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Antibody-mediated protection against *Staphylococcus aureus* dermonecrosis and sepsis by a whole cell vaccine

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ABSTRACT

Staphylococcus aureus is a very important human pathogen that causes significant morbidity and mortality worldwide. Several vaccine clinical trials based on generating antibody against staphylococcal surface polysaccharides or proteins have been unsuccessful. A killed whole cell lysate preparation (SaWCA) was made by lysing a USA 300 strain with lysostaphin followed by sonication and harvest of the supernatant fraction. Immunization with SaWCA and cholera toxin (CT) generated robust IL-17A but relatively modest antibody responses, and provided protection in the skin abscess but not in the dermonecrosis or invasive infection model. In contrast, parenteral immunization with SaWCA and alum produced robust antibody and IL-17A responses and protected mice in all three models. Sera generated after immunization with SaWCA had measurable antibodies directed against six tested conserved surface proteins, and promoted opsonophagocytosis activity (OPA) against two *S. aureus* strains. Passive transfer of SaWCA-immune serum protected mice against dermonecrosis and invasive infection but provided no demonstrable effect against skin abscesses, suggesting that antibodies alone may not be sufficient for protection in this model. Thus, immunization with a SA lysate preparation generates potent antibody and T cell responses, and confers protection in systemic and cutaneous staphylococcal infection models.

1. Introduction

The Gram-positive bacterium *Staphylococcus aureus* is a common pathogen of humans that causes a wide range of infections, which can involve the skin (such as boils or cellulitis), as well as many other organs (including the lungs, heart, bone and joints, among others) or cause shock syndromes. The rise of methicillin-resistance in *S. aureus* strains (MRSA), as well as the emergence of vancomycin-intermediate/resistant strains (VISA/VRSA) [1], increases the complexity and cost of treatment of these infections. It is estimated that, annually, 10 billion US dollars are spent treating hospital-associated infections (HAI), such as surgical site infections, central line associated bloodstream infections, ventilator associated pneumonia and catheter associated urinary tract infections. In the US, over 10% of HAI are likely due to infection by *S. aureus* [2,3]. While *S. aureus* can certainly cause disease in healthy individuals, those undergoing surgery, who are on dialysis, in intensive care units or with underlying immunocompromising conditions are at particularly high risk [4].

S. aureus vaccine development efforts have not been successful so far. While generating opsonophagocytic (OPA) antibody against the capsular polysaccharide of a microorganism has long been the vaccine strategy against pathogenic bacteria such as *Haemophilus influenzae* type b, *Streptococcus pneumoniae* and *Neisseria meningitidis*, this approach has not been useful in the case of *S. aureus:* a candidate vaccine comprising two staphylococcal capsular polysaccharides (type 5 and 8) conjugated to recombinant exoprotein A showed partial protection in an early clinical trial but failed in a phase III clinical trial [5,6]. Similarly, a passive immunization trial using pooled human immunoglobulin preparations from donors with high antibody titers against staphylococcal CP 5 and 8 gave disappointing results [7]. Other vaccine approaches that have been tested but failed include active immunization with or passive transfer of either polyclonal or monoclonal antibodies to individual proteins of *S. aureus* [8–11]. Thus, there is a major and urgent unmet need for vaccine development against *S. aureus.*

There is strong evidence to suggest that mechanisms other than antibodies alone may mediate resistance to staphylococcal infections. Indeed, whereas individuals with congenital agammaglobulinemia do not seem to be at particularly high risk for staphylococcal infections, children with complete DiGeorge syndrome (who lack a thymus and T cell responses) tend to have recurrent infections with *S. aureus*. Similarly, adult patients with HIV

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infection and CD4+ T cell deficiency are very susceptible to staphylococcal infection [12]. It was long known that patients with autosomal dominant hyper-IgE syndrome (Job's syndrome) have a propensity to develop recurrent staphylococcal skin or pulmonary infections, but the underlying immunodeficiency has only been identified recently. It is now established that Job's syndrome patients have mutations in STAT3, a signalling protein that is critical for the development of memory Th17 cells [13].

These data, while consistent with a role of T cells in immunity to staphylococcus, do not prove that CD4+ T cells, or Th17 cells in particular, play an independent role in preventing infection however, since CD4+ T cell deficiency or STAT3 mutations can also have important effects on antibody production or other aspects of the immune response. More recently, evidence supporting an independent role of IL-17A-mediated protective mechanisms against *S. aureus* infection has been obtained in mouse models. IL-17A-deficient mice are more prone to staphylococcal infection [14,15] and clearance of nasal staphylococcal carriage in mice is IL-17A dependent [16]. In support of this hypothesis, several protein-based vaccines (such as those containing IsdB or ClfA [17,18]) confer Th17-dependent protection against *S. aureus* infection in preclinical models. Furthermore, adoptive transfer of immune Th17 cells can protect against infection [19,20], suggesting that these cells are sufficient to protect mice. More recently, studies in mice have also suggested a potential role of Th1 cells in protection [21,22]. Given the lack of success so far of immunization strategies relying exclusively on antibody generation and the growing evidence that T cells can provide protection against other extracellular respiratory pathogens [23–27], we hypothesize that an effective *S. aureus* vaccine strategy may require the generation of both antibody and T cell (specifically Th17 and Th1) responses to the organism.

