Anti-Alpha-Hemolysin Monoclonal Antibodies Mediate Protection against Staphylococcus aureus Pneumonia

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Staphylococcus aureus pneumonia is one of the most common invasive diseases caused by this human pathogen. S. aureus alpha-hemolysin, a pore-forming cytotoxin, is an essential virulence factor in the pathogenesis of pneumonia. Vaccine-based targeting of this toxin provides protection against lethal staphylococcal pneumonia in a murine model system, suggesting that a monoclonal antibody-based therapy may likewise prove to be efficacious for prevention and treatment of this disease. We report the generation of two distinct anti-alpha-hemolysin monoclonal antibodies that antagonize toxin activity, preventing human lung cell injury in vitro and protecting experimental animals against lethal S. aureus pneumonia. Each of these two monoclonal antibodies recognized an epitope within the first 50 amino acid residues of the mature toxin and blocked the formation of a stable alpha-hemolysin oligomer on the target cell surface. Active immunization with the first 50 amino acids of the toxin also conferred protection against S. aureus pneumonia. Together, these data reveal passive and active immunization strategies for prevention or therapy of staphylococcal pneumonia and highlight the potential role that a critical epitope may play in defining human susceptibility to this deadly disease.

Staphylococcus aureus is a gram-positive human pathogen that causes a myriad of diseases ranging from minor skin infections to life-threatening deep tissue infections and toxinoses (19). Pneumonia is among the most prominent S. aureus-mediated diseases, accounting for approximately 15% of documented invasive staphylococcal infections (14), and there are an estimated 50,000 cases per year in the United States alone (17). In addition to being one of the most common causes of ventilator-associated pneumonia, S. aureus is increasingly recognized as an important cause of community-acquired pneumonia, affecting previously healthy adults and children (8, 16). This is particularly notable in association with influenza infection, where concomitant staphylococcal pneumonia is often a lethal complication (7, 8, 12). Up to one-half of staphylococcal pneumonia isolates are classified as methicillin (meticillin)-resistant S. aureus (MRSA), confounding the delivery of appropriate treatment and resulting in reported mortality as high as 56% (1, 17, 24). The combination of an increasing disease burden and declining potency of traditional antimicrobials to combat S. aureus pneumonia heightens the need for novel prophylactic and therapeutic strategies.

We have defined an essential role of alpha-hemolysin (Hla) in S. aureus pneumonia, as strains lacking this pore-forming cytotoxin are avirulent in a murine model of disease (4). Drawing on this knowledge, we have demonstrated that vaccine-based approaches targeting Hla provide protection from lethal pneumonia in experimental animals (5). The ability of Hla to injure the lung and other tissues rests on the ability of the toxin to form a 2-nm heptameric pore in the plasma membrane of susceptible cells (2, 26). This chromosomally encoded toxin is secreted as a water-soluble monomer by the majority of S. aureus strains (22). Membrane binding of the monomer permits a series of well-defined intermolecular interactions between neighboring monomers, resulting in the formation of a barrel-shaped oligomeric pore that penetrates the membrane (9, 13). Residues located at the N terminus of the mature toxin are essential for assembly of the lytic oligomer, as point mutations or truncations within this region disrupt the formation of an active toxin (21, 27, 28).

In addition to its role in the lung, Hla is central to pathogenesis in other tissues, as hla mutants are less virulent in animal models of intraperitoneal (i.p.), intramammary, and corneal infection (3, 6, 23). Supporting this role for Hla in disease, immune sera generated against a single point mutant with a mutation that disrupts pore formation, termed HlaH35L, provide a high degree of protection against pneumonia, i.p. infection, and challenge with purified active toxin (5, 20). We therefore built upon these observations by generating mouse monoclonal antibodies (MAbs) following immunization with inactive HlaH35L to investigate whether an antibody with a single specificity could provide protection against S. aureus pneumonia.

