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Review

Cationic antimicrobial peptide resistance mechanisms of streptococcal pathogens[☆]Christopher N. LaRock^{a,*}, Victor Nizet^{a,b,c,**}^a Department of Pediatrics, University of California, San Diego, La Jolla, CA, USA^b Skaggs School of Medicine and Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA, USA^c Rady Children's Hospital, San Diego, CA, USA

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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 asymptotically, 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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asymptotically 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 enterococci [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.

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 wall. 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.

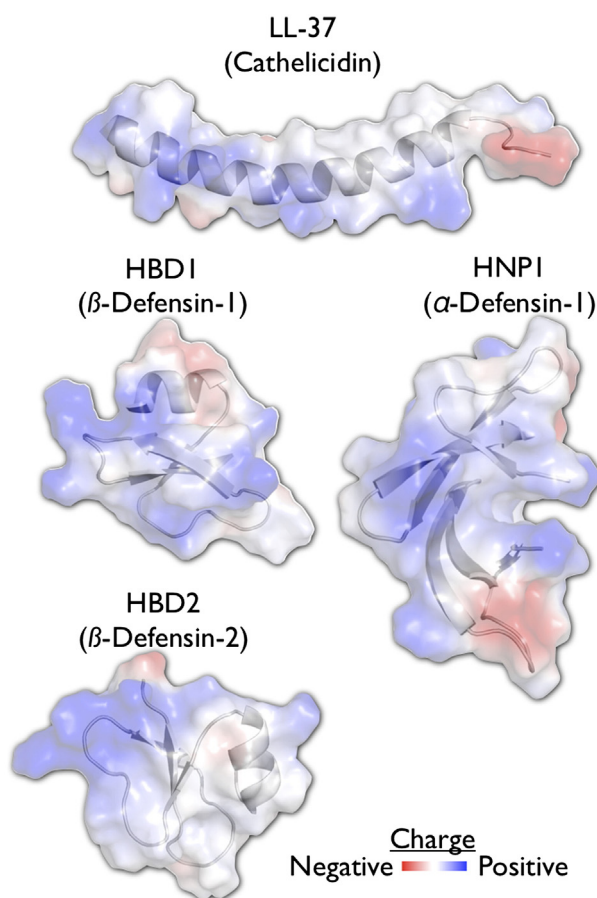


Fig. 1. Common electrostatic properties of antimicrobial peptides. The mature fragment of human cathelicidin, LL-37, is an α -helical peptide (pdb: 2K6O), while the defensins can have a number of different folds (pdb: 1FD3, 1E4S, 2PM1). These peptides have in common a strongly cationic face that mediates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

