



Review

Preventing Staphylococcal Disease by Disarming the Immune Responses to Infection

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Abstract. Use of experimental models of staphylococcal infections clarified several bacterial virulence factors as well as many hematopoietic cell types and their products that are involved in the pathogenesis of infection. For many decades it has been believed that antibody mediated response to staphylococci and their products was the major, if not the only one, hallmark of immune reactivity during infection. Recent studies have documented that T cell mediated responses to superantigens produced by staphylococci are not only prominent but also decisive with respect to sequels. Also the nonantigen specific immune responsiveness to staphylococcal infection is reviewed including roles of neutrophils, complement system and nitric oxide. The knowledge gained regarding staphylococcal virulence factors and the host immune responses has prompted researchers to develop new strategies how to interact *in vivo* with the infectious process. Some of these approaches are commented in this review regarding e.g. vaccination procedures in order to prevent severe infections as well as therapeutic procedures to minimize organ damage during an ongoing infectious process.

Key words: *Staphylococcus aureus*; vaccination; virulence immunity; T cells; B cells; cytokines; CpG oligonucleotides.

Staphylococcus aureus infections remain associated with high morbidity and mortality, a permanent threat to mankind. Staphylococci give rise to a diverse spectrum of infectious manifestations ranging from cutaneous affection to life-threatening conditions such as sepsis, endocarditis and arthritis. A study in the USA reported *S. aureus* as the most common cause of nosocomial infections during the period of 1990–1996⁹. The increasing prevalence of immunocompromised subjects and appearance of methicillin-resistant staphylococci should prompt researchers to reach a better understanding of the host-bacterium relationship, a prerequisite for better therapeutic and preventive measures. Studies of pathogenetic mechanisms in human *S. aureus*

infections have met with shortcomings, mostly related to uncertainty as to the exact time of the onset of the disease. Use of experimental animals overcomes this and a number of other problems related to use of genetically modified bacterial strains. In this respect, mice are the most suitable species due to: a) the development of spontaneous staphylococcal infections⁷, b) the availability of many inbred and genetically well characterized mouse strains, c) the availability of large numbers of strains either lacking certain gene(s) of potential interest (so called knock-out mice) or having overexpression of certain gene(s) (so called transgenic mice), and d) last, but not least, the current knowledge of the murine immune system.

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The aim of this review is to discuss experimental models of *S. aureus* infections with emphasis on the pathogenetic mechanisms governing bacterial virulence and host responsiveness in septic arthritis and sepsis. Also, certain aspects regarding the treatment and prophylaxis of staphylococcal infections will be covered.

Determinants of Staphylococcal Virulence

Staphylococci are among the most resistant non-spore-forming bacteria able to survive many extreme environmental conditions such as extended periods of dryness and high salt concentrations. Staphylococcal virulence depends on the relative inefficiency of the innate host defence in dealing with a wide variety of extracellular toxins and enzymes, components of the cell wall and, as is the case for many strains, the expression of a capsule and a slime layer.

There are 11 recognized types of capsular polysaccharides. However, type 5 and type 8 capsular polysaccharides comprise more than 80% of all isolates obtained in cases of bacteremia. It has been demonstrated that encapsulation is a virulence determinant, since the capsule acts as a physical barrier preventing contact between the cell wall-associated complement factor C3 and the membrane of the phagocyte^{12, 31}, thereby inhibiting phagocytosis and intracellular killing of bacteria²². Indeed, a recent study has demonstrated that staphylococcal strains isogenic with respect to expression of polysaccharide capsule type 5 expressed distinctly different patterns of virulence in experimental models of sepsis and septic arthritis²².

Inside the capsule there are major cell wall components, i.e. peptidoglycan, teichoic acid and different proteins which bind to host tissue structures, i.e. adhesins. Different types of staphylococcal adhesins determine the ability of *S. aureus* strains to colonize host tissues, enabling the bacteria to initiate infections in wounds, bone tissue, heart valves or joint cartilage. It is evident that each *S. aureus* strain is able to express several types of adhesins, some of which may be environmentally regulated, while others are expressed constitutively. The process of bacteria interacting with host tissues is highly dynamic and an object of modulation by the regulatory mechanisms of the bacteria¹⁹, environmental factors, host defence mechanisms as well as anti-bacterial therapy¹⁵. For example, in wound infections staphylococci may bind to fibrinogen or fibronectin exposed in the wound³⁵, whereas in osteomyelitis expression of the receptor for bone sialoprotein seems to predispose for disease²⁶. In staphylococcal arthritis,

staphylococcal collagen adhesin was found in essentially all isolates compared to only one-third of the strains isolated from soft tissue infections³². Further experimental studies clearly demonstrated that collagen adhesin is an important virulence determinant in *S. aureus* arthritis²⁵. Interestingly, systemic vaccination with a recombinant fragment of collagen adhesin A-subunit protects mice from severe septic arthritis and sepsis triggered death²³. This protective effect is mediated by induction of collagen adhesin-specific antibodies triggered by the vaccination procedure. In contrast, infection with a collagen adhesin-expressing strain will not trigger specific antibody response to this structure²³, which is a possible explanation for the recurrent pattern of staphylococcal infections.