MATERIALS AND METHODS

Bacterial strains and culture. For mouse lung infection, S. aureus strains Newman and LAC/USA300 were grown at 37°C in tryptic soy broth to an optical density at 660 nm of 0.5. Culture aliquots (50 ml) were centrifuged and washed in phosphate-buffered saline (PBS) prior to resuspension. For mortality studies, S. aureus Newman was resuspended in 750 μl (3 × 10⁸ to 4 × 10⁸ CFU per 30 μl), while LAC/USA300 was resuspended in 1,250 μl (2 × 10⁸ CFU per 30 μl). For bacterial load and histopathology experiments, S. aureus Newman was resuspended in 1,250 μl (2 × 10⁸ CFU per 30 μl). For cytotoxicity studies, 5 ml of a culture prepared as described above was resuspended in 10 ml F12K medium (Invitrogen). A 100-μl suspension was used for each assay well.
Plasmid construction. PCR products encoding serial 50-amino-acid fragments of Hla, amplified from S. aureus Newman chromosomal DNA, were cloned into pGEX-6P-1 (GE Healthcare) and transformed into Escherichia coli. Each construct was sequenced for verification. A plasmid encoding glutathione S-transferase (GST)-HlaH35L was generated as previously described (5). To generate a polyhistidine-tagged version of full-length, active Hla, a PCR product encoding the mature polypeptide was amplified from S. aureus Newman chromosomal DNA, cloned into pET24b (Novagen), and then transformed into E. coli BL21/D3.

M Abs. M Abs to Hla were generated by the Frank W. Fitch Monoclonal Antibody Facility at the University of Chicago. Splenocytes derived from mice immunized with full-length Hla were utilized to generate hybridomas. Control M Abs of isotypes immunoglobulin G2a (IgG2a) and IgG2b were purified and supplied by the Frank W. Fitch Monoclonal Antibody Facility.

Animals and procedures. Animal experiments were reviewed, approved, and supervised by the Institutional Animal Care and Use Committee at the University of Chicago. For lung infection, 7-week-old C57BL/6J mice (The Jackson Laboratory) were anesthetized before inoculation of 30 µl of an S. aureus suspension prepared as described above into the left naris. Animals were placed into a cage in a supine position for recovery and were observed for the time courses of bacterial recovery from lungs and red cell hemolysis. For molecular modeling, atomic coordinates were retrieved from the Protein Data Bank (PDB ID 7ahl) based on the data of Song et al. (26). Models were activated with 0.2 M N-ethyl-N-(3-dimethylaminopropyl)carbodiimide and 0.05 M N-hydroxy succinimide. Antibody was bound to the chip by passing 1 µM antibody in HBS-P buffer (20 mM HEPES [pH 7.4], 150 mM NaCl, 0.005% [vol/vol] surfactant P20) over the activated chip. Free amines were then neutralized using 1 M ethanolamine hydrochloride. The control surface was prepared similarly, except that running buffer was injected instead of Hla. We measured the rates of association and dissociation of purified Hla at concentrations ranging from 5 to 100 nM. All measurements were performed in triplicate. After each binding experiment the sensor chip was regenerated using 10 mM NaOH. Derived sensorsgrams were analyzed using BLAevaluation 4.1. Affinity constants were estimated by curve fitting using a 1:1 binding model.

Enzyme-linked immunosorbent assay (ELISA). Serum antibody titers were determined with immunooplates (MaxiSorp; Thermo Fisher Scientific) coated with 1 µg/ml purified HlaGST or GST-HlaGST. Dilutions of either mouse or human sera prepared in PBS were incubated in the appropriate plates, which were developed with horseradish peroxidase-conjugated secondary antibodies and a 3,3’5,5’-tetramethylbenzidine substrate kit (Thermo Scientific) and examined with a spectrophotometer (GENios; Tecan). All human sera were collected and in accordance with a human subject protocol that was reviewed, approved, and supervised by the Institutional Review Board at the University of Chicago.

