Adaptive Immunity Against *Staphylococcus aureus*

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Abstract A complex interplay between host and bacterial factors allows *Staphylococcus aureus* to occupy its niche as a human commensal and a major human pathogen. The role of neutrophils as a critical component of the innate immune response against *S. aureus*, particularly for control of systemic infection, has been established in both animal models and in humans with acquired and congenital neutrophil dysfunction. The role of the adaptive immune system is less clear. Although deficiencies in adaptive immunity do not result in the marked susceptibility to *S. aureus* infection that neutrophil dysfunction imparts, emerging evidence suggests both T cell- and B cell-mediated adaptive immunity can influence host susceptibility and control of *S. aureus*. The contribution of adaptive immunity depends on the context and site of infection and can be either beneficial or detrimental to the host. Furthermore, *S. aureus* has evolved mechanisms to manipulate adaptive immune responses to its advantage. In this chapter, we will review the evidence for the role of adaptive immunity during *S. aureus* infections. Further elucidation of this role will be important to understand how it influences susceptibility to infection and to appropriately design vaccines that elicit adaptive immune responses to protect against subsequent infections.

Contents

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1 Introduction

Staphylococcus aureus is a major human pathogen. Data from the USA and Europe indicate it is the predominant cause of both cutaneous and invasive infections and is the leading cause of infectious morbidity and mortality in the industrialized world (Tong et al. 2015). Strain-specific virulence strategies and acquisition of resistance against a variety of antibiotics reflect the adaptive capabilities that have shaped its ability to cause continually shifting patterns of disease (Chambers and Deleo 2009; Tong et al. 2015). Despite its clear pathogenic potential, *S. aureus* has the ability to coexist with its human host as a commensal, with 20–30 % of the population colonized at mucocutaneous surfaces and significantly higher proportions exposed at least intermittently (Verhoeven et al. 2014). The success of *S. aureus* as a human commensal and pathogen suggests the evolution of a complex and intricate interplay between host and bacterial factors.

S. aureus has a plethora of virulence factors that evade and modulate components of the human innate and adaptive immune system (Nizet 2007; Lowy 1998; Rooijakkers et al. 2005). Much attention has been rightly focused on interactions with the innate immune system, in particular neutrophils, which play a central role in host defense against *S. aureus*. However, the readily detectable antibody and T cell responses in humans and the extensive mechanisms for staphylococcal evasion of antibody and T cell-mediated host defense suggest an important contribution of adaptive immunity that may influence host susceptibility and will need to be invoked by a successful vaccine. In this chapter, we will highlight the major findings related to adaptive immune responses induced by *S. aureus* and the evasion mechanisms it uses to escape this aspect of host defense.

2 Immunological Overview

The immune response against *S. aureus* involves activation of both the innate and the adaptive immune systems. As the first line of defense against infections, the innate immune response is rapidly activated by pathways that detect pathogen-associated molecular patterns. A key result of this is activation of phagocytic cells such as macrophages and neutrophils. Neutrophils are recognized as a key component of the acute response and centrally important against *S. aureus*,

as declared by the susceptibility of humans and mice with inherited and acquired neutrophil defects to deep-seated infections. The adaptive immune response kicks in later during the course of infection, dependent on the presentation of bacterial antigens by antigen-presenting cells (APCs) and influenced by the cytokine milieu generated by the innate response. Through T cell activation and B cell production of antibodies, the adaptive immune response targets specific bacterial antigens and can be recalled during subsequent infections to provide 'memory' against that particular pathogen. Antibodies and T cells can have direct activity against bacteria, but also amplify the activity of innate immune cells, e.g., by increasing phagocyte killing and recruitment. The prevalence of recurrent infections with *S. aureus* suggests the adaptive memory response is not completely effective, although it could be argued that the relative paucity of systemic infections despite the high rate of colonization may be evidence for its protective role. Understanding the contribution of the adaptive immune response in determining *S. aureus* susceptibility may help identify risk factors and therapeutic strategies, and will be essential to harness for successful vaccine development.