S. aureus secretes a large number of enzymes and toxins, many of which have been implicated as potential virulence factors. For example, *S. aureus* produces five membrane-damaging toxins – four hemolysins (α , β , γ , δ) and leukocidin. These toxins may all give rise to damage of erythrocytes, but they differ in their mechanisms of action. It has recently been shown that combination of α and γ hemolysins jointly promote virulence of staphylococci in septic arthritis²¹. Finally, a group of staphylococcal toxins display superantigenic properties, i.e. they have the capacity to stimulate a large fraction of T lymphocytes expressing particular T cell receptor V β sequences. These toxins include the enterotoxins (A, B, C1-3, D, E, and G), toxic shock syndrome toxin-1 (TSST-1), and exfoliatin A and B³⁰. Superantigens cause fever, hypotension and other acute toxic shock-like symptoms by release of proinflammatory cytokines, such as interferon γ (IFN- γ) and tumor necrosis factor¹⁰. Apart from their systemic effects, enterotoxins cause gastroenteritis with diarrhoea and vomiting, whereas TSST-1 gives rise to toxic shock syndrome characterized by fever, desquamative skin rash, hypotension and multiple organ failure. We have recently demonstrated that staphylococcal superantigens are important virulence determinants in experimental septic arthritis⁸. Thus, *S. aureus* mutant strains isogenic for TSST-1 show clearly different patterns as to their capacity to trigger arthritis³. Furthermore, selective interaction with the T cell receptor V β family that recognizes TSST-1 will downregulate the severity of arthritis and the frequency of septic death caused by staphylococci².

The genes that code for the production of exoproteins and cell wall proteins are controlled by different regulatory loci, such as agr (accessory gene regulator), and sar (staphylococcus accessory regulator). The synthesis of the majority of toxins and extracellular

enzymes is enhanced by the agr system, whereas the production of protein A and coagulase is suppressed. The sar locus normally activates the production of hemolysins as well cell wall proteins. We have recently demonstrated that both agr and sar are important virulence determinants in experimental septic arthritis^{1, 20}.

A recently discovered and potentially important virulence factor is bacterial CpG rich (unmethylated) DNA. Nucleotide sequences containing CpG motifs, originating for example, from *S. aureus*, give rise to macrophage activation leading to release of proinflammatory cytokines, including IL-1 β , IL-6, IL-12 and TNF- α . These events, if present in the joint, as in the case of localised staphylococcal growth, will promptly lead to arthritis¹⁰. Thus, induction of arthritis and septic shock during *S. aureus* septicemia might be triggered by the concerted action of both superantigens activating T lymphocytes and free bacterial DNA (originating from disrupted bacteria) triggering macrophages to release proinflammatory cytokines.

Mechanisms of Host Defence

Host protective mechanisms – the role of the innate immune system

The normal host is protected against staphylococcal infections by a triad of a) intact host tissues, b) immunologic and c) non-immunologic defense mechanisms. With respect to the integrity of host tissues it is clear that subjects displaying skin defects, damaged joints (e.g. due to rheumatoid arthritis) or defective heart valves (e.g. due to rheumatic fever) will be at risk of acquiring *S. aureus* infection. Also, patients with installed prostheses or having indwelling catheters will be more prone to develop staphylococcal infections originating at these sites. However, even in cases of organ integrity defects in the innate immune system may be an important determinant of susceptibility to infection. Experimentally, selective deletion of neutrophils will lead, within a few days, to sepsis-mediated death in experimental animals inoculated i.v. with *S. aureus*³⁴. Also, selective inhibition of nitric oxide synthase will invariably lead to aggravation of severe *S. aureus* infections, including both sepsis and septic arthritis^{17, 18}. As might be expected from human case reports regarding subjects with complement deficiencies, depletion of the complement system in experimental animals will give rise to increased severity of sepsis and septic arthritis²⁹.

Thus, as judged from the above examples it is clear that innate immune system is defending the host against the invading staphylococci.