RESULTS AND DISCUSSION

Anti-Hla M Abs prevent Hla-mediated cell injury. The ability to neutralize Hla-mediated S. aureus lung injury through the development of M Abs offers an exciting new strategy for the prevention and treatment of pneumonia. To this end, we generated m Abs to the nontoxic HlaGST mutant. Immunoreactive M Abs were examined to determine their ability to prevent injury to cultured alveolar epithelial cells (data not shown). Two M Abs, M Abs 7B8 and 1A9, provided a high degree of protection against Hla-mediated injury. Uninfected human A549 alveolar epithelial cells retain a green fluorophore when they are examined with a live (green)/dead (red) staining reagent (Fig. 1A). Infection of cells with S. aureus Newman, a methicillin-sensitive clinical isolate, resulted in cell death after 3 h of coculture in the presence of PBS, as demonstrated by an increased number of red fluorescent cells (Fig. 1B). Treatment of A549 cells with IgG2a, an isotype-matched control for 7B8, was similar to treatment with PBS (Fig. 1C). In contrast, treatment with 7B8 provided nearly complete protection from S. aureus-induced death (Fig. 1D). Nonspecific IgG2b did not confer protection (Fig. 1E), while isotype-matched MAb 1A9 provided robust protection (Fig. 1F). The percentages of cell death are shown in Fig. 1.

To quantify the ability of M Abs 7B8 and 1A9 to antagonize the effects of Hla on A549 cells, we examined concentration-dependent protection using an LDH release assay. Both M Abs conferred a significant degree of protection at concentrations ranging from 0.5 to 5 µg/ml (3 to 33 nM) when they were

phosphatase [monobasic], 150 mM NaCl [pH 7.4], 1 mg/ml bovine serum albumin, 1 mM β-mercaptoethanol) was incubated with 30 µl of the radiolabeled Hla mixture in the presence of 0.1 to 5 µM M Ab 7B8 or 1A9 for 1 h at 20°C. The controls were analysed in the absence of added antibody or in the presence of 5 µM isotype-matched M Abs as indicated below. Following incubation, samples were centrifuged at 13,000 rpm for 5 min and then washed with 500 µl K-PBSA/JME and centrifuged as described above. Samples were then resuspended in 90 µl 1× Laemmli buffer and incubated at 37°C for 10 min before 12-µl portions of the samples were loaded onto 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) gels for electrophoresis. The gels were dried, and then the results were visualized using a phosphorimager.

Surface plasmon resonance (SPR). The affinity and rates of association and dissociation between 7B8 and Hla and between 1A9 and Hla were measured using a BIAcore 3000. The carboxyl groups on the sensor surface of a CM5 chip were activated with 0.2 M N-ethyl-N-(3-dimethylaminopropyl)carbodiimide and 0.05 M N-hydroxy succinimide. Antibody was bound to the chip by passing 1 µM antibody in HBS-P buffer (20 mM HEPES [pH 7.4], 150 mM NaCl, 0.005% [vol/vol] surfactant P20) over the activated chip. Free amines were then neutralized using 1 M ethanolamine hydrochloride. The control surface was prepared similarly, except that running buffer was injected instead of Hla. We measured the rates of association and dissociation of purified Hla at concentrations ranging from 5 to 100 nM. All measurements were performed in triplicate. After each binding experiment the sensor chip was regenerated using 10 mM NaOH. Derived sensorsgrams were analyzed using BLAevaluation 4.1. Affinity constants were estimated by curve fitting using a 1:1 binding model.

Statistical analysis. In mortality studies statistical significance was determined using the Fisher exact test; the significance of LDH release assay results and the results of bacterial recovery from lungs and red cell hemolysis was calculated using the two-tailed Student’s t test.

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added to cocultures of A549 cells with live *S. aureus* (Fig. 1G and H) \((P < 0.02\) for all conditions). The calculated 50% inhibitory concentration for 7B8 was 2.83 \(\mu\)g/ml, while that for 1A9 was 1.92 \(\mu\)g/ml. These results reveal the in vitro protective efficacy of two novel anti-Hla MAbs which protect human lung epithelial cells from *S. aureus*-mediated injury. The low concentrations at which these antibodies have protective effects in vitro highlight their potential therapeutic relevance.