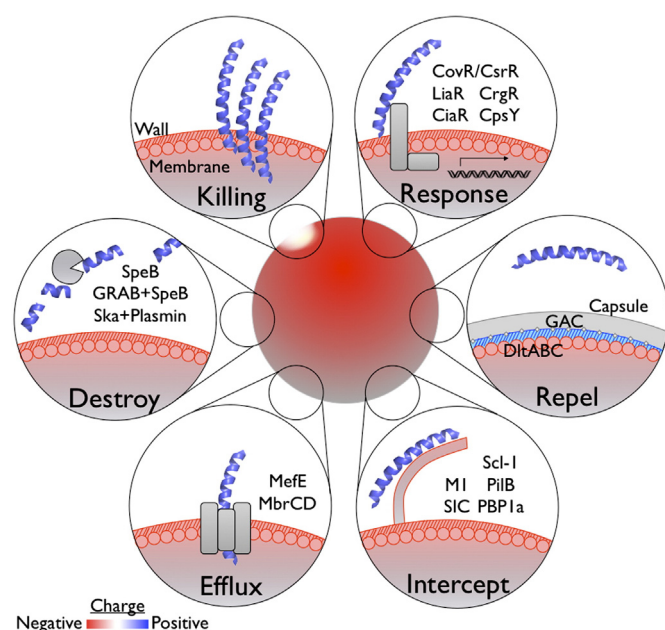


Fig. 2. Models of streptococcal resistance to cationic antimicrobial peptides. CAMPs attracted to the cell surface form pores that disrupt membrane integrity (Killing). Pathogens, including the streptococci, are able to detect CAMPs either directly or indirectly via sensors. Detection of sub-inhibitory concentrations of CAMPs can induce resistance mechanisms protective for when higher CAMP concentrations are encountered (Response). Examples of these resistance mechanisms are cell surface modification (Repel), competitive binding (Intercept), removal or secretion (Efflux), or proteolytic inactivation (Destroy).

It is not yet known if any of the pathogenic streptococci can alter their lipid cell membrane in a manner providing resistance to CAMPs. Several Gram-positive pathogens have been reported to modify the acidic membrane lipid phosphatidylglycerol with the cationic amino acid, L-lysine [21], reducing the net negative charge of the membrane, thereby decreasing attraction of CAMPs. This L-lysinylation reaction is carried out by a lysyl-phosphatidylglycerol synthase encoded by *mprF*, a gene absent from the GAS and pneumococcal genomes. GBS do possess *mprF*, but deletion of the gene did not significantly alter resistance to CAMPs [22]. The *mprF* gene is present in the sequenced genomes of other veterinary/zoootic pathogens including *S. iniae* and *S. suis*, but any contribution to CAMP resistance has yet to be demonstrated.

The Gram-positive cell membrane is covered by the cell wall, a rigid porous mesh composed of heavily cross-linked peptidoglycan. The backbone of peptidoglycan consists of *N*-acetylglucosamine and *N*-acetylmuramic acid linked by short peptides. Two modifications that streptococci can make to this base structure are *N*-deacetylation of the *N*-acetylglucosamine subunit, carried out by PgdA, and *O*-acetylation of the *N*-acetylmuramic acid, catalyzed by OatA (Adr). Despite increasing surface charge and lysosome resistance, *N*-deacetylation does not alter CAMP resistance in *S. pneumoniae* [23] nor *S. suis* [24]. *O*-acetylation also increases surface charge and decreases binding of cationic proteins. However, for *S. iniae* this did not translate to altered CAMP susceptibility [25]. In *S. pneumoniae*, *O*-acetylation does increase resistance to lysosome, which is cationic [26], but the effect on CAMP resistance was not evaluated. The same *N*-deacetylation and *O*-acetylation mechanisms confer CAMP resistance in other species, suggesting that they may still act in streptococci in manners yet to be evaluated.

Teichoic acids make up a second major component of the Gram-positive cell wall. These glycerophosphate polymers are anchored to the peptidoglycan and cell membrane, and like both, are also acidic. The *dlt* operon encodes enzymes mediating D-alanylation of wall teichoic acids, a modification nearly ubiquitous among Gram-positive pathogens. The highly cationic side chain of alanine increases cell surface charge; cell wall density is concurrently increased by a mechanism

that is not yet entirely clear [22]. Together, these changes increase resistance to CAMPs, as well as bacteriocins, lysozyme, acid, and NETs, and thereby the major immune cells producing these antimicrobial factors, neutrophils [27–29]. Thus, *dlt* is important for the virulence of GAS [29], GBS [30], *S. pneumoniae* [27], *S. suis* [31], and *S. mutans* [32]. The conservation of the phenotype of *dlt* mutants between these species clearly illustrates its advantageous role in direct resistance to CAMPs, however, some care must be taken to avoid over interpreting results from mutations like this that alter fundamental cell structures. For example, the increased CAMP susceptibility of GAS *dlt* mutants may not be solely a consequence of failing to modify cell teichoic acids, but also due to lower expression levels of M protein and SIC [33], two important virulence factors that also contribute to CAMP resistance in this species [34,35].

