus suis* (swine) and *Streptococcus iniae* (fish).

A critical first line of host innate defense against invasive infections by pathogenic streptococci is provided by endogenous cationic antimicrobial peptides (CAMPs). CAMPS are produced by epithelial cells and by circulating immune cells including neutrophils and macrophages, and are among the first immune effectors encountered by an invading microbe [7,8]. CAMP expression is greatly induced during infection; it is also induced in sterile models of injury that compromise the epithelial barrier, indicating that it can function as a prophylactic measure against imminent pathogen invasion [9].

Two major classes of the CAMPs present in mammals are the cathelicidins and the defensins (Fig. 1). Both represent small, cytotoxic pore-forming peptides that contain regions of strong cationic charge that intersperse solvent-exposed hydrophobic residues. This amphiphilicity is a key source of their antimicrobial activity; the positive charge attracts them to a microbe's surface and their hydrophobic surfaces insert into and permeabilize the bacterial membrane. An additional target of CAMPs is the ExPortal, an organelle dedicated to the biogenesis of secreted proteins in streptococci and entercocci [10]. Since many of the CAMP resistance mechanisms that will be discussed rely on the secretion of proteins through this system, this may represent a way to counter these resistance mechanisms. In addition to directly targeting the pathogen, CAMPs can also coordinate the immune response to infection by contributing to cytokine signaling, immune cell chemotaxis, and wound healing [11–13]. Therefore, some microbial mechanisms for counteract CAMPs can also impact these downstream immune pathways.

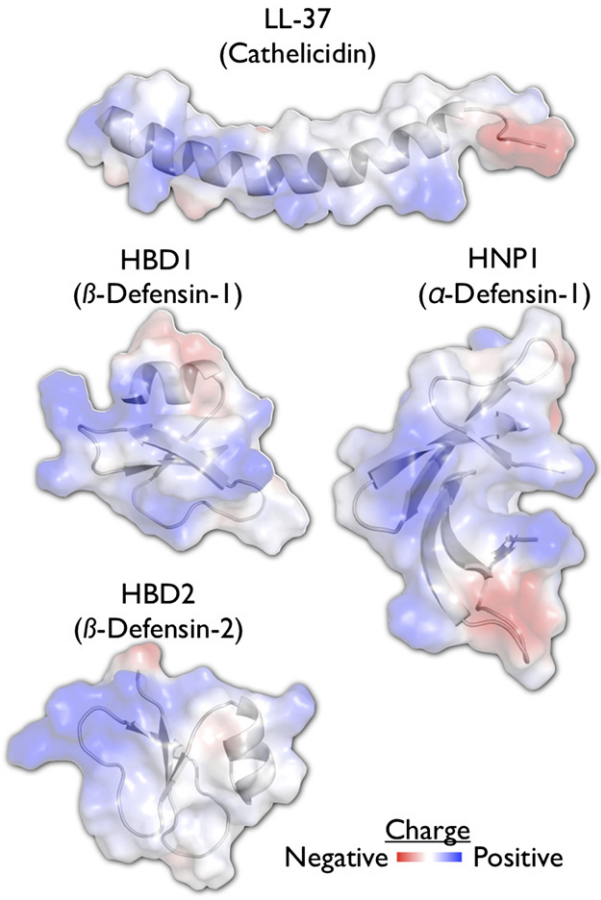

Fig. 1. Common electrostatic properties of antimicrobial peptides. The mature fragment of human cathelicidin, LL-37, is an α-helical peptide (pdb: 2K6O), while the defensins can have a number of different folds (pdb: 1FD3, 1E4S, 2PM1). These peptides have in common a strongly cationic face that mediates the electrostatic attraction to the cell surface of the Streptococci and other microbes.

Defensins are highly polymorphic, with numerous alleles expressed by various immune cell types. In contrast, mice and humans express only one cathelicidin: hCAMP18 (human) or mCRAMP (murine). These proteins are made of a conserved amino-terminal cathelin (protease inhibitor) domain and a highly charged alpha-helical carboxy-terminus. Cathelicidins are not antimicrobial until the cathelin pro-domain is proteolytically removed, freeing the remaining peptide, in humans named LL-37, to act against the microbe [14]. In addition to these classical CAMPs, cathelicidins and defensins, several other proteins and their degradation products are cationic and antimicrobial. These proteins include lysozyme, histones, thrombocidin, lactoferrin, cathepsins, myeloperoxidase, kininogen, and heparin-binding proteins. Many of these proteins are found in neutrophil extracellular traps (NETs), web-like structures in which these cationic antimicrobials are embedded within an extruded (acidic) DNA matrix [15]. NETs can potentiate killing of extracellular microbes, which will be trapped and exposed to a higher local concentration of cathelicidin and other CAMPs. While neutrophils are critical in controlling streptococcal infections, additional cells make NET-like structures that may also function in pathogen defense [16].

While mechanisms of resistance to cathelicidin/LL-37 or defensins do not always correlate with one another [17], many of the virulence factors the pathogens employ to evade one CAMP can be cross protective against others. Nearly any virulence factor of a pathogen may contribute, at least indirectly, to a pathogen's resistance or susceptibility to CAMPs. For example, pore-forming toxins induce cell death that can eliminate CAMP-producing cells [18], secreted DNases can facilitate escape from NETs [19], and any number of mechanisms that shield potential pathogen-associated molecular patterns can work to lessen the induction of CAMP expression via TLRs [20]. In this review, we focus primarily on molecular mechanisms that directly target CAMPs, by the common themes of repulsion, sequestration, export, and destruction (Fig. 2). We further discuss how streptococcal pathogens detect and regulate their gene expression to resist these CAMPs, and emerging strategies toward combatting infection by boosting or supplementing CAMP defenses.

2. Repel

One of the first mechanisms recognized by which pathogens evade killing by CAMPs is directed at one of their rudimentary properties — charge. The outer leaflet of the mammalian cell contains zwitterionic phospholipids and carries little negative charge that would attract CAMPs, which protect cells from toxicity from these molecules. CAMPs are attracted to bacterial membranes, which are abundant in acidic phospholipids. Streptococci, like other Gram-positive bacteria, are additionally coated with an acidic polymer of teichoic acids on their cell well. When a bacterium can increase its net surface charge to more closely resemble the charge profile of a mammalian cell, it can decrease the affinity of cationic molecules like CAMPs to its surface. This can be accomplished by several chemical modifications possible for each layer of the cell surface: the lipid membrane, the peptidoglycan and teichoic acid-containing cell wall, and the capsular polysaccharides.