**Passive immunization with anti-Hla MAbs protects mice from *S. aureus* pneumonia.** To examine the ability of anti-Hla MAbs to prevent *S. aureus*-induced pneumonia, we passively immunized 7-week-old C57BL/6J mice with 7B8 or 1A9 delivered via i.p. injection 24 h prior to intranasal (i.n.) infection with \(3 \times 10^8\) to \(4 \times 10^8\) CFU of *S. aureus* Newman. Groups of 15 mice received 10, 5, 1, or 0.1 mg/kg of 7B8 (Fig. 2A) or 1A9 (Fig. 2B); control animals received 10 mg/kg of the corresponding control MAb. Mice were observed for acute lethal disease secondary to *S. aureus* pneumonia. For animals receiving as little as 1 mg/kg of 7B8 there was a significant reduction in mortality (Fig. 2A) \((P = 0.015)\) compared to animals receiving the isotype control. The protective efficacy of 1A9 was less prominent; a reduction in mortality over 72 h was evident with a minimum MAb dose of 5 mg/kg (Fig. 2B) \((P = 0.039)\). Concentrations of 7B8 that resulted in a significant decrease in mortality resulted in half-maximal serum antibody titers greater than 1:547 \(\times 218\), similar to anti-Hla titers that we have reported to confer protection in prior immunization studies (5). Interestingly, 1 mg/kg of 1A9 resulted in a half-maximal titer of 1:606 \(\times 143\), yet it was not protective, suggesting that 1A9 has either a lower affinity for Hla or a diminished capacity for functional neutralization.

To evaluate the pathological correlates of 7B8 and 1A9 protection, we examined *S. aureus* recovery from lungs 24 h after infection for mice that received 10 mg/kg of each MAb or a control. Immunization with 7B8 and 1A9 led to marked reductions in the number of CFU recovered from the right lung compared to controls (Fig. 2C) \((P = 0.027\) and \(P = 0.02\), respectively). Gross inspection of infected lungs revealed more focal disease in animals that received 7B8 or 1A9, as indicated by a reduction in the dense, red appearance of the tissue (Fig. 2D). These observations were confirmed by histopathologic analysis, which showed that the majority of the airspace in control mice was obliterated by inflammatory cell infiltrates or aggregates of *S. aureus* (Fig. 2E, left panels); in contrast, in animals treated with 7B8 or 1A9 the lung tissue was predominately unaffected (Fig. 2E, right panels).

As an increasing number of severe *S. aureus* pneumonias are caused by MRSA isolates, we examined the efficacy of 7B8 and 1A9 for protecting mice against the highly virulent MRSA strain LAC/USA300. We have previously shown that LAC/USA300 secretes approximately twice as much Hla as *S. aureus*.
Newman, which directly correlates with increased virulence in the lung (5). Treatment of mice with 7B8 or 1A9 24 h prior to i.n. infection with $2 \times 10^8$ CFU of LAC/USA300 resulted in a marked reduction in mortality compared to the controls (Fig. 3A) ($P = 0.013$ and $P = 0.002$, respectively), confirming the ability of these MAbs to protect against both methicillin-sensitive \textit{S. aureus} and MRSA isolates upon prophylactic administration.

The clinical utility of MAbs that prevent \textit{S. aureus} pneumonia, especially disease caused by virulent strains such as LAC/USA300, is readily appreciated when surgical and intensive care patients that are highly susceptible to staphylococcal ventilator-associated pneumonias are considered (15, 25). A more far-reaching impact, however, would be possible if these MAbs confer protection following the onset of infection. To examine therapeutic efficacy, mice were immunized via the i.p. route with 10 mg/kg control IgG2a or 7B8. Anti-Hla MAb-treated animals received doses 24 h prior to infection or at 4, 8, or 12 h after infection with \textit{S. aureus} Newman. Treatment up to 8 h following infection, when mice exhibit prominent signs of disease, conferred significant protection from 24-h mortality ($P = 0.021$) (Fig. 3B). This protection was not durable, however, as the late mortality was similar to that observed for the control. While the absolute 24-h mortality was reduced in mice vacci-
nated 12 h after infection, the results were not statistically significant. These data suggest that Hla may be essential for an early stage of pathogenesis when the toxin may be crucial for inciting lung injury. Once this injury has occurred, antagonism of the toxin may be of little benefit. An extrapolation of these findings to human disease implies that treatment with such MAbs early in the course of *S. aureus* pneumonia has the potential to delay disease progression, providing a much-needed window of opportunity to enhance the utility of antimicrobial and supportive therapies.

