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Understanding how leading bacterial pathogens subvert innate immunity to reveal novel therapeutic targets

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***Staphylococcus aureus* (SA) and group A *Streptococcus* (GAS) are prominent Gram-positive bacterial pathogens, each associated with a variety of mucosal and invasive human infections. SA and GAS systemic disease reflects diverse abilities of these pathogens to resist clearance by the multifaceted defenses of the human innate immune system. Here we review how SA and GAS avoid the bactericidal activities of cationic antimicrobial peptides, delay phagocyte recruitment, escape neutrophil extracellular traps, inhibit complement and antibody opsonization functions, impair phagocytotic uptake, resist oxidative burst killing, and promote phagocyte lysis or apoptosis. Understanding the molecular basis of SA and GAS innate immune resistance reveals novel therapeutic targets for treatment or prevention of invasive human infections. These future therapies envision alternatives to direct microbial killing, such as blocking disease progression by neutralizing specific virulence factors or boosting key innate immune defenses. (J Allergy Clin Immunol 2007;120:13-22.)**

Key words: Staphylococcus aureus, *Group A* Streptococcus, *innate immunity, virulence factors, antimicrobial peptides, complement, phagocytosis, neutrophil, macrophage*

Abbreviations used

AMP: Antimicrobial peptide
C4BP: C4b-binding protein
CHIPS: Chemotaxis inhibitory protein of staphylococci
CR: Complement receptor
FH: Factor H
FHL-1: Factor H–like protein 1
FI: Factor I
GAS: Group A *Streptococcus*
HIF-1α: Hypoxia-induced transcription factor 1α
NET: Neutrophil extracellular trap
PVL: Panton-Valentine leukocidin
MRSA: Methicillin-resistant *Staphylococcus aureus*
SA: *Staphylococcus aureus*
SCIN: Staphylococcal complement inhibitor

Innate immunity in human beings and other higher animals represents an integrated and highly effective system of molecules and cellular systems that defend the host against infection, despite continual encounter with potential pathogens in a complex environment. In addition to the physical barrier function of skin and mucosal epithelium, innate immunity is composed of soluble effectors such as cationic antimicrobial peptides (AMPs) and complement proteins. Sophisticated pattern recognition systems are deployed by the innate immune system to activation and target inflammatory responses. Finally, innate immunity gains a critical contribution from phagocytic cell types such as neutrophils and macrophages capable of directed migration, microbial uptake, and production of a variety of bactericidal compounds.

The clinical specialty of infectious diseases largely reflects the spectrum of medical conditions resulting from failures of innate immunity. Often the etiology is intrinsic to the host, including developmental immaturity or senescence of defense functions at extremes of age, genetic or acquired (eg, chemotherapy) immunodeficiencies, loss of

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barrier integrity (eg, surgical wounds), high-risk exposures and behaviors, or debilitation caused by chronic illness or malnutrition. In other cases, no obvious predisposing host condition can be defined; nevertheless, serious infection develops requiring antibiotic therapy and perhaps surgical drainage and additional supportive measures. On initial presentation, the empiric diagnostic and therapeutic approach to such patients is appropriately focused toward a relatively short list of likely etiologic agents.

The Gram-positive bacteria *Staphylococcus aureus* (SA) and group A *Streptococcus* (GAS) are preeminent human pathogens responsible for a wide spectrum of superficial and invasive disease conditions. SA accounts for >10 million skin and soft tissue infections annually in the United States alone[1] and is the single leading cause of hospital acquired infections.[2] Each year worldwide, GAS is responsible for more than 700 million cases of pharyngitis or skin infection and more than 650,000 invasive infections.[3] Both pathogens can produce infections in essentially every human organ or tissue, including severe life-threatening conditions such as necrotizing fasciitis, endocarditis, sepsis, and toxic shock syndrome. The propensity of SA and GAS to produce systemic infections, often in otherwise healthy children and adults, defines a capacity of each pathogen to resist host innate immune clearance mechanisms that normally function to prevent microbial dissemination beyond epithelial surfaces.

This review focuses on the multiple virulence factors of SA and GAS capable of interfering with the host innate immune defenses, placing a particular emphasis on recent discoveries established through molecular analysis of the pathogens. The multifaceted basis of SA and GAS resistance to cationic antimicrobial peptides (AMPs), complement, and host phagocytic cell function is outlined. Our enhanced understanding of the mechanisms of SA and GAS pathogenicity and the subtle limitations of innate immunity they exploit reveal novel avenues for infectious disease therapy.