The third component of the streptococcal cell wall is the Lancefield group-specific carbohydrate. These carbohydrates compose up to half the cell wall by weight, and provide the basis for the serological separation of streptococcal groups. In groups A and C streptococci, the antigen consists of a polyribose backbone and immunodominant side-chain specific to each group. The functional role of these carbohydrates had long remained a mystery, but recently, it was shown that the group A carbohydrate of GAS has an *N*-acetylglucosamine side-chain which blocks LL-37 binding, thereby providing resistance to CAMPs as well as killing by neutrophils and NETs [36].

Another method to repel CAMPs is the recruitment of factors to increase the net charge of the bacteria surface, rather than to directly modify it. An example of this mechanism can be found in *S. pneumoniae* where the LytA protein decorates the bacterial surface with highly charged choline [37]. This masking could decrease attraction of CAMPs, as in the respiratory pathogen *Haemophilus influenzae*, recruitment of choline to its surface, albeit by a different mechanism, does afford significant protection against CAMPs [38]. Separate from providing protection from CAMPs, pneumococcal cholate binding does contribute NET resistance [28]. Lipoteichoic acid D-alanylation did not affect NET trapping of pneumococci, but did provide the pathogen protection against killing by CAMPs embedded within the NET [28].

The most distal surface on many Gram-positive bacteria is an exopolysaccharide capsule. This family of critical virulence factors shields the bacterial surface and promotes resistance to phagocytosis and complement [39]. This also masks susceptible underlying cell layers from CAMP action, as GAS lacking their hyaluronic acid capsule is more susceptible to CAMPs, neutrophils, and NETs [40]. Mutations altering capsule can similarly sensitize *S. iniae* [41] and *S. pneumoniae* [42] to killing by CAMPs. Interestingly, capsule expression can instead sensitize a bacterium to killing by some CAMPs; as some strains of encapsulated *S. pneumoniae* are more sensitive to defensins [43], possibly a result of increased attraction of CAMPs to the bacterium by a particularly anionic capsule composition. Yet, during infection this interaction could also translate into a virulence mechanism; several pathogens including *S. pneumoniae* dynamically shed their negatively charged capsular polysaccharide in vivo, which can intervene and neutralize CAMPs before they can bind the underlying cell structures [42]. This mechanism of binding CAMPs before they can find their cellular target introduces the second method of protection against CAMPs; interception.

3. Intercept

Streptococci decorate their outer surface with numerous proteins that inhibit immune functions ranging from phagocytosis to cytokine signaling. Some of these proteins encode for more than one activity, and one of the common accessory functions of these proteins is providing CAMP resistance. One of the earliest of these identified proteins is the serum inhibitor of complement (SIC), first characterized for its role in protection against killing by the membrane attack complex [44], but later found to also bind and inhibit killing by defensins and LL-37 [45]. Consequently, SIC is required for the full virulence of GAS

[35]. Several proteins with homology to SIC have similar activity, including another GAS protein, distantly related to SIC (DRS) [46], and the *Streptococcus dysgalactiae* subsp. *equisimilis* (GCS/GGS) protein DrsG [47].

One of the major surface proteins of GAS is the fimbrial M protein. This immunodominant surface anchored protein is multifunctional during infection, and can variably promote or inhibit adhesion and invasion of mammalian cells, inhibit complement and opsonization, induce inflammation, and act as a superantigen [48]. These broad activities are not universal among isolates, due to both a varying modular arrangement of domains and a region of hyper-variability, which serves as the basis for the serological classification of isolates from this species. One M protein variant encoded by the isolates most commonly associated with infection, M1, can directly bind CAMPs like LL-37 and mCRAMP [34]. This sequestration can significantly increase the resistance of M1 GAS strains to CAMPs, and such isolates with higher CAMP resistance are more commonly associated with severe disease [34]. Another common M-type, M49, did not bind CAMPs, but since more than one hundred M-types have been identified, but it is not yet clear how pervasive M-mediated CAMP resistance might be among GAS.