**SPR reveals similar affinities of MAbs for Hla.** To quantify the affinity of 7B8 and 1A9 for Hla, we performed SPR studies. Purified toxin (5 to 100 nM) was passed over each MAb, which was amino coupled to a CM5 chip, allowing determination of the kinetic rate constants for association and dissociation and the affinity constant. Both 7B8 and 1A9 have affinities in the low-nanomolar range (8.54 nM and 6.49 nM, respectively) (Fig. 4A), indicating that these MAbs are able to form a tight association with Hla, a feature that likely enhances their capacity to neutralize toxin activity.

**MAb recognition of an N-terminal epitope prevents Hla oligomerization.** An appreciation of the role of specific amino acids and regions of Hla in toxin assembly and function has been obtained through studies of the crystal structure of the heptameric toxin and a series of elegant biochemical studies (21, 26, 27). To examine the mechanism by which 7B8 and 1A9 neutralize Hla, we generated a series of GST fusion proteins comprised of overlapping 50-amino-acid segments of the mature toxin to map the epitope(s) recognized by each MAb (Fig. 4B). Dot blot analysis of these fusion proteins revealed that both MAbs bind within the first 50 amino acids. The Coomassie blue-stained SDS-PAGE gel shows the integrity of each fusion protein. (C) Structure of Hla as a monomer (left structure) and as a heptamer (middle and right structures), in which the first 50 amino acids recognized by the MAbs are indicated by black lines.

**FIG. 4. Anti-Hla MAbs bind to epitopes within the first 50 amino acids of Hla.** (A) SPR was performed by amino coupling 7B8 and 1A9 to a CM5 chip. Binding of purified Hla revealed that the affinities of each MAb for Hla were in the low-nanomolar range. $k_a$, kinetic rate constant for association; $k_d$, kinetic rate constant for dissociation; $K_D$, affinity constant. (B) Overlapping, serial 50-amino-acid segments of Hla were purified as GST fusion proteins and used in a dot blot analysis to map the epitopes recognized by 7B8 and 1A9, which revealed that both MAbs bind within the first 50 amino acids. The Coomassie blue-stained SDS-PAGE gel shows the integrity of each fusion protein. (C) Structure of Hla as a monomer (left structure) and as a heptamer (middle and right structures), in which the first 50 amino acids recognized by the MAbs are indicated by black lines.

**FIG. 3. Anti-Hla MAbs protect against highly virulent *S. aureus* LAC/USA300 and have therapeutic value.** (A) Immunization with 7B8 and 1A9 conferred protection against 48- and 72-h mortality in mice infected via the i.n. route with *S. aureus* LAC/USA300 ($P = 0.013$ and $P = 0.002$, respectively; 15 animals per group). (B) Mice were passively immunized with 7B8 24 h prior to i.n. challenge with *S. aureus* Newman or 4, 8, or 12 h postchallenge. A significant decrease in 24-h mortality was observed for animals treated up to 8 h postinfection ($P = 0.021$; 15 animals per group). Statistical significance ($P < 0.05$) is indicated by an asterisk.

**To quantify the capacity to neutralize toxin activity.** Interestingly, the first 50 amino acids comprise a region of the mature toxin that is required for the formation of a functionally active oligomeric pore. The structure of this region of Hla can be appreciated by examination of these residues (Fig. 4C) in the context of the full-length monomer (based on a predicted structure) and the assembled heptamer derived from the known crystal structure (26).