SA AND GAS SUBVERT HOST INNATE IMMUNITY

Resistance to cationic antimicrobial peptides

Recent discoveries using knockout mice have confirmed that cationic AMPs such as cathelicidins and β-defensins play a crucial role in restricting microbial proliferation to skin and mucosal surfaces and in preventing spread to the deep tissues, where serious infection may develop. For example, cathelicidin-deficient mice are more susceptible to necrotizing GAS skin infection,[4] and β-defensin knockout mice exhibit reduced clearance of SA from the urinary tract.[5] Skin keratinocytes and mucosal epithelial cells produce very low levels of AMPs under baseline conditions, but their expression of AMPs can be induced dramatically in response to injury or infectious stimuli. These epithelial barrier functions are further supplemented by AMPs produced by leukocytes (eg, neutrophils) recruited during an inflammatory response. Defects in local AMP production are noted in atopic dermatitis and may help explain the clinical predisposition for bacterial superinfection in individuals with this condition.[6]

Relative resistance to efficient AMP killing is increasingly recognized as a discriminating feature of important human pathogens, including SA and GAS. The AMP avoidance mechanisms deployed by these 2 pathogens are diverse and include charge modifications of the cell membrane, proteolytic degradation, and the AMP binding and inactivation activities of specific bacterial surface or secreted proteins. By incorporating positively charged residues into their cell wall lipoteichoic and teichoic acid, SA and GAS increase electrostatic repulsion of the defense peptides. For example, D-alanylation of teichoic acids mediated by the *dlt* operon is present in both pathogens, promoting resistance to AMP and neutrophil killing.[7,8] In addition, positively charged lysyl-phosphatidylglycerol modifications of teichoic acids are encoded in the functions of the SA *mpr*F or *lys*C genes and contribute to human AMP resistance.[9,10] SA mutants defective in Dlt and MprF show reduced virulence in small animal infection models.[11,12]

The SA metalloprotease aureolysin can cleave and inactivate human cathelicidin LL-37, thereby contributing to the bacterium's resistance to innate immune clearance.[13] Similarly, the secreted cysteine protease SpeB of GAS can be retained on the bacterial surface, where it proves capable of degrading LL-37 and protecting the bacteria against its antimicrobial action.[14] Complex binding of human neutrophil α-defensins by staphylokinase neutralizes their bactericidal effect against SA,[15] and the streptococcal inhibitor of complement protein binds and inactivates both α-defensins and cathelicidin LL-37.[16]

Impairment of phagocyte recruitment

Circulating leukocytes respond to chemotactic signals to leave the vasculature and migrate to the site of infection. Although chemoattractants include products from the bacteria cell wall (eg, N-formyl peptides), the strongest and most specific stimuli are host-derived, including the CXC chemokine IL-8 and the complement-derived anaphylotoxin C5a. By pathogen interference with host chemokine functions, the kinetics of the innate immune response are delayed favoring bacterial survival. Interestingly, GAS appears to target the chemotactic peptides directly, whereas SA blocks essential host receptor functions.

IL-8 acts as a potent chemoattractant[17] and can be found tethered to the luminal surface of the microvasculature, where it provides a stop signal to rolling neutrophils.[18,19] GAS produce a protease (SpyCEP, also known as ScpC) that specifically cleaves the C-terminus of IL-8, leading to functional inactivation of the chemokine.[20] Mutation of SpyCEP dramatically reduces GAS virulence in the mouse necrotizing fasciitis model and is correlated with increased neutrophil influx to the site of infection.[21] C5a is an 11-kd fragment of the complement cascade with multiple inflammatory properties including the recruitment of

neutrophils and stimulation of their bactericidal capacity.[22] GAS sheds surface dehydrogenase, which binds and inactivates human C5a[23] and expresses an endopeptidase, ScpA, which cleaves this chemoattractant within the critical domain for leukocyte receptor recognition.[24]

Many SA strains produce the chemotaxis inhibitory protein of staphylococci (CHIPS), which binds with high avidity to the leukocyte receptors for C5a and N-formyl peptides, thereby blocking functional engagement of the respective chemoattractants.[25] SAs also express the extracellular adherence protein that binds and inhibits intercellular adhesion molecule-1 (ICAM-1), the endothelial receptor required to initiate leukocyte adhesion and diapadesis.[26]