Another major cell surface structure provides CAMP resistance for GBS through an interception mechanism: the pilus. GBS pili have been primarily characterized for their role in adhesion, but the major pilin subunit of this complex, PilB, can also sequester CAMPs like LL-37 and mCRAMP, [49]. As with M protein and SIC, the surface charge of the bacteria is not appreciably altered by the presence or absence of the protein. Also like M protein and SIC, inhibition of CAMPs seems to be highly variable and not all isolates of GAS and GBS make pilin, and not all pilin have this activity [50]. The GBS penicillin binding protein PBP1a also likely acts through competitive binding [51]. PBPs are surface localized and often important for cell wall biosynthesis, but many have unknown or unrelated cellular roles. PBP1a itself is not necessary for critical cellular pathways such as cell wall biosynthesis, as a mutant can be made and is fully viable, but has a virulence defect *in vivo* [52]. This attenuation may be largely due to a vulnerability to CAMPs, since a PBP1a mutant is more sensitive to killing by LL-37, CRAMP, and defensin [51]. Since all of these proteins have other clear virulence functions, CAMP resistance might be considered to be more of an accessory function secondary to their other contributions to the cell, similar to many members of the next category of CAMP resistance factors, those that affect export.

4. Export

Efflux pumps are an important contributor to virulence in several Gram-positive pathogens. However, efflux pumps that provide resistance to CAMPs have most extensively been studied in Gram-negative pathogens [53]. *S. pneumoniae* encodes an efflux pump for macrolides, MefE, that also provides protection against defensins, LL-37, and CRAMP [54]. This cross-protection would suggest that many such efflux pumps might provide resistance, and *S. pneumoniae* encodes several additional possible transporters [55]. Mutation of some of these putative transporters increases pneumococcal CAMP susceptibility, suggesting they are expressed and active [56]. The homolog of one of these, SP0912–0913 (MbrCD) has also been found in *S. mutans* to contribute to resistance against defensins [57]. While not well studied in other streptococci, many of these genes are found in additional species; if they are confirmed to act as CAMP efflux pumps, this could represent significant contributor to CAMP resistance throughout the genus *Streptococcus*.

5. Destroy

One last, very direct, mechanism for resisting killing by CAMPs is to destroy them. This can be accomplished by secreted proteases that hydrolyze the peptides before they can exert their cytotoxic activities. Gram-positive pathogens, in particular, are prolific secretors of proteases

that target host proteins. The factors can promote virulence by inactivating antibodies, cytokines, complement proteins, autophagy machinery, and many other immune factors, including CAMPs. However, targeting of CAMPs is not a universal property of even highly active bacterial proteases, suggesting that these molecules are not generally protease-sensitive; the virulence factor needs to specifically target the CAMP. For instance, even though the IgG protease of GAS, Mac/IdeS, is very highly expressed, it provides no resistance to LL-37 [58].

However, GAS does secrete at least one protease active against CAMPs: SpeB. This intensively studied cysteine protease has been found to cleave numerous host proteins in both extracellular and intracellular locations [59,60] including the CAMPs LL-37 [61] and β -defensin [62]. In addition to directly hydrolyzing CAMPs into inactive or less active forms, SpeB can target other factors in ways that interfere with killing. SpeB degradation of tissue proteoglycans releases glycosaminoglycans like dermatan sulfate, a charged molecule which can bind and inhibit CAMPs including α -defensin [63] and LL-37 [64].

SpeB-mediated CAMP protection can be amplified even further through the activity of a second GAS virulence factor, G-related α 2-macroglobulin-binding protein (GRAB) [65]. This surface protein forms a complex with the circulating α 2-macroglobulin, an abundant broad-spectrum protease inhibitor with numerous roles in maintaining homeostasis. Proteases trapped by α 2-macroglobulin lose the ability to process large substrates, however, peptides small enough to penetrate the α 2-macroglobulin:GRAB:SpeB complex are still cleaved [65]. Thus, this complex can both retain SpeB on the bacterial surface where it can be most efficacious against CAMPs, while also maintaining the proteolytic activity of SpeB, and possibly even amplifying it by antagonizing hydrolysis of other possible substrates of SpeB.