Tissue destruction during staphylococcal infection is mediated by adaptive immune responses

During the last few years it has become increasingly evident that, whereas the innate immune system is protective in the case of staphylococcal infection, the adaptive (or specific) immune system participates in the destructive process. Indeed, elimination of T lymphocytes from the host will, quite surprisingly, significantly decrease the severity of staphylococcal arthritis⁶. This autoreactive process is mediated preferentially by certain populations of CD4⁺ T cells, being specific for superantigens. Indeed, elimination of either the entire CD4⁺ population or V β 11-expressing T lymphocytes clearly downregulates both septic arthritis as well as sepsis-triggered mortality². Even more surprisingly, B lymphocyte deficiency will also lead to amelioration of septic arthritis³⁶ despite decreased antibody production. This outcome may be due to a decreased capacity for antigen/superantigen presentation by B cells. Alternatively, resistance to *S. aureus* infection in B cell deficient mice correlated with increased mRNA expression for IFN- γ , a cytokine of importance for bacterial defence (see below). Not astonishingly, early neutralization of this cytokine in B cell deficient mice led to significantly increased mortality as compared to sham treated controls³⁶.

Another set of cells participating in the adaptive immune responses is a heterogeneous group of antigen-presenting cells. Most of the antigen presentation is mediated by major histocompatibility complex (MHC) molecules. Whereas MHC class I molecules are present on all nucleated cells, MHC class II expression is predominantly found on B lymphocytes, monocytes/macrophages, and dendritic cells. The major function of these molecules is to bind intracellularly processed and degraded proteins and to present them to T lymphocytes. This MHC mediated antigen recognition leads to the formation of a trimolecular complex between antigenic peptides, MHC molecule and T cell receptor. The role of MHC class II (the class of importance in presenting exogenous antigens and superantigens) in the case of staphylococcal sepsis and septic arthritis has also been studied. The results indicate that a total absence of MHC class II abrogates the expression of disease. In addition, a different class II MHC expression in congenic mice encodes for a different susceptibility to joint inflammation induced by staphylococci⁴. These differen-

ces have been shown to depend on the antigen-presenting capacity of different MHC class II molecules rather than differential activation of responding T lymphocytes⁴.

As a part of both specific and innate immune response there will be production and secretion of cytokines and chemokines. These low molecular weight proteins may be protective, for example by activating cells participating in defence during in the infectious process, but they may also either “overactivate” or “paralyse” many cell types, leading to deficient recognition of the invading bacterium or, even worse, the destruction of host tissue. Below, only a limited number of cytokines of interest in *S. aureus* infection will be discussed. IFN- γ is a cytokine secreted mainly by activated T cells and NK cells. It has a great array of physiologic functions in the body, including upregulation of MHC expression, activation of phagocytic cells and regulation of T and B cell activity. One reason to study this cytokine is the observation that both IFN- γ mRNA and protein expression are clearly increased during the course of *S. aureus* infection³⁷. To study the role of IFN- γ one should preferably do several independent manipulations of the host. One such manipulation is to inactivate the receptor for IFN- γ by which this cytokine mediates its effects. Such an inactivation is readily performed by so-called gene knock-out technology. Mice lacking functional IFN- γ receptor and inoculated with *S. aureus* display more frequent and severe arthritis compared with wild-type littermates. Also, sepsis-triggered mortality is increased in the early (but not late) stage of the infection³⁹. In contrast, supplementation of normal mice with extrinsic IFN- γ did significantly decrease staphylococcal sepsis triggered mortality but also enhanced the development of arthritis³⁸. Interleukin 4 is another cytokine produced by T lymphocytes. Mice not able to produce this compound are protected against septic arthritis and sepsis-triggered mortality. Our data suggest that this protection seems to be due to the role of IL-4 as an inhibitory factor regarding phagocytosis, decreasing the clearance of bacteria during ongoing infection¹⁴. Yet another cytokine produced predominantly by monocytes/macrophages is the tumor necrosis factor. This cytokine is known to be one of the major causative factors involved in degradation of cartilage and subchondral bone in aseptic arthritis, e.g. rheumatoid arthritis. In the case of septic arthritis this cytokine increases joint destruction but ameliorates the severity of sepsis¹³. These few examples show that the same cytokine may display different properties depending on its local concentration, synergy with other cytokines, the type of responding cells, and the stage of the disease process.