Killed staphylococcal whole cells have been tested as vaccine candidates in mice for protection against *S. aureus* infection but were not very successful [28,29]. A formalin-killed whole cell vaccine failed to protect animals in a rabbit endocarditis model. Whole cell vaccines made with irradiated wild-type and Spa mutant strains did not protect against subsequent intravenous infection with *S. aureus*. Recently, different whole cell preparations using mutant strains showed more promise in animal models. Immunization with an UV-irradiated preparation of a serine/threonine phosphatase mutant strain protected against systemic *S. aureus* infection [30]. Another study showed that previous immunization with a live strain containing a sortase A deletion protected against systemic infection [31]. In addition to intact whole cells, lysed cells have been shown to be protective in animal models [32–34]. It is important to note that these attempts were focused on antibody production against whole bacteria, and therefore T-cell (including Th17) responses, and their potential role in protection, were not evaluated.

Here, we present evidence that a lysed *S aureus* whole cell vaccine preparation induces both antibody and Th17/Th1 responses when given with an adjuvant and can provide protection against *S. aureus* in three disease models. We show that while anti-SaWCA antibody is sufficient for protection against skin dermonecrosis and invasive infection, passive transfer of antibodies did not protect against focal skin abscesses, arguing for an important and complimentary role of T-cells in protection following immunization with this vaccine.

2. Material and methods

2.1. Material

Aluminum hydroxide (alum) was from Brenntag North America (2% Alhydrogel). Saline was from B. Braun Medical Inc. (Bethlehem, PA). Cholera toxin (CT) was purchased from List Biological Laboratories. DMEM and FCS were from ThermoFisher scientific. Lysostaphin and other chemical reagents were purchased from Sigma.

2.2. Bacterial strains

Staphylococcus aureus strains USA 300 TCH959 [35] and ATCC 29213 were purchased from ATCC. Bacteria were grown on blood agar plate overnight and then inoculated into Tryptic soy broth (TSB) to grow overnight at 37 °C with shaking. Cells were re-inoculated into fresh TSB medium and incubated at 37 °C with shaking for 3 h. Cells were washed twice with saline and adjusted to concentrations as noted in animal models (described below) in saline before use.

2.3. SaWCA preparation

The USA300 TCH959 strain was grown overnight on blood agar and resuspended into PBS. Cells were washed twice with PBS and resuspended to OD_{600} = 20 in PBS. Lysostaphin was added to the suspension and cells were shaken at 37 °C for 30 min. Cells were then lysed with sonication and then exposed to chloroform (1/40 vol/vol) and kept stirring at 4 °C for 2 h. Initially, we also included a preparation of cells that were only exposed to chloroform, without the initial lysing step. Either preparation was then plated on blood agar to confirm that no live bacteria was detectable before being frozen and then lyophilized. Lyophilized vials were reconstituted in the same volume of water and centrifuged for 5 min at 16,000*g* before use. For the lysed preparation, the supernatant fraction was isolated and defined as SaWCA. Protein concentrations were determined using the BCA kit with bovine serum albumin as standard (Thermo Scientific).

2.4. Immunization and challenge of mice

All animal protocols were approved by the Institutional Animal Care and Use Committee at Boston Children's Hospital (protocol number 16-03-3133). Female C57BL/6 J mice (Jackson Laboratories, Bar Harbor, Maine) were used for all experiments. The age at time of first immunization was between 4 and 6 weeks. Two types of adjuvants were used in mice immunizations. Cholera toxin (CT) was used as an adjuvant in intranasal immunizations to promote T cell responses to SaWCA whereas aluminium hydroxide (alum) was used for subcutaneous (s.c.) immunizations to promote both antibody and T cell responses [36–39]. For intranasal immunization, SaWCA was mixed with CT and the dose of immunization was 64 μg (protein content) of SaWCA and 1 μg of CT per mouse per immunization. Mice were immunized twice one week apart and bled three weeks after last immunization. Peripheral blood samples were stimulated with SaWCA and assayed for IL-17A production; plasma samples were analyzed for antibody production by ELISA.

For s.c. immunization, vaccines were prepared as follows. One day prior to immunization, SaWCA was diluted to the appropriate concentration, and mixed with alum at the indicated concentration; the mixture was then rotated end-over-end overnight at 4 °C to allow for adsorption. The immunization dose in s.c. experiment was 100 μg of SaWCA (protein content) per mouse. Gently restrained, nonanesthetized mice received 3 s.c. injections of 200 μl of adjuvant with or without antigen in the back at 2-week intervals. Blood was drawn 2 weeks after the last immunization; plasma and whole blood were assayed for antibody and for IL-17A and INF-γ production as noted above.

Mice were challenged in three different models as described previously with some modifications [40–42]:

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