Escape from neutrophil extracellular traps

It has recently been appreciated that, apart from their phagocytic function, neutrophils can efficiently capture and kill microbes in the extracellular space. This process involves neutrophil extrusion of a matrix of DNA and histones known as neutrophil extracellular traps (NETs) that ensnare bacteria, even in septic blood.[27,28] The trapped bacteria are then subjected to microbicidal effectors including peptidoglycan recognition protein S and the granule proteases elastase and myeloperoxidase.[27,29] With chromatin representing the principal scaffold of NETs, bacterial degradation of DNA represents a potential mechanism for pathogens to escape this aspect of innate immunity. A GAS strain with mutations in 3 encoded deoxyribonucleases (DNases) is significantly more susceptible to neutrophil killing and attenuated in murine skin and systemic infection models as well as pharyngeal infection of cynomolgus macaques.[30] The most prominent of these DNases was the highly active bacteriophage-encoded Sda1, present in the secreted proteome of the virulent M1T1 GAS clone associated with severe, invasive infections.[31] Targeted mutagenesis and heterologous expression of DNase Sda1 reveal that the enzyme is necessary and sufficient for promoting GAS NET degradation and resistance to neutrophil killing *in vitro* and *in vivo*.[32]

Interference with complement function

After activation of the classic, lectin, or alternative complement pathways, opsonization of foreign microbes occurs through deposition of C3b and its cleavage fragment iC3b on their surface. Complement receptors (CRs) on neutrophils and macrophages engage the bound C3b (CR1) or iC3b (CR3 and CR4) to facilitate phagocytosis. Because the complement system is capable of efficient self-amplification, potential host cell damage is mitigated by the counterregulatory proteins such as C4b-binding protein (C4BP), factor H (FH), and factor I (FI) that dampen the activity level of complement. SA and GAS exhibit the capacity to manipulate key host complement regulatory pathways selectively to thwart efficient opsonophagocytosis.

Cleavage of C3 to opsonically active C3b is accomplished after assembly of C3 convertase complexes C4bC2a (classic/lectin pathways) or C3bBb (alternative pathway) on the bacterial surface. The secreted ~10-kd SA protein known as staphylococcal complement inhibitor (SCIN) binds and stabilizes both convertases on the bacterial surface, preventing generation of additional convertases, impairing their enzymatic activities, and effectively inhibiting all 3 complement pathways.[33] Host regulatory protein C4BP interferes with the assembly of the C4bC2a C3 convertase of the classic/lectin pathway. GAS is able to acquire C4BP selectively from human serum through the action of the hypervariable regions of several M-protein family members, thereby inhibiting classical pathway activation[34,35]; a strong correlation can be established between C4BP acquisition on the GAS surface and evasion of phagocytosis.[36]

Factor H and its splice variant FH-like protein 1 (FHL-1) are central fluid-phase regulators of the alternative complement pathway, functioning to accelerate the decay of the C3bBb C3 convertase and acting as cofactors for FI-mediated degradation of C3b.[37] The cell wall anchored M protein has long been known to restrict deposition of C3b on the GAS surface, a function that can be correlated to resistance to phagocytosis.[38] Many GAS M proteins were found capable of binding FH and FHL-1 through their conserved C-repeat region and/or hypervariable N-terminal regions,[39] but the overall significance of M protein binding to FH and FHL-1 to complement resistance remains controversial. In GAS strains of the M1 serotype, the M protein is dispensable for FH and FHL-1 binding; instead, the surface-anchored protein Fba mediates interactions with these complement regulatory factors. Fba promotes M1 GAS survival in human whole blood and prevents deposition of C3b on the bacterial cell surface.[40] SA is also capable of manipulating host FI function, thereby shedding opsonically active C3b from the bacterial surface and impeding phagocytosis.[41]

Deposition of complement on the SA or GAS surface can also occur via the classic pathway under nonimmune conditions, but can be blocked by the ability of each pathogen to bind fibrinogen, which reduces accumulation of the C4bC2a C3 convertase. Binding of fibrinogen to the B and C repeats of GAS M protein plays an important role in restricting the number of epitopes that can serve as targets for opsonization.[42] The M-related protein Mrp, expressed by more than half of GAS strains, also recruits fibrinogen to the bacterial surface in a fashion that impairs complement deposition.[43] The surface-anchored SA fibrinogen-binding protein known as clumping factor acts in a similar fashion, effectively reducing opsonophagocytosis by macrophages and neutrophils.[44,45] The secreted SA fibrinogen-binding protein Efb-C can bind free C3, altering the solution conformation of this critical complement component such that it is unable to participate in its downstream opsonization functions.[46]