3 Role of B Cells and Antibodies

The major function of B cells is to secrete immunoglobulins (antibodies) that neutralize the function of target proteins (e.g., toxins and other virulence factors) or opsonize pathogens to optimize phagocytosis and clearance. The importance of antibody-mediated protection against infectious agents is clearly demonstrated by patients with X-linked agammaglobulinemia (XLA), in whom lack of appropriate B cell maturation leads to susceptibility to infections with a variety of viruses and encapsulated bacteria that is largely reversed with the periodic administration of pooled donor immunoglobulins (Bruton 1952; Conley and Howard 1993). The apparent lack of increased susceptibility in this patient population to invasive *S. aureus* infection argues that antibodies are unimportant in protection against *S. aureus* infection. Although these patients have a recognized susceptibility to cellulitis, this has also not been clearly attributed to *S. aureus*. The lack of increased susceptibility to *S. aureus* infection in B cell- or antibody-deficient mice (Gjertsson et al. 2000; Schmaler et al. 2011; Gaidamakova et al. 2012) parallels the observations in patients with XLA. However, recent work has revealed that primary *S. aureus* cutaneous infection can induce antibody-mediated protection against a subsequent infection in certain mouse strains (Montgomery et al. 2014), and numerous preclinical studies have shown at least partial protection from subsequent infection after induction of antibodies by vaccination (see below). Furthermore, the ubiquitous presence of antibodies after *S. aureus* exposure in humans and animal models, and the virulence strategies of *S. aureus* that have evolved to evade antibodies, suggests antibodies may have a role in modulating susceptibility to infection. Evidence for this potential role will be examined in further detail here.

3.1 Preexisting Antibodies as Immunologic Correlates for Protection

The immune correlates of protection from and susceptibility to staphylococcal infections are still not well understood. A few reports have suggested that preexisting antibodies toward certain staphylococcal virulence factors can correlate with clinical outcome in humans. Adhikari et al. (2012a) measured serum antibodies to an array of staphylococcal exotoxins and observed that low antibody titers correlated with a higher risk for the development of sepsis. Another study found that elevated serum titers against *S. aureus* α-hemolysin (Hla) correlated with the protection from subsequent infection, and invasive infections elicited a more durable antibody response when compared to cutaneous infections (Fritz et al. 2013). This study also reported high titer anti-staphylococcal antibodies in colonized individuals without a history of overt infection (carriers), which may explain the enhanced recovery from infection observed in carriers despite their increased risk of developing infection compared to non-carriers (Wertheim et al. 2005; von Eiff et al. 2001a).

3.2 Role of Antibodies in Vaccine-Mediated Protection

S. aureus has been generally regarded as an extracellular pathogen. Consequently, complement and antibodies with neutralizing and opsonizing qualities were considered major players not only in mediating neutralization of secreted virulence factors, but also in facilitating uptake and clearance of the pathogen by innate immune cells (van Kessel et al. 2014; Verbrugh et al. 1982; Leijh et al. 1981). Because most vaccines in use today are thought to work through the elicitation of protective antibody responses, it is also not surprising that most of the vaccine candidates against *S. aureus* to date were chosen and evaluated based heavily on their ability to generate opsonizing and neutralizing antibodies (Pozzi et al. 2012; Fattom et al. 1990; Ohlsen and Lorenz 2010).

The conjugate vaccine StaphVax (Nabi Biopharmaceuticals) was the first *S. aureus* vaccine candidate to enter a phase III clinical trial. It targeted clinically prevalent capsular polysaccharide (CP) serotypes 5 (CP5) and 8 (CP8), emulating the successful strategy of targeting CPs to prevent infections with *Streptococcus pneumonia* and *Haemophilus influenzae*. Preclinical studies demonstrated that CP-specific antibodies protected mice from lethal *S. aureus* challenge and bacterial dissemination (Fattom et al. 1990, 1996), and an initial phase III clinical trial in hemodialysis patients suggested modest reductions in bacteremia early after vaccination (Shinefield et al. 2002). However, a booster dose in a subsequent phase III study failed to prevent bacteremia despite augmenting antibody titers (Fattom et al. 2004; Schaffer and Lee 2008). The reasons for this failure remain incompletely understood, but the outcome highlighted that *S. aureus* virulence is not solely dependent on CP production, a fact exemplified by the lack of capsule production in some highly virulent strains such as USA300.

