Staphylococcus aureus Protein A Promotes Immune Suppression

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ABSTRACT *Staphylococcus aureus* **is a prominent cause of human infections worldwide and is notorious for its ability to acquire resistance to antibiotics. Methicillin-resistant** *S. aureus* **(MRSA), in particular, is endemic in hospitals and is the most frequent cause of community-associated bacterial infections in the United States. Inasmuch as treatment options for severe MRSA infections are limited, there is need for a vaccine that protects against such infections. However, recent efforts to generate a staphylococcal vaccine have met with little success in human clinical trials. These failures are somewhat puzzling, since the vaccine antigens tested promote opsonophagocytosis** *in vitro* **and confer protection in animal infection models. One possibility is that the pathogen inhibits (and/or fails to elicit) the development of protective immunity in humans. Indeed,** *S. aureus* **produces numerous molecules that can potentially promote immune evasion, including protein A (SpA), an immunoglobulin (Ig)-binding protein present on the bacterial surface and freely secreted into the extracellular environment. SpA binds the Fc region of antibody and the Fab regions of the B-cell receptor, processes that are known to block opsonophagocytosis and cause B-cell death** *in vitro.* **In a recent study, Falugi et al. [F. Falugi, H. K. Kim, D. M. Missiakas, and O. Schneewind, mBio 4(5):e00575-13, 2013] showed that vaccination with** *spa* **mutant** *S. aureus* **strains lacking antibody Fc- and/or Fab-binding capacity protects against subsequent challenge with the USA300 epidemic strain. The findings provide strong support for the idea that SpA promotes** *S. aureus* **immune evasion** *in vivo* **and form the foundation for a new approach in our efforts to develop a vaccine that prevents severe** *S. aureus* **infections.**

*S*taphylococcus aureus is a ubiquitous human pathogen and a leading cause of infections worldwide. The pathogen is capable of causing a diversity of syndromes ranging in severity from common skin and soft tissue lesions to highly invasive and systemic disease. The high prevalence of staphylococcal infection is facilitated by the commensal lifestyle of the bacterium, which is frequently associated with the skin and anterior nares of healthy individuals. *S. aureus* is a predominant cause of nosocomial infections, which often occur in individuals with predisposing risk factors, such as hemodialysis or surgery. Historically, the success of *S. aureus* as a human pathogen has been influenced by a strong propensity to develop antibiotic resistance, and methicillin-resistant *S. aureus* (MRSA) now ranks as a leading cause of hospital-associated infections (1). Multidrug-resistant *S. aureus* strains are endemic in hospitals, and the pathogen has developed mechanisms to negate virtually all antibiotics of clinical value. To further obfuscate *S. aureus* epidemiology, one of the most notable developments in recent bacterial infectious disease history was the rapid emergence of community-associated MRSA (CA-MRSA). First reported in the 1990s, CA-MRSA rapidly emerged worldwide, and a strain known as USA300 is the most abundant cause of community-associated bacterial infections in the United States (2).

The outlook for new therapeutic options to treat *S. aureus* is confounded by a paucity of new classes of antimicrobial agents in the drug discovery pipeline (3). Considering the penchant of *S. aureus* to rapidly develop antibiotic resistance, there is a clearly defined need for an effective vaccine. Unfortunately, the overwhelming majority of attempts to develop a clinically useful vaccine have failed (4). The lack of success is largely attributed to use of conventional strategies directed at enhancing the process of opsonophagocytosis, which is problematic since the vast majority of adults are already endowed with a repertoire of opsonic antibodies and serum complement. Indeed, vaccines comprised of *S. aureus* surface antigens, such as iron surface determinant B (IsdB) and polysaccharide capsular antigens CP5 and CP8, failed to protect against *S. aureus* infection according to results from phase III clinical trials (5, 6). Although *S. aureus* vaccines designed to enhance bacterial uptake by phagocytes have had limited utility, it remains to be determined if alternative vaccine strategies will prove useful. For example, *S. aureus* secretes several toxins that collectively contribute to pathogenesis, and toxins such as alpha-hemolysin (Hla) are under evaluation as vaccine candidates in early clinical trials.

To gain an enhanced understanding of the mechanisms by which *S. aureus* causes disease, Falugi et al. investigated the role of SpA in virulence and host immune evasion (7). The authors generated Newman strains with deletion of *spa* (Δspa) and mutations in the antibody Fc- and/or F(ab')$_2$-binding domains of *spa* (spa_{KK}, spa_{AA}, and spa_{KKAA}), and compared the ability of wild-type and mutant strains to circumvent and/or alter innate and adaptive immune responses in the mouse (7). The authors show that (i) the Fc-binding domain of SpA is important for *S. aureus* survival in mouse blood *in vivo* and *in vitro*, (ii) infection or vaccination with the spa_{KKAA} strain elicits a pronounced anti-*S. aureus* antibody response not present following infection the wild-type strain, and (iii) vaccination or infection with the spa_{KKAA} protects mice from death caused by subsequent USA300 infection. These SpA-mediated phenomena were absent in mice lacking B cells and antibody. One important implication of these findings is that spa_{K}-