Another mechanism of interference with opsonization derives from bacterial co-optation of host proteolytic activities. The SA surface receptor staphylokinase binds plasminogen from host serum and converts the zymogen to the active protease, plasmin. Surface-bound plasmin can then cleave human C3b and C3bi from the bacterial cell wall and impair neutrophil phagocytosis.[47] GAS

expresses streptokinase, a protein with similar plasminogen-binding and zymogen activator capacities. The interaction of streptokinase with complement factors has not been studied in detail, but its role in plasmin accumulation on the bacterial surface is critical to development of GAS invasive infection.[48,49]

Interference with antibody-mediated opsonization

Immunoglobulins generated against specific bacterial epitopes provide a second effective form of opsonization, promoting engagement and uptake by host neutrophils and macrophages expressing surface receptors for the Ig Fc domain. SA and GAS confound this branch of host defense though specific proteins that degrade Ig molecules or bind them in a nonopsonic fashion not recognized by phagocyte Fc receptors.

The broad-spectrum SA serine protease SSP and GAS cysteine protease SpeB have been shown to cleave IgG, IgM, and IgA antibodies *in vitro*.[50,51] Mac-1, a second GAS cysteine protease, cleaves IgG *in vitro* and *in vivo*, targeting the lower Fc region,[52] and also binds FcγRIIIB (CD16) on the surface of neutrophils inhibiting phagocytosis and activation of the oxidative burst.[53] The closely related IgG endopeptidase Mac-2 likewise binds Fcγ receptors and competitively inhibits host phagocyte recognition of IgG on the bacterial surface.[54] The secreted GAS protein EndoS hydrolyzes the chitobiose core of the asparagine-linked glycan on IgG, preventing recognition of IgG by phagocyte Fc receptors, blocking complement activation through the classic pathway, and impairing opsonophagocytosis.[55] Staphylokinase-mediated acquisition and activation of host plasmin can also cleave IgG and remove the entire Fc fragment from the SA surface.[47]

The effector function of Ig is also thwarted when the pathogen binds its Fc region, effectively decorating the bacterial surface with the host molecule in a backward, nonopsonic orientation. This Fc-binding activity is classically associated with protein A of SA, which serves to block Fc-receptor mediated phagocytosis and contributes to animal virulence.[56] The surface-expressed GAS fibronectin-binding protein I also binds the aFc region of IgG, preventing antibody-dependent cell cytotoxicity by macrophages.[57] Several M protein types and protein H of GAS bind the Fc domains of IgG,[58-60] inhibiting IgG-dependent complement activation on the bacterial cell surface. It should be noted that there is no direct evidence that Fc binding proteins of SA or GAS can specifically bind antibacterial IgG; rather, these proteins may act to cloak the bacterial surface with abundant IgG of any specificity.

Polysaccharide capsules

The majority of SA clinical isolates express surface capsules composed of serotype 5 or 8 polysaccharide.[61] The presence of SA capsule is associated with reduced opsonophagocytic uptake of the pathogen by neutrophils and increased virulence in a mouse bacteremia model.[62,63] Analogous functions can be ascribed to an additional SA surface polysaccharide, poly-N-acetylglucosamine.[64] All GAS express a capsule composed of a homopolymer of hyaluronic acid; variants with increased encapsulation are linked epidemiologically to greater invasive disease potential.[65] Capsule-deficient GAS produced by targeted mutagenesis of the hyaluronic acid biosynthesis biosynthetic operon or through hyaluronidase treatment become susceptible to phagocytic clearance and become less virulent in animal challenge models.[66] Neither the SA nor the GAS exopolysaccharides directly inhibit deposition of complement factors on the bacterial surface; rather, they appear to serve as a superficial cloak that restricts access of phagocytes to the opsonins.[67,68]

Resistance to phagocyte intracellular killing

After phagocytic uptake of the target bacteria, neutrophils and macrophages deploy an array of bactericidal mechanisms such as vacuole acidification, generation of reactive oxygen and nitrogen species, and production of cationic molecules including AMPs (discussed above), myeloperoxidase, and lysozyme.[69] Recently it has been shown that SA and GAS can escape from the phagosome into the cytoplasm of neutrophils.[70,71] Because viable SA and GAS can be isolated from inside host phagocytic cells both *in vitro* and *in vivo*,[71,72] the traditional interpretation of these Gram-positive species as extracellular bacterial pathogens is undergoing re-evaluation. Consequently, increased attention has been focused on the molecular basis of SA and GAS survival within phagocytes.