A more recent vaccine candidate for which preclinical promise failed to translate into clinical trial success targeted the *S. aureus* iron-binding surface determinant B (IsdB). This protein was identified as a vaccine candidate by screening patients with high antibody titers against *S. aureus* surface antigens displayed by an *E. coli* expression library (Etz et al. 2002). Immunization with this protein in preclinical and phase I clinical studies showed protection in mouse models of sepsis and antibody induction in mice, macaques, and humans (Kuklin et al. 2006; Stranger-Jones et al. 2006; Kim et al. 2010; Harro et al. 2010, 2012). However, a phase IIB/III clinical trial with the IsdB vaccine (Merck V710) in cardiothoracic surgery patients was stopped prematurely when, despite induction of IsdB antibodies, excessive deaths were noted in the vaccine group among subjects who developed postoperative *S. aureus* infections (Fowler et al. 2013). Subsequent serum cytokine analysis showed that low IL-2 and IL-17 levels post-vaccination correlated with mortality in subjects who later developed *S. aureus* infections, consistent with a potential T cell-based mechanism although immune analysis after infection to further characterize the associated immune response has not been reported (McNeely et al. 2014).

Instead of targeting cell surface antigens to promote opsonophagocytic clearance of organisms, other vaccine approaches have attempted to generate neutralizing antibodies against secreted *S. aureus* virulence factors. Active and passive immunization studies have validated this strategy in experimental models. The clearest rationale for this approach has been against toxic shock syndrome, which is driven by superantigen toxins such as staphylococcal enterotoxin A (SEA), SEB, or toxic shock syndrome toxin 1 (TSST-1). Several studies have reported that immunization of mice and rhesus macaques with recombinant superantigen toxoids devoid of their superantigenic activity induces toxin-specific antibodies and protects from lethal shock induced by the targeted wild-type toxins (Bavari et al. 1996; Lowell et al. 1996; Stiles et al. 1995; Boles et al. 2003). Furthermore, active immunization with recombinant SEA and TSST-1 toxoid vaccines, as well as adoptive transfer of immune sera, protected mice from systemic *S. aureus* infection (Hu et al. 2003; Nilsson et al. 1999).

The option of targeting other secreted toxins that contribute to *S. aureus* infection became apparent when antibodies generated against Hla_{H35L}, a non-pore-forming mutant of Hla, were shown to protect mice from lethal pneumonia (Bubeck Wardenburg and Schneewind 2008) and from skin and soft tissue infections (Kennedy et al. 2010; Mocca et al. 2014). Similarly, antibodies raised against a recombinant fusion protein (AT-62) designed to mimic key topographic features of the Hla heptamer protected mice from bacteremia and lethal pneumonia (Adhikari et al. 2012b). Antibodies raised against attenuated recombinant LukF-PV and LukS-PV, subunits of the bicomponent Panton–Valentine leukocidin (PVL), showed protective efficacy in a mouse bacteremia model and appeared to have cross-neutralizing activity toward other leukocidins in PVL-deficient strains (Karauzum et al. 2013), a potentially important characteristic given the complex redundant and antagonistic interactions between these bicomponent toxins (Yoong and Torres 2015). Considering the multiple virulence strategies employed by