Instead of producing its own proteases, a bacterial pathogen can also recruit and utilize host proteases. The virulence factor streptokinase (Ska) allows GAS to recruit and activate host plasminogen on the bacterial surface [66]. Recruitment of plasminogen is relatively common strategy of pathogens; plasminogen is the precursor zymogen of plasmin, a serine protease that degrades fibrin clots, connective tissue, and extracellular matrix. Plasmin activated by streptokinase can contribute to the virulence of GAS by dissolving fibrin deposits to promote dissemination throughout a host tissue [66]. In addition to this activity targeting the host fibrinolytic system, plasmin activated by streptokinase is able to cleave the cathelicidins LL-37 and CRAMP, which increases the bacterium's resistance to these CAMPs [67].

The role of proteases in providing CAMP resistance to other streptococcal species is not yet fully clear. Other important human pathogens including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Porphyromonas gingivalis* all make proteases that target CAMPs [61]. Therefore, even though obvious homologs of SpeB are not found in the other pathogenic streptococci, a common activity from dissimilar and unrelated proteases suggest that other proteases may have a CAMP neutralizing function. Obvious candidates would be proteases that are highly expressed and are secreted. For GBS, this would include ScpB and CspA, and for *S. pneumoniae*, the serine protease PrtA. In addition to making their own CAMP proteases, streptococci other than GAS may utilize streptokinase-activated plasmin for this purpose; GBS, GCS, and pneumococcus can all activate plasminogen [68].

One caveat for a pathogen resisting CAMPs through proteolysis is the possibility of working at cross-purposes and inactivating other important virulence factors. In addition to cleaving CAMPs, SpeB also degrades streptokinase, M1 protein, and host plasminogen; consequently, SpeB can be a barrier to invasive disease [69]. These proteins all contribute to CAMP resistance, thus providing a very clear example of the shared benefits and liabilities of SpeB expression. Since it is highly expressed *in vivo* and maintained within the GAS pan-genome, the virulence tradeoff to SpeB is context-dependent. Nonetheless, SpeB is observed to colocalize with LL-37 in tissue samples from patients with invasive GAS infection, suggesting that it may remain a dominant virulence factor for the degradation of CAMPs [70].

Another consideration for the effect of bacterial proteases on CAMPs is the possibility of crosstalk with their activating proteases. As an example, cathelicidin is synthesized as a propeptide and requires protease cleavage to release the antimicrobial carboxy-terminus (LL-37 and related peptides). Cleavage of a CAMP by a microbial protease in the wrong site could end up liberating a CAMP, rather than destroying one. Furthermore, the remaining amino-terminal cathelin domain of cathelicidin has homology with the cystatin family of cysteine protease inhibitors [14]. Since this will be present in the same tissue as the active CAMP, it could inhibit microbial attempts to destroy CAMPs. Additionally, protease inhibitors in general are some of the most common circulating proteins. In an infection context, the presence of inhibitors that can target bacterial proteases could dampen the ability of bacteria to destroy CAMPs, despite experiments that readily observe hydrolysis of CAMPs in vitro [9].

6. Regulation

The ability of a microbe to respond to environmental stimuli and stressors can allow expression of costly or conditionally detrimental proteins in only the contexts that are beneficial for the microbe. Response regulators sensitive to CAMPs that allow a pathogen to selectively induce any of the resistance mechanisms mentioned above could greatly increase the adaptability and virulence potential. The archetype of this response is the PhoPQ two-component regulatory system of *Salmonella*. The sensor PhoQ contains an acidic patch that binds CAMPs, subsequently signaling through PhoP to induce CAMP-protective cell surface modifications [71]. It is unknown whether pathogenic streptococci encode sensors that directly recognize CAMPs, but several response regulator components are responsive to CAMPs.