Catalase production is a diagnostic tool for SA in the clinical laboratory, and its ability to neutralize hydrogen peroxide generated during oxidative burst may promote phagocyte resistance and virulence.[73] The golden pigment for which SA is named is a carotenoid molecule with potent antioxidant properties that is necessary and sufficient to promote bacterial neutrophil resistance and virulence in a subcutaneous infection model.[74,75] SA also resists oxidative stress through superoxide dismutases, as confirmed by diminished *in vivo* survival of mutants lacking these enzymes.[76] The multifaceted antioxidant capacities of SA likely underpin its prominent role as an opportunistic pathogen in patients with chronic granulomatous disease, where defects in nicotinamide dinucleotide phosphate (NADPH) oxidase lead to marginal oxidative burst function.

Because GAS lacks catalase or pigmentation present in SA, the bacterium has generally been considered more susceptible to host oxidative burst killing. However, a recent study revealed that GAS expression of glutathione peroxidase GpoA allows the organism to adapt to oxidative stress and contributes to virulence in several animal models of GAS infection.[77] GAS M protein was found to inhibit the fusion of azurophilic granules with the phagosome and other membrane trafficking events required for phagosome maturation.[78,79]

Leukocidal toxins

Staphylococcus aureus and GAS elaborate a variety of potent cytotoxins, and another important mechanism for innate immune resistance may involve triggering the death of the phagocytic cell types before bacterial killing can be accomplished. SA produces a family of 2-subunit

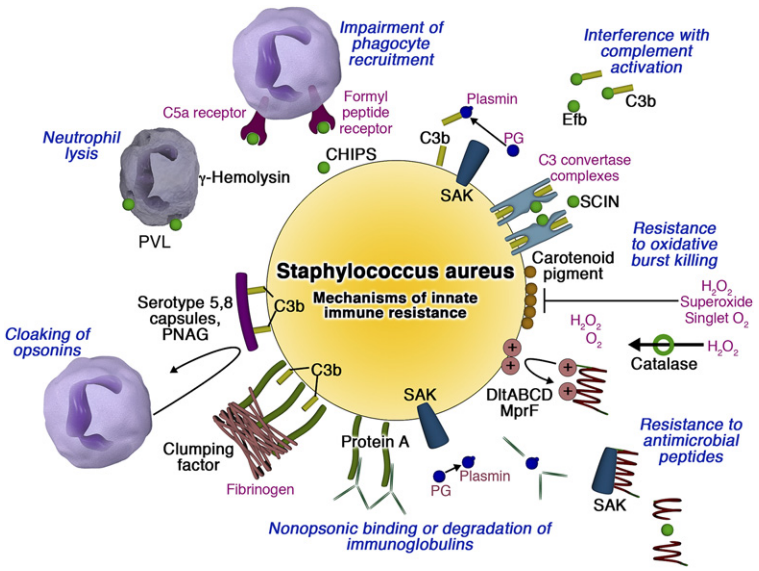

FIG 1. Mechanisms by which the bacterial pathogen SA subverts host innate immune defense. Phagocyte recruitment is limited by binding of CHIPS to chemokine receptors. Complement activation is blocked by protein Efb binding of soluble C3 and inhibition of the both the classic/lectin and alternative C3 convertases by SCIN. Golden carotenoid pigment provides an antioxidant shield whereas catalase detoxifies hydrogen peroxide. Resistance to cationic antimicrobial peptides is afforded by positive charge modifications of the cell wall, aureolysin-mediated proteolysis, and binding/inactivation by staphylokinase. Protein A binds Fc domains of Igs in a nonopsonic manner, whereas fibrinogen binding clumping factor and the surface polysaccharide capsule and poly-N-acetylglucosamine *(PNAG)* act to cloak surface bound opsonins from phagocyte recognition. The heptameric pore-forming toxins γ-hemolysin and PVL preferentially target leukocyte membranes. The plasminogen *(PG)* binding protein staphylokinase *(SAK)* activates the zymogen to the active protease plasmin, which can degrade complement opsonin C3b and the immunoglobulin Fc domain.