Prophylactic Measures

The prevalence of antibiotic multiresistance among clinical isolates of *S. aureus* spurs the development of vaccination strategies. An ideal vaccine candidate should induce responses which prevent bacterial adherence, promote opsonophagocytic killing by leukocytes and neutralize secreted toxic proteins as suggested recently by LEE¹⁶. Such a vaccine has thus far not been developed. Infection with *S. aureus* itself does not *per se* provide any protection against subsequent infections. Indeed, serological studies have, in general, failed to reveal a correlation between bacterial antibody titers and protective capacity. In recent times 3 distinct approaches to achieve immunization have been assayed. The first of these involves vaccination with staphylococcal polysaccharides alone or conjugated to a protein carrier. Results from experimental trials show that this approach clearly decreases the severity of infection in models of *S. aureus* peritonitis, endocarditis, bacteraemia, and renal abscess formation¹⁶. One potential obstacle in these immunizations is the multitude of different capsular polysaccharide serotypes ($n = 11$) that can be used by various staphylococcal strains. An encouraging note however is that two polysaccharide serotypes (numbers 5 and 8) comprise about 75% of all disease-inducing staphylococcal strains.

A second possibility recently explored is to trigger protective immunity against staphylococcal superantigens. Inducing immunity using native superantigens is not advisable, since these molecules will trigger severe inflammation, potentially leading to septic shock. An alternative approach might be the use of enterotoxins devoid of their superantigenic properties but still expressing major antigenic determinants. Vaccination of mice with staphylococcal enterotoxin A mutated in a hydrophobic loop dominating the interface with MHC class II locus induced protective immunity against sepsis caused by SEA producing staphylococci²⁴. However, even here the high number of different toxins which may be produced by various virulent staphylococcal strains makes the task of developing a universal staphylococcal vaccine difficult.

S. aureus binds to numerous components of the extracellular matrix, including collagens, laminin, bone sialoprotein, fibronectin, fibrinogen and vitronectin. Antibodies directed to staphylococcal adhesins, thus interfering with bacterial binding to the host tissue, might prevent successful colonization. Indeed, experiments involving immunization of the host with fibronectin binding protein provided partial protection against experimental endocarditis and mastitis in rodents¹⁶. Re-

cently, we²³ have demonstrated that immunization with recombinant collagen-binding adhesin significantly alleviates the outcome of severe, life threatening *S. aureus* sepsis. Here, vaccination-induced antibodies displayed protective properties, as proved by passive transfer experiments. Since collagen adhesin is expressed on the majority of staphylococci involved in invasive infections it is another promising vaccine candidate that should merit evaluation in clinical trials.

A recent study has described the successful attempt to use the staphylococcal surface polysaccharide poly-N-succinyl β -1-6-glucosamine (PNSG) as a vaccine¹⁸. Mice immunized with PNSG were protected against kidney infections and death. An advantage of such a vaccine is that PNSG is expressed by both *S. aureus* and *S. epidermidis*.

Treatment of Already Ongoing Infection

Antibiotics are and will continue to be the standard treatment of systemic staphylococcal infections. However, despite adequate use of antibiotics and consequent eradication of staphylococci, continuous tissue destruction may occur. This can be clearly seen in the case of, for example, septic arthritis and is mediated by the exaggerated activation of the host immune response (see above). In addition, septic shock caused by *S. aureus* will not successfully respond to antibiotic treatment alone. Thus, there is a room for improvements with respect to the treatment of already ongoing *S. aureus* infections. We have recently shown that concomitant corticosteroid and antibiotic treatment will, despite the inhibitory role of steroids on neutrophil function, clearly downregulate the severity of septic arthritis and nephritis^{27, 33}. A recent report⁵ suggests targeting RNA III, a molecule regulating production of staphylococcal toxins. Mice vaccinated with RNA III activating protein prior to infection or treated with RNA III inhibiting peptide at the time of bacterial inoculation were protected from *S. aureus* mediated skin infections.

Some other possible, but thus far unproven, ways to improve the outcome of *S. aureus* infection are the use of either anti-inflammatory cytokines (e.g. IL-10, IL-4, and/or TGF- β) or antagonists to proinflammatory cytokines (e.g. IL-1 receptor antagonist). Yet another way would be to introduce antagonists to NF- κ B, an intracellular messenger mediating the activation of many proinflammatory cytokines.

Final Comments

During the last decade work involving the use of experimental models of staphylococcal infections clarified several bacterial virulence factors as well as many hematopoietic cell types and their products that are involved in the pathogenesis of infection. Much still remains obscure, for example how bacterial virulence factors act *in vivo* and the many details of the host-bacterium interplay. More information is required about the risk factors for acquiring staphylococcal infection in order to perform a proper selection of subjects for vaccination procedures. In addition, new modalities should be developed to treat already ongoing infections with combinations of anti-inflammatory agents, passive immunization and antibiotics to minimize the risks of sequels.

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