S. aureus, Spaulding et al. (2014) demonstrated that vaccination with a cocktail of 7 secreted virulence factors, consisting of superantigens and cytolysins, induced antibody-mediated protection against lethal pneumonia in a rabbit model. Interestingly, in the same report, vaccination with a cocktail of surface antigens enhanced lethality in a rabbit model of infective endocarditis, an outcome suggested to be due to antibody-mediated bacterial aggregation (Spaulding et al. 2014). This highlighted the potential for deleterious antibody responses that may be elicited depending on the antigenic targets and the model of infection. Based on the role of antibody shown in such active immunization studies, the therapeutic potential of passive immunization has also been demonstrated in mouse models using mouse, human, and/or chimeric monoclonal antibodies targeting secreted or surface-bound virulence factors such as clumping factor A (ClfA) (Domanski et al. 2005), lipoteichoic acid (LTA) (Weisman et al. 2009, 2011), Hla (Ragle and Bubeck Wardenburg 2009; Tkaczyk et al. 2012), and SEB (Larkin et al. 2010; Karauzum et al. 2012; Varshney et al. 2014).

3.3 *Evasion Mechanisms from the Humoral Immune Response*

S. aureus has developed evasion mechanisms that combat the B cell antibody response. In particular, staphylococcal protein A (SpA) and the second immunoglobulin binding protein (Sbi) (Zhang et al. 1998; Smith et al. 2011) are virulence factors that bind immunoglobulins. SpA is a highly expressed, cell wall-anchored surface protein that binds to the complement-binding Fcγ portion of mammalian IgG. Decoration of the staphylococcal surface with IgG molecules bound in this reverse manner interferes with the complement activation and opsonophagocytosis. In addition, SpA in its secreted form acts as a B cell superantigen, binding the $F(ab)_2$ portion of the B cell receptor to induce B cell proliferation and death (Kobayashi and DeLeo 2013). Beyond its direct effects on opsonophagocytosis and B cell survival, SpA activity has been shown to inhibit the development of antibody responses against other staphylococcal antigens in mouse models and in humans (Kim et al. 2011; Falugi et al. 2013; Pauli et al. 2014). In contrast to SpA, baseline expression of Sbi on the cell surface is low but increases in the presence of IgG, suggesting a highly specific mechanism of immune evasion (Zhang et al. 2000). Like SpA, Sbi can act as both a cell wall-anchored or secreted virulence factor, binding the Fcγ portion of IgG on the cell surface and the soluble complement factor C3, respectively (Smith et al. 2011).

In addition to these specific mechanisms, *S. aureus* can also abandon its usual niche as an extracellular pathogen and evade the humoral immune response as a facultative intracellular organism. For example, it can resist killing and grow within neutrophils (Voyich et al. 2005), or persist in epithelial cells in the form of small colony variants (SCVs). Persistence as SCVs enables the bacterium to avoid

antimicrobial treatment, promote disease pathogenesis, and facilitate recurrent infections (Tuchscherr et al. 2011; Proctor et al. 1995; von Eiff et al. 2001b; Gresham et al. 2000). Furthermore, certain antibody responses generated against *S. aureus* can promote its virulence. For example, treatment with anti-PVL antibodies increased bacterial loads in mouse skin abscesses and inhibited in vitro killing of *S. aureus* by human neutrophils (Yoong and Pier 2010). Further highlighting the unpredictable potential for negative effects of antibody responses, the combination of two antibodies against surface polysaccharides (CP and poly-N-acetyl glucosamine) interfered with the beneficial effects of each individually on opsonophagocytic activity and protection in mouse models of bacteremia and skin infection (Skurnik et al. 2010).

In sum, antibody deficiency in mice and humans shows us that antibodies are not necessary for protection against *S. aureus* infections. However, they may very well contribute to the protective response as suggested by the modulation of antibody responses by *S. aureus* virulence factors, the ubiquitous presence of anti-staphylococcal serum antibodies, antibody-mediated protection after active and passive immunization in preclinical models, and human data correlating antibody titers with protection. Published data also support the possibility of ineffective or deleterious antibody responses, emphasizing the need to better understand the characteristics of a protective antibody response in order to elucidate contributions to natural immunity and implications for vaccine design.