The GAS CovRS (CsrRS) two-component system is the most characterized of these response regulators. CovRS-regulated genes are activated by the CAMP LL-37 and repressed by high Mg^{2+} [72], similar to the PhoPQ paradigm where CAMP activates and Mg^{2+} represses gene expression [71]. CovS is the sensor of this pair, and CovR a transcriptional repressor that is inactivated by CovS signaling [73]. Mutation of CovR and CovS, singly or in combination, appears to only modestly alter resistance of GAS to the CAMP LL-37, a difference that was significant in one study [74] but not another [75]. Nonetheless, virulence factor induction through LL-37 dependent CovRS signaling is protective during infection, indicating that it may be more potent at counteracting other immune mechanisms [72,76]. Clear homologs of CovRS are present in other streptococci including GBS where it shares the same name; their role in the detection of and resistance to CAMPs is not yet clear, but these two-component systems similarly regulate virulence factor expression and impact pathogenesis [77].

Which CovRS-regulated proteins contribute to CAMP resistance is not fully clear; 10% of the entire GAS genome is differentially regulated by CovRS [78]. Many CovRS-regulated genes encode virulence factors, including several known to provide resistance to CAMPs. SpeB expression is abolished in a CovRS mutant, while SIC and the operon encoding hyaluronic acid capsule biosynthesis (*has*) are more highly expressed. The counterbalance of these CAMP resistance mechanisms might explain why CovRS mutants are not more highly sensitive to CAMPs than mutants for any particular resistance factors, like M1, SIC, capsule, or SpeB. This interplay is further complicated by SpeB degradation of other CAMP resistance factors, counterbalanced by the naturally occurring CovS mutants that arise during invasive infection [79].

CovRS is active in GBS, but it is unknown whether it is also responsive to CAMPs. However, GBS do induce several known and putative CAMP resistance factors in response to sub-inhibitory concentrations of LL-37 [56]. Two different two-component system response regulators are known to be involved: LiaR [80] and CiaR [81]. Both systems induce resistance to CAMPs like LL-37 and CRAMP, but it is not known whether the sensor kinases of the systems, LiaS and CiaS, directly detect the CAMPs or whether they transduce signals downstream of some effect

that CAMPs have on the cell or its environment. LiaR-mutant bacteria are less piliated, express less of the penicillin-binding protein Pbp2b, and less peptidoglycan cross-linking enzymes, which could all relate to this CAMP susceptibility [80]. LiaRS is also active in *S. pneumoniae* and *S. mutans*; it is not known whether it mediates CAMP resistance in these species, but it shows sensitivity to many of the same stressors as the GBS system [82,83]. Genes regulated by CiaR are more cryptic in function, but include several proteases, which might be responsible for increased CAMP resistance if they were capable of degrading the molecules. However, CiaR also regulates resistance to stressors like hydrogen peroxide and hypochlorite, which could suggest a more broad regulation of protective modifications of the cell surface. Another regulator of GAS, CrgR, strongly increases resistance of GAS to the murine CRAMP [84]. While the factors mediating this resistance are not known, this mutant only outcompetes wild-type GAS in the presence of CRAMP and during infection CRAMP knockout mice, indicating that the CrgR virulence phenotype is highly specific to CAMP resistance.