heteroheptameric toxins capable of oligomerizing in the membrane of target leukocytes to produce pores and promote hypo-osmotic cell lysis. These include γ-hemolysin, leukocidin, and the bacteriophage encoded Panton-Valentine leukocidin (PVL).[80] The last toxin has gained notoriety because of its strong epidemiologic association with severe cases of community-acquired methicillin-resistant SA (MRSA) infections.[81] The true contribution of the PVL toxin to SA virulence remains uncertain. Phage transduction of PVL into a previously naive SA background appeared to increase virulence in murine necrotizing pneumonia model[82]; however, the more direct test of isogenic deletion of PVL in the epidemic USA300 and USA400 clones associated with severe community-acquired MRSA infections had no effect on neutrophil lysis or virulence in skin abscess and systemic infection models.[83]

The pore-forming GAS β-hemolysin streptolysin S exerts cytocidal activity on host neutrophils and thereby promotes GAS resistance to phagocytic killing.[84,85] The structurally unrelated cholesterol-binding cytolysin streptolysin O is also toxic to human neutrophils, impairs their phagocytic capacity, and allows GAS to avoid lysosomal localization.[86-88] Consequently, both the streptolysin S and streptolysin O toxins are key virulence factors in the pathogenesis of invasive GAS infection.[84,89,90]

On phagocytosis, GAS also mediate a program of accelerated neutrophil apoptosis that can be correlated to enhanced phagocyte resistance relative to a variety of other common human pathogens.[91] Although the GAS virulence factors involved in the neutrophil apoptosis differentiation program and their cellular targets remain to be elucidated, epithelial cell models suggest GAS can induce a unique apoptosis pathway based on caspase-9 release, mitochondrial dysfunction, and calcium regulation.[92,93]

CAN WE LEVEL THE PLAYING FIELD AGAINST THESE FORMIDABLE PATHOGENS?

In the preceding section, we reviewed 2 large arsenals of molecular mechanisms deployed by leading human pathogens SA (Fig 1) and GAS (Fig 2) to avoid clearance by the innate immune system. Although the individual virulence factors have different names, encoding genes, and chemical structures, each pathogen interferes with host defense at multiple points, from AMPs at the epithelial surface, to initial phagocyte recruitment, to the processes of opsonization, to bacterial entrapment and uptake, to the intracellular effectors of bacterial killing. The accumulated repertoire of virulence factors finds corroboration in the high prevalence of human SA and GAS infections and their potential to produce severe disease in patients of all ages, even those previously healthy. Vaccine strategies to combat each pathogen have progressed slowly in

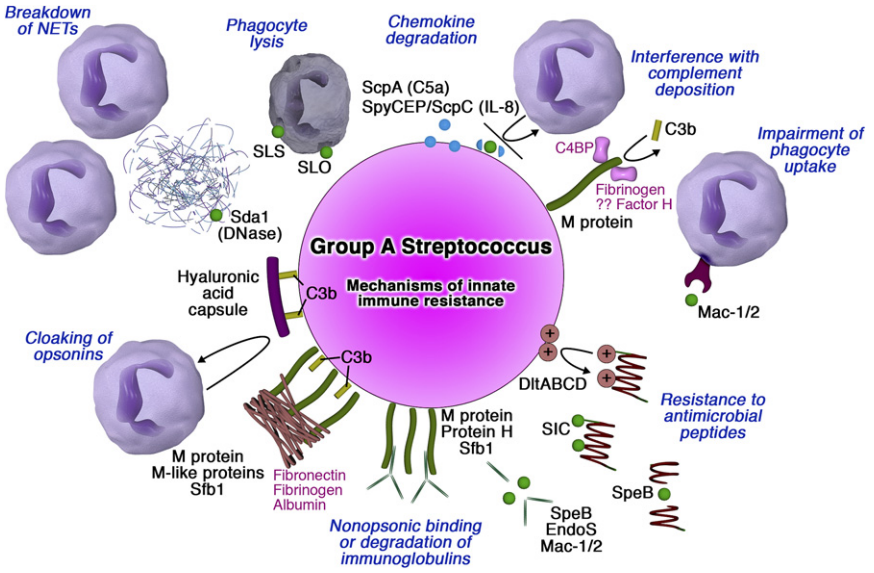

FIG 2. Mechanisms by which the bacterial pathogen GAS subverts host innate immune defense. Phagocyte recruitment is reduced by peptidases ScpA and SpyCEP/ScpC that degrade the chemokines C5a and IL-8, respectively. Complement deposition is limited by M protein binding of host counterregulatory factors C4BP and FH. Phagocytic uptake is reduced by Mac-1/2 binding of phagocyte Fc receptors. Resistance to cationic antimicrobial peptides is afforded by D-alanyl modification of cell wall teichoic acids, cysteine protease SpeB-mediated degradation, and binding/inactivation by protein streptococcal inhibitor of complement *(SIC)*. M protein and protein H bind Fc domains of Igs in a nonopsonic manner, and proteolytic inactivation of Ig is a property of SpeB, Mac-1/2, and endopeptidase S. DNase production facilitates escape from NETs, and the pore-forming cytolysins streptolysin S *(SLS)* and streptolysin O *(SLO)* exhibit lytic activity against host neutrophils and macrophages.

clinical development and face considerable technical and biological challenges.[94,95] Moreover, the rising epidemic of MRSA in hospital and community settings poses a serious threat to the medical community and public health.