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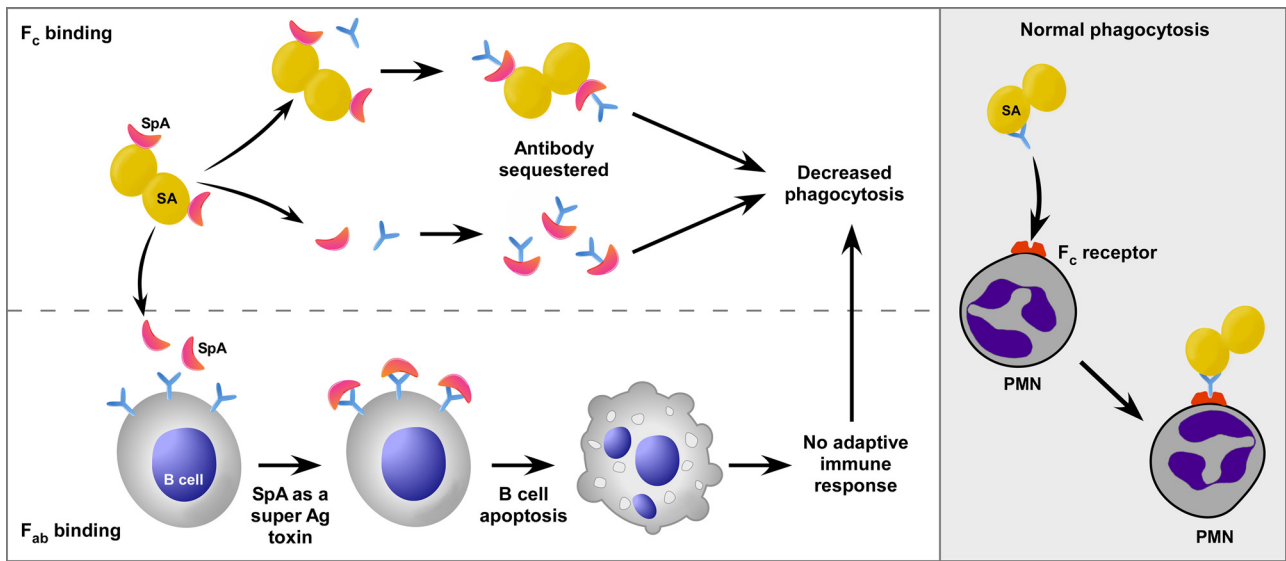
FIG 1 Mechanisms of SpA-mediated immune evasion. (Left panel) SpA (red crescent shape) present on the surface of *S. aureus* (SA) or SpA that is freely secreted binds the Fc region of antibody (Ab), thereby preventing normal phagocytosis (right panel). Alternatively, SpA binds the Fab regions of the B-cell receptor (lower left panel), which induces B-cell death and prevents the production of antibody specific for *S. aureus*. Ag, antigen; PMN, polymorphonuclear leukocyte.

KAA could be used—at least in part—in a vaccine approach for *S. aureus* infections.

SpA has long been known to bind inhibit opsonophagocytosis *in vitro* (8), and the ability of the protein to block phagocytosis is dependent on the presence of host antibody (9). In addition to its Fc-binding capacity, SpA binds Fab regions of the B-cell receptor (membrane-anchored IgM) (10, 11), and in doing so, it functions as a B-cell superantigen that induces programmed cell death (12) (Fig. 1). Thus, SpA can potentially alter the innate and adaptive immune responses to *S. aureus*. In their original studies of the ability of SpA to inhibit phagocytosis, Dossett et al. proposed that SpA "may play a role in the pathogenesis of staphylococcal infections." (8). This idea is borne out in the work by Falugi and colleagues (7).

The mechanisms for SpA-mediated virulence seem clear—i.e., inhibition of opsonophagocytosis mediated by specific antibody and inhibition of B-cell responses that lead to production of opsonic and neutralizing antibodies (Fig. 1). Although these processes likely contribute to the success of *S. aureus* as a human pathogen (as Falugi et al. suggest [7]), several other factors—some highlighted by the authors—must be considered. First, whether humans have protective immunity to *S. aureus* is a question that remains to be resolved. The observation that a select group of individuals succumb to recurrent infections provides strong support to the idea that at least some people lack or fail to develop immunity to *S. aureus* infections. On the other hand, most adults have been exposed to *S. aureus* or other staphylococci, and 30% of noninstitutionalized individuals are asymptomatically colonized by the bacterium (13), but the vast majority fail to develop—or have never had—serious invasive *S. aureus* infections. Also, many skin and soft tissue infections resolve without treatment. These findings appear at variance with the idea that humans fail to develop immunity or lack immunity (which includes innate immunity) to *S. aureus*.

Indeed, the innate immune system is widely regarded as the primary defense against *S. aureus* infections in humans. Previous studies have demonstrated that *S. aureus* cells (including USA300) opsonized with normal human serum or those present in human blood are bound and/or ingested rapidly by phagocytes *in vitro* (14, 15). Consistent with these findings, David Rogers first reported that *S. aureus* is rapidly cleared from the bloodstream of rabbits by neutrophils, which ultimately traffic the pathogen to distal tissues (16). Whether antibody is required for phagocytosis of *S. aureus* has also been brought into question in past studies. For example, early work by Shayegani and Kapral demonstrated that *S. aureus* can be ingested by leukocytes in the absence of antibodies under conditions now known to prime neutrophils for enhanced phagocytosis (17). Notably, adherence primes neutrophils for enhanced functions, such as phagocytosis, and the vast majority of community-associated *S. aureus* infections are those of skin and soft tissue—conditions under which phagocytes are adherent. Thus, although specific antistaphylococcal antibodies can promote phagocytosis *in vitro* and in mouse infection models, the relative importance of such antibodies in the protection of humans from infection remains to be determined.

Despite these caveats, the work by Falugi et al. (7), coupled with earlier studies by the same group (18), represents a significant step forward in our efforts to design a staphylococcal vaccine. SpA has been considered an *S. aureus* virulence molecule for decades, but the application of nontoxigenic SpA as a vaccine antigen is an innovative and refreshing approach. One of the long-standing problems with *S. aureus* vaccines and vaccine antigens is that protection can be generated in mice, but such protection has failed to translate successfully to humans. Thus, if the protection generated in mice can be translated to humans, then this approach has tremendous potential for success.

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