Several regulators important for CAMP resistance in other species are present in the pathogenic streptococci but their contribution to virulence is not yet fully characterized. *Staphylococcus epidermidis* uses ApsXRS to induce the *dlt* operon and MprF in response to defensin and LL-37 [85]. *dlt* and MprF also contribute to CAMP resistance in many streptococcal species, and clear homologs to the Aps system are present in some of the strains. Similarly, the BceRS (MbrAB) bacitracin sensor and response regulator can detect and initiate a protective response against CAMPs for *Bacillus subtilis* [86]. This system is found in several streptococcal pathogens. BceRS has been studied in *S. mutans* where it was still found to work against bacitracin, but no cross-protection against other CAMPs was found [57]. The CpsY (MetR/MtaR) regulator of *S. iniae* modulates expression of genes involved in peptidoglycan acetylation [25]. Mutation of CpsY led to an increase in surface charge and susceptibility to lysozyme, though no difference in LL-37 killing was observed [25]. Since decreased surface charge generally inhibits CAMP attraction, this could still mediate resistance to other CAMPs, especially if a CpsY inducer were uncovered that could lead to greater induction of this pathway.

7. Conclusion

The pathogenic streptococci employ a large, often redundant, array of CAMP resistance mechanisms (Table 1). While highlighting a persistent evolutionary selective pressure to escape CAMP killing, it is telling that streptococci have not found a complete solution; the high CAMP concentrations and diverse CAMP repertoire that are detected in vivo [87] still restrict streptococci employing their full complement of resistance mechanisms in the vast majority of host–pathogen encounters. Since CAMPs are ancient molecules present in all forms of life and remain critical in the human immune system, high level-resistance, as seen for most modern pharmaceutical antibiotics, just might not be achievable for a bacterium. This recognition suggests that enhancing endogenous CAMP function could provide an attractive avenue for the treatment of bacterial infections.

Low levels of defensin and LL-37 correlate with atopic dermatitis and susceptibility to bacterial infection [87]. Mice deficient in the cathelicidin CRAMP are hyper-susceptible to GAS infection [84], while transgenic mice expressing additional CAMP are more resistant to infection [88,89]. Therefore, boosting the expression of endogenous CAMPs might be seen to have both prophylactic and therapeutic potentials in combating streptococcal infection. Vitamin D receptor signaling induces expression of human cathelicidin, *camp*, and β -defensin, *defB2*, but not the murine *cramp* [90,91]. This pathway can be further amplified by TLR signaling in response to pathogen-associated molecular patterns [20] or during injury, likely in response to damage-associated molecular patterns [92]. Similarly, infection can induce HIF, a global regulator in response to hypoxia that is important for CRAMP expression [93–95]. HIF can also be boosted pharmacologically, boosting cathelicidin

Table 1

Summary of the CAMP resistance mechanisms of streptococcal pathogens. GAS, group A *Streptococcus*; GBS, group B *Streptococcus*; GCS, Group C (and G) *Streptococcus*; SPN, *Streptococcus pneumoniae*; Sm, *Streptococcus mutans*; and Ss, *Streptococcus suis*.

Category	Specific mechanism	References
<i>Repel</i>		
DltABC	Lipoteichoic acid D-alanylation	GAS [29], GBS [30], SPN [27], Ss [31], Sm [32]
HasABC	Hyaluronic acid (capsule)	GAS [40]
GacA-L	Glucuronic-B-1,3-N-acetylglucosamine (group A carbohydrate)	GAS [36]
<i>Intercept</i>		
M1	Competitive binding	GAS [34]
SIC/DRS	Competitive binding	GAS [45], [46], GCS [47]
Scl-1	Competitive binding	GAS [97]
PilB	Competitive binding	GBS [49]
PBP1a	Competitive binding	GBS [51]
Dermatan sulfate	Competitive binding by host proteoglycans released by bacterial proteases	GAS [63]
<i>Efflux</i>		
SP0785-7	ABC transporter (putative)	Sp [56]
MbrCD	ABC transporter (putative)	Sm [57], Sp [56]
MefE	Efflux pump	Sp [54]
<i>Destroy</i>		
SpeB	Direct proteolysis	GAS [61]
GRAB	Protease recruitment/redirection	GAS [65]
Ska	Activation of proteolysis by plasmin	GAS [67]

expression, and providing a potential avenue for adjunctive treatment of invasive streptococcal infections [96].

Transparency document

The Transparency document associated with this article can be found, in online version.

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