Recently, the National Research Council National Academy of Sciences convened 2 Workshops on Novel Antimicrobial Therapeutics entitled ''Treating Infectious Diseases in a Microbial World.''[96] The committees singled out 2 particular areas of basic research that, although high-risk, represent genuinely novel approaches that have the potential to reap great benefit in the long term: (1) alternatives to direct killing of microbes that disable or confuse the pathogen, and (2) mechanisms to boost innate immunity against a wide array of infectious agents. The emerging mechanistic knowledge on the interplay between specific SA and GAS virulence determinants and individual components of human innate immunity provides a framework for rational drug design in each category. Such innovative therapies are now beginning to be explored in terms of proof of principle, and the subsequent sections provide a few illustrative examples.

NEUTRALIZING BACTERIAL VIRULENCE FACTORS THAT THWART INNATE HOST DEFENSE

The concept of antimicrobial therapy targeting individual pathogen virulence factors conceivably provides 2 theoretical advantages over conventional antibiotic therapy. First, because the therapy seeks not to kill the pathogen but rather to render it harmless, selection pressure for evolution of resistance may be lessened. Second, because the therapy is focused on an specific virulence factor of a particular pathogen, undesired broad-spectrum activities against the normal flora (and attendant side effects) would be diminished. As reviewed, genetic inactivation of several individual SA or GAS virulence factors produces isogenic mutants with increased susceptibility to innate immune clearance and reduced disease potential in animal models; these gene-encoded pathogenicity factors represent pharmacologic targets in this vein.

Recently, therapeutic approaches to virulence factor neutralization have been demonstrated as proof of principle in animal models. The golden carotenoid pigment promotes SA virulence by providing a shield against oxidant-based neutrophil killing.[74,75] A chemical inhibitor of the enzymatic pathway for carotenoid biosynthesis has no effects on SA growth rate but yields a colorless bacterium that is sensitive to hydrogen peroxide and singlet oxygen and susceptible to neutrophil killing in whole blood.[74] DNase Sda1 promotes GAS escape from extracellular killing in NETs.[30,32] G-actin, a natural pharmacologic inhibitor of type I DNases including Sda1, prevented Sda1 degradation of NETs *in vitro* and *in vivo*, promoting neutrophil killing and reducing lesion development in the mouse necrotizing skin infection model.[32] DNA immunization of mice with the gene for clumping factor ClpA produced

serum neutralizing antibodies that reduced the pathogen's ability to bind fibrinogen, resist macrophage killing, and establish infection in a mouse mastitis model.[97]

A companion concept is to target pharmacologically regulatory pathways that control the pathogen's expression of key virulence determinants. A synthetic thiolactone-containing peptide capable of inhibiting the SA *agr* quorum sensing regulatory system reduced the development of abscesses in the mouse skin infection model[98] but must be approached with caution because *agr* repression can in some cases promote biofilm formation or antibiotic-resistant small colony variants. Salicylic acid, the principal *in vivo* metabolite of aspirin, activates the SA stress response gene *sigB*, which inhibits the global transcriptional regulons of *sarA* and *agr*, reducing expression of fibronectin binding and cytolytic proteins, abrogating SA virulence in a rabbit endocarditis model.[99] Exogenous administration of a synthetic form of the competence-stimulating pheromone GAS peptide silCR reduced the pathogen's production of the chemokine protease SpyCEP, promoting a robust neutrophil response, and prevented systemic spread of infection.[100]

PHARMACOLOGIC AUGMENTATION OF INNATE HOST DEFENSE

A current limitation of infectious disease therapy is that our efforts are essentially focused on only 1 side of the host-pathogen equation, the microbe itself. The concept that the mammalian innate immune system is by necessity not as good as it could be is illustrated by genetic studies in mice, such as those showing enhanced resistance to GAS infection after transgenic overexpression of cathelicidin AMPs[101] or increased bactericidal activity of macrophages in which the counterinflammatory factor IκB kinase (IKK) catalytic subunit IKKα is removed.[102] Molecular dissection of SA and GAS virulence mechanisms for innate immune evasion coincidentally highlight those defense factors that could, if expressed at sufficient levels and in the right microenvironment, effectively restrict spread of the pathogen. Our innate immune system must be tightly regulated to allow rapid response to microbial threats while limiting collateral inflammatory damage to tissues. Understanding this balance means additional bactericidal capacities can be tapped by stimulating activation pathways or removing regulatory brakes governing expression of immune effector molecules.