4 Role of T Cells

T cells are thymic-derived cells that express unique T cell receptors (TCRs) that recognize antigen-derived peptides in the context of major histocompatibility complex (MHC) molecules on APCs. Similar to B cells and antibodies, a case can be made for a role for T cells during *S. aureus* infection based on the presence of detectable T cell responses in humans (Zielinski et al. 2012; Kolata et al. 2015) and the ability of the bug to modulate T cells as exemplified by its expression of a multitude of T cell superantigens (Spaulding et al. 2013). However, it has been reported that T cells are not essential for protection against *S. aureus* in mice (Schmaler et al. 2011). Furthermore, *S. aureus* shows up only occasionally as a cause of infection in evaluations of humans with T cell deficiencies (Stephan et al. 1993), although the severe susceptibility of these patients to other organisms confounds our ability to fully assess the contribution of T cells to staphylococcal immunity in this context. Various subsets of T cells have differing functions, and a more nuanced role for these subsets has become evident in mouse studies and with the recognized susceptibility to staphylococcal infections of patients with HIV and other partial T cell disorders (Hidron et al. 2010; Cook and Tangye 2009). These will be discussed in further detail below.

The majority of T cells are comprised of CD4+ and CD8+ T cells that have long been recognized to be the major cellular arm of adaptive immunity. The major

function of CD8+ T cells is to target intracellular pathogens by cytolytic killing of the infected host cell. Consistent with *S. aureus* being a primarily extracellular pathogen, a clear role for CD8+ T cells has not been reported, although CD8+ T cell activation can be detected during *S. aureus* infection and staphylococcal superantigen exposure. Naïve CD4+ T cells are polarized toward different effector functions depending on the cytokine milieu in which activation of their TCR occurs. These helper T cell (Th) subsets are functionally characterized by their cytokine expression profiles, which will be detailed below. A percentage of these polarized cells will persist in the host as memory cells awaiting re-activation by subsequent antigen exposure. The role of these different subsets of effector CD4+ T cells in the context of *S. aureus* infection will be reviewed below. In addition to CD4+ and CD8+ T cells, more recently described subsets of T cells, such as γδ T cells, innate lymphoid cells (ILC), and NK T cells, contribute mainly to the innate immune response at mucosal sites rather than antigen-specific memory, although recent reports have suggested the potential for γδ T cells to contribute to a memory response under certain circumstances (Murphy et al. 2014).

4.1 Th1 Cells

TCR-mediated activation of naïve CD4+ T cells in the presence of IL-12 signaling via STAT4 leads to the generation of Th1 effector cells. Although capable of producing multiple inflammatory cytokines, including IL-2, TNFα, and GM-CSF, Th1 cells are defined by the secretion of their signature cytokine interferon (IFN)-γ and expression of the transcriptional regulator T-bet (O'Shea and Paul 2010; Schmitt and Ueno 2015; Raphael et al. 2014). Th1 cells are not the only source of IFNγ, with various innate immune cells, including NK cells and ILC being notable producers. Among its functions, IFNγ activates phagocytic cells such as macrophages and neutrophils to promote killing of intracellular pathogens. Its role in protection against these organisms is highlighted by the susceptibility of patients with hereditary defects in IFNγ signaling to infections with *Mycobacteria*, *Salmonella*, and certain viruses (Rosenzweig and Holland 2005). Unregulated IFNγ production can contribute to immunopathology and autoimmunity (Feldmann et al. 1998).

In the context of *S. aureus* infections, it appears Th1 cells and IFNγ can have both beneficial and detrimental roles. Guillen et al. reported a protective role of an enhanced Th1 response in a mouse model of septicemia and septic arthritis in mice transgenic for lactoferrin. The enhanced production of IFNγ and TNFα in these mice during infection resulted in higher bacterial clearance and lower mortality compared to their wild-type littermates (Guillen et al. 2002). An overproduction of this cytokine, however, can be associated with immunopathology. An early study evaluating the role of T cells in *S. aureus*-induced arthritis indicated that Th1 cells, stained positive for the IL2R and intracellular IFNγ, infiltrated the synovium of joints of infected mice, and depletion of CD4+ but not CD8+ T cells in the infected

animals ameliorated disease (Abdelnour et al. 1994). However, intravenous inoculation of mice deficient in T-bet, which may have deficiencies beyond a defect in Th1 cell IFNγ production (Lazarevic et al. 2013), had increased severity of septic arthritis that was associated with increased weight loss, mortality, and kidney bacterial burden (Hultgren et al. 2004). Consistent with the potential duality of roles for Th1 cells during *S. aureus* infection, Th1 cells and IFNγ production were reported to promote chemokine-mediated neutrophil recruitment in a wound infection model, but this resulted in a paradoxical increase of bacterial burden, potentially due to the ability of *S. aureus* to persist in neutrophils (McLoughlin et al. 2006, 2008).

Th1 cells appear to be able to contribute to vaccination-induced protection against subsequent *S. aureus* infection. CD4+ T cell IFNγ production was required for the protection against subsequent systemic infection after vaccination with a recombinant protein derived from Als3p, a *Candida* protein that cross-protected against *S. aureus* (Lin et al. 2009). Similarly, vaccination with extracellular vesicles released from *S. aureus* induced a Th1 response, and protection in a pneumonia model was dependent on CD4+ T cells and IFNγ (Choi et al. 2015). However, protection after vaccination against cutaneous infection with a lethally irradiated whole-cell vaccine was not associated with an IFNγ response (Gaidamakova et al. 2012), and increased mortality in similarly vaccinated mice after intravenous challenge was dependent on CD4+ T cell IFNγ production (Karauzum and Datta, unpublished data). Another study also hinted at potential detrimental effects of vaccine-induced Th1 responses by showing that mice vaccinated with heat-killed *S. aureus* had significant disease burden after intravenous infection despite detectable CD4+ T cell IFNγ production (Schmaler et al. 2011); however, lack of direct comparison to an unvaccinated control group prevents conclusive interpretation of these results.

In sum, it appears Th1 cells can have protective, detrimental, or non-contributory roles against *S. aureus* infection, likely dependent on factors such as route of infection, organism burden, antigenic targets, level of induction, and balance with other immune mechanisms. Clarification of the conditions under which Th1 cells exert these apparently contradictory effects will better guide approaches to interventions aimed at therapy and prevention.

4.2 Th2 Cells

Activation of naïve CD4 T cells in the presence of IL-4 via STAT6 signaling leads to the priming of Th2 cells. This subset of CD4 T cells is characterized by its signature transcription factor GATA-3, which promotes induction of Th2 cytokines that include IL-4, IL-5, and IL-13. Th2 cells play an important role in host defense against extracellular parasites, driving various aspects of cellular and humoral immunity to promote parasite clearance and tissue repair (Allen and Sutherland 2014). Their dysregulation contributes to allergic and atopic diseases (Raphael et al. 2014; Geginat et al. 2013). Of particular relevance to staphylococcal disease is

atopic dermatitis (AD), a prevalent inflammatory skin disorder that is characterized by the overexpression of Th2 cytokines (Hamid et al. 1994), which contribute to barrier permeability issues and other features of AD. Skin colonization and infection with *S. aureus* is almost a universal feature of AD (Boguniewicz and Leung 2011). The propensity of Th2 cytokines to inhibit antimicrobial gene programs, including induction and mobilization of antimicrobial peptides such as human beta-defensin (HBD)-3, are thought to contribute to this susceptibility (Kisich et al. 2008; Nomura et al. 2003; Howell et al. 2006). Th2 cytokines may not only drive aspects of AD and staphylococcal susceptibility, but *S. aureus* colonization may further promote this Th2-driven milieu. Staphylococcal cell wall components, such as peptidoglycan (Matsui and Nishikawa 2012) and lipoteichoic acid (Matsui and Nishikawa 2002), were shown to induce Th2 cells and may contribute along with other secreted toxins toward the inflammatory environment in the skin of AD patients (Schlievert et al. 2010; Nakamura et al. 2013; Brauweiler et al. 2014).