The pharmacologic administration of an individual effector molecule of human innate immunity such as a cationic AMP raises an interesting quandary. Because certain bacterial species have been known to mutate to AMP resistance on serial exposure to peptides,[103] we might risk selection for pathogens of enhanced virulence and public health concern because of their ability to avoid innate immune clearance in subsequent infectious encounters. Use of nonhuman AMPs such as the bovine cathelicidin BAP-28, shown to reduce lethality of SA in a mouse sepsis model,[104] represents 1 viable alternative. A second appealing strategy is to augment cellular production of AMP in the context of the appropriate physiologic response to infection. Very recently, vitamin D was shown to increase cathelicidin expression in skin by enabling keratinocytes to recognize and respond to microbial stimuli via Toll-like receptor 2, promoting killing of SA and protecting wounds against infection.[105,106] This pharmacologic strategy could prove of clinical benefit in prophylactic therapy of human surgical wounds or eczematous lesions known to be prone to SA superinfection.

The transcriptional regulator hypoxia-induced transcription factor 1α (HIF-1α) has recently been recognized to respond directly to bacterial stimuli including SA and GAS and promote transcription of macrophage and neutrophil genes governing expression of antimicrobial effectors such as cathelicidin, granule proteases, and nitric oxide.[107] Mice deficient in HIF-1α in their myeloid lineage are more susceptible to GAS infection; conversely, phagocyte killing by host macrophages can be augmented by genetic or pharmacologic augmentation of HIF-1α levels.[107] A promising new candidate for therapeutic enhancement of innate immunity is innate defense regulator peptide 1, which signals through mitogen-activated protein kinase pathways to stimulate release of monocyte/macrophage chemokines specifically yet dampens release of undesired proinflammatory cytokines.[108] Pretreatment regimens with innate defense regulator peptide 1 were helpful in controlling a number of bacterial infections in the murine model, including MRSA.[108] An advantage of targeting host molecules that enhance phagocyte recruitment or activate array of phagocyte bactericidal mechanisms is lack of selective pressure for resistance—the bacterial pathogen cannot evolve to combat a drug that targets the host and effectively deploys a multifaceted combination therapy of natural antimicrobial molecules.

CONCLUSION

Bacterial pathogens associated with significant human infection such as SA and GAS often reside in the transient microflora of healthy individuals in the context of asymptomatic colonization. Experimental analysis of SA and GAS interactions with the human innate immune system represents a useful paradigm for discovery and understanding of the underlying mechanisms dictating the development or prevention of serious bacterial infection. Of the many SA and GAS factors reviewed herein, still only a subset have been definitely proven to contribute to innate immune subversion *in vivo*, whereas others have been shown to promote phagocyte resistance in *ex vivo* systems, and still others have simply been shown to interact *in vitro* with host effector molecules in a fashion that could be predicted to promote the pathogen's survival. Ongoing investigations will begin to define variations in host phagocyte defense dictated by genetic polymorphisms that may render individual patients particularly vulnerable to SA or GAS infection.

From *The Art of War*, the 6th century treatise by Chinese philosopher Sun Tzu, derives the military axiom,

"Know your enemy and know yourself." In the modern war against infectious disease, this demands both a careful understanding of the key components of innate immunity and the molecular mechanisms by which certain pathogens avoid effective clearance. In the future, pharmacologic approaches can be envisioned in which the drug does not kill the infectious microbe directly, but rather blocks disease progression by neutralizing specific virulence factors or boosting key host innate immune defenses. Such approaches could be useful adjuncts to conventional therapy in patients with altered host immunity (eg, cancer chemotherapy or AIDS) or defects in essential barrier functions (eg, surgical wounds or burns). Finally, these therapies should prove equally effective against antibiotic-resistant bacteria such as MRSA, because these strains depend on the same virulence attributes as their antibiotic-sensitive counterparts to subvert innate immunity and produce disease.

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