The role of Th2 responses during *S. aureus* infection outside the setting of AD is less clear. *S. aureus* footpad infection was less severe in the Th2-biased DBA and BALB/c mouse strains than in Th1-biased C57BL/6 mice (Nippe et al. 2011). A protective role for Th2 cells was also suggested in an ocular keratitis model where unexpectedly high Th2 responses in C57BL/6 mice correlated with the protection compared to less robust responses seen in more susceptible BALB/c mice (Hume et al. 2005). However, these correlative observations do not definitively address whether the Th2 response is driving resistance to infection or whether other immunological parameters are responsible. A recent study in a model of persistent biofilm infection did show STAT6-dependent clearance in BALB/c mice that suggested a contribution of Th2 responses to protection (Prabhakara et al. 2011). In the same study, neutrophilic inflammatory responses worsened infection and this effect could be reversed by neutralization of IL-12p40 or IL-6, treatments that would be predicted to dampen Th1 and Th17 responses, respectively, and skew toward Th2 responses (Prabhakara et al. 2011).

The complexity of Th2 responses and their downstream effects may potentially trigger both beneficial and detrimental responses. It seems clear that Th2 responses contribute to a vicious cycle of inflammation and *S. aureus* susceptibility at the skin in the context of AD. However, Th2 effects may play a role in achieving the appropriate balance between inflammatory and anti-inflammatory responses in other situations, particularly during chronic infection.

4.3 Th17 Cells

Th17 cells are a relatively recently recognized subset of effector CD4+ T cells. They are defined by their expression of Rorγt and secretion of inflammatory cytokines, including IL-17A, IL-17F, and IL-22 (Liang et al. 2006; Chung et al. 2006; Ivanov et al. 2006). These cytokines predominantly act on epithelial cells to enhance barrier function, antimicrobial properties, and neutrophil recruitment

(Ouyang et al. 2008). Initially discovered in the context of autoimmunity, they have been shown to play a protective role in mouse models of extracellular bacterial and fungal infections, especially at mucosal sites (Ouyang et al. 2008).

A protective role for Th17 cells against *S. aureus* was first suggested by a report that mice deficient in both IL-17A and IL-17F spontaneously developed mucocutaneous *S. aureus* infections (Ishigame et al. 2009). Subsequent work clarified that induction of IL-17A from γδ T cells in the skin played a critical role in controlling *S. aureus* burden and abscess size after subcutaneous inoculation (Cho et al. 2010). In this context, γδ T cells functioned in their traditional role as a cytokine-activated component of innate immunity, implicating an important role for innate immune cell-derived IL-17A. Hla-dependent Th17 induction (Frank et al. 2012) and influenza-mediated antagonism of Th17-dependent protection (Kudva et al. 2011) indicated a role for Th17 cells in a model of *S. aureus* pneumonia. Interestingly, CD4+ T cell depletion in the context of this Th17-inducing pneumonia model improved outcomes in another study, hinting at a delicate balance within the CD4+ T cell compartment between protective immunity and immunopathology (Parker et al. 2015). Deficiency in IL-17A also enhanced susceptibility to *S. aureus* joint infection, although the relevant cellular source was not identified (Henningsson et al. 2010). Deficiency in IL-17A did not increase susceptibility to systemic challenge with *S. aureus* in multiple studies (Ishigame et al. 2009; Lin et al. 2009; Narita et al. 2010; Henningsson et al. 2010), consistent with a primary role for this cytokine at skin and mucosal sites. However, a protective function for IL-17A from vaccination-induced Th17 cells has been shown against skin (Gaidamakova et al. 2012) and systemic *S. aureus* infections (Lin et al. 2009; Narita et al. 2010; Joshi et al. 2012). Consistent with this, antibody-dependent protection against multiple models of infection by a four-component vaccine was further enhanced by Th1 and Th17 cell induction with inclusion of a TLR7 agonist adjuvant (Bagnoli et al. 2015). The Merck IsdB vaccine also showed contribution of IL-17A, but not IL-22 or IFNγ, to protection in a mouse model of sepsis (Joshi et al. 2012), and, as mentioned previously, low IL-2 and IL-17 levels post-vaccination correlated with mortality in *S. aureus*-infected human subjects (McNeely et al. 2014). Of note, the Th17-associated cytokine IL-22 seems to have either no or minimal effects on the course of acute cutaneous infection in mice (Myles et al. 2013; Chan et al. 2015), but can independently contribute to protection against pneumonia (Kudva et al. 2011) and vaccine-induced protection against skin and systemic infection (Yeaman et al. 2014).

Autoantibody- and genetically mediated dysfunction of the IL-17 pathway predisposes to mucocutaneous *Candida* infections (Burbelo et al. 2010; Kisand et al. 2010; Puel et al. 2010, 2011). Only a striking minority of these patients reported *S. aureus* infections, making it unclear whether IL-17 is a critical element for human anti-staphylococcal responses. However, patients with HyperIgE (or Job's) Syndrome, who are susceptible to staphylococcal skin and lung infections, lack normal Th17 generation due to STAT3 dysfunction (Minegishi et al. 2007; Holland et al. 2007; Milner et al. 2008). The role for Th17 cytokines in promoting keratinocyte and epithelial antimicrobial function (Minegishi et al. 2009) is also consistent with an IL-17-dependent basis for their susceptibility specifically to skin and lung infections,

although other functions of STAT3, including its direct role in antimicrobial peptide production (Choi et al. 2013), very likely contribute. Consistent with a potential contribution of Th17 cells to human immunity is the Th17 depletion seen in HIV-infected patients early in the course of disease that correlates with their increased likelihood of *S. aureus* skin and soft tissue infections (Hidron et al. 2010; Prendergast et al. 2010). Patients with AD also have decreased IL-17 pathway cytokines and decreased antimicrobial peptides in lesional skin, potentially contributing to their staphylococcal susceptibility (Guttman-Yassky et al. 2008). The relative induction of these pathways in psoriasis has been postulated to contribute to the relative resistance of these patients to *S. aureus* (Guttman-Yassky et al. 2008).

In sum, IL-17 from either innate or adaptive sources plays an important role against *S. aureus* in mouse models of infection at skin and mucosal sites. Induction of Th17 cells by vaccination can enhance protection at these sites and also against bacteremia. Th17 cells appear to be potential key players in immunity against *S. aureus*; however, their exact contribution to the control of human staphylococcal infection remains to be fully elucidated and their potential for autoimmune inflammation will need to be kept in check if they are to be targeted by clinical vaccines.

4.4 Regulatory T Cells

Regulatory T cells (T_{reg}) display contact-dependent and cytokine-mediated immunosuppressive functions that counteract inflammatory responses and maintain immune homeostasis. *S. aureus* may exploit these immunosuppressive functions by inducing T_{reg} responses that contribute, along with other immunosuppressive mechanisms, to diminished effector T cell responses during models of persistent infection (Ziegler et al. 2011; Tebartz et al. 2015). Increased T_{reg} numbers may also contribute to the immune dysregulation and *S. aureus* susceptibility seen in the skin of patients with AD (Ou et al. 2004). However, depletion of T_{reg} exacerbated a model of chronic biofilm infection, suggesting that an appropriate balance between inflammatory and anti-inflammatory responses is needed for optimal bacterial control (Prabhakara et al. 2011). Further studies will be needed to increase our nascent understanding of the role of T_{reg} in modulating the response to *S. aureus* infection and how this may influence susceptibility.

5 Conclusion

Immune control of acute *S. aureus* infection is critically dependent on the innate immune system. However, adaptive immunity in the form of B cell and T cell responses may influence this control and is potentially of particular importance in determining the outcomes of chronic persistent infections. The search for a protective vaccine will depend on our ability to induce an effective adaptive immune

response. Recent studies suggest that induction of an antibody response alone may not be sufficient, and an appropriate vaccine-induced T cell response will be needed to confer protective immunity. The potential for eliciting deleterious adaptive immune responses has become apparent in both animal models and clinical vaccination trials. This highlights the need for further elucidation of the components of an effective immune response, a task complicated by the multiple virulence strategies and sites of infection employed by this bug that will each likely require targeting by unique strategies for effective prevention and therapy.

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