To investigate whether the interaction of each MAb with Hla disrupts the formation of fully assembled Hla, we utilized a RRBC hemolysis assay in which [35S]methionine-labeled Hla is visualized by SDS-PAGE in its monomeric and heptameric forms (27). To confirm the functional ability of 7B8 and 1A9 to protect against hemolysis, we incubated $3 \times 10^8$ cells with 100 nM purified active Hla and each MAb at concentrations of 0.1 to 5 µM. Dose-dependent prevention of hemolysis was observed for both MAbs (Fig. 5A); a significant reduction in hemolysis was observed with concentrations of 7B8 as low as 0.5 µM ($P \leq 0.005$) and with concentrations of 1A9 as low as 0.1 µM ($P \leq 0.033$). An examination of the radiolabeled monomeric toxin (Hla) (Fig. 5B) demonstrated that the binding of Hla to RRBC was not impaired by addition of 7B8 or 1A9. Importantly, only Hla that is bound to the RRBC membrane was revealed in the autoradiogram. Thus, the similar signal intensities observed for the monomer in lanes treated with the MAb and in lanes without antibody treatment or in the presence of control antibody strongly suggest that the
Mabs do not function merely by preventing toxin binding to target cells. The monomeric form of Hla produced in vitro migrated as two bands, as seen in previous studies (13). Each MAb, however, prevented the assembly of Hla into an SDS-stable oligomer (Hla7) in a concentration-dependent fashion (Fig. 5B). The inhibition of heptamer formation in the presence of 7B8 and 1A9 correlates with protection from hemolysis, defining a potent molecular mechanism by which these MAbs exert their protective effects. While the affinity constants of these MAbs for Hla were similar, 7B8 prevents the formation of the heptamer more effectively than 1A9 does, which correlates with protection from hemolysis, defining a potent molecular mechanism by which these MAbs exert their protective effects. While the affinity constants of these MAbs for Hla were similar, 7B8 prevents the formation of the heptamer more effectively than 1A9 does, which correlates with protection from hemolysis, defining a potent molecular mechanism by which these MAbs exert their protective effects.

Active vaccination with Hla1-50 prevents mortality due to S. aureus pneumonia. These observations led to the hypothesis that active immunization with a purified antigen consisting of the first 50 amino acids of Hla may prevent disease. Groups of 15 4-week-old mice were vaccinated via the i.m. route on day 0 with 20 μg of GST, GST-HlaH35L, or GST-Hla1-50 emulsified in Freund’s adjuvant and then were boosted on day 10 with antigen emulsified in incomplete Freund’s adjuvant. On day 21, animals were infected via the i.n. route with 3 × 10⁸ S. aureus Newman CFU and evaluated for protection from lethal pneumonia. In agreement with our previously published observations, immunization with GST-HlaH35L led to complete protection, in contrast to immunization with GST alone, after which the majority of mice succumbed to infection (P < 0.001) (Fig. 6) (5). Immunization with Hla1-50 resulted in significant protection over the entire course of the assay, and full protection was evident at 24 h (P < 0.002). The half-maximal serum antibody titers for each immunogen at the time of infection were 1:3,178 ± 1,468 (GST-HlaH35L) and 1:692 ± 669 (GST-Hla1-50). These observations, along with our description of protective Hla MAbs, have important implications for the development of clinically relevant immunologic tools to combat S. aureus pneumonia. First, these studies highlight the potential to utilize only a portion of Hla as a component of a multisubunit S. aureus vaccine. This may simplify vaccine design, allowing generation of a chimeric antigen in which a segment of Hla can be fused to a second candidate vaccine antigen. Further, a greater margin of safety can be achieved by avoiding the use of a full-length variant of a potent human toxin. Second, the humanization of MAbs, notably MAb 7B8, with specificity for the N-terminal region of Hla may provide an efficient means by which to translate these observations to the clinical arena.

Serum antibody titers to Hla1-50 vary within the human population. Implementation of an effective Hla-based immunotherapy for S. aureus pneumonia is predicated on the notion that some humans do not harbor a preexisting, effective host response to this toxin, rendering them more susceptible to disease. Previous studies have clearly documented that serum antibodies to Hla are commonly found within populations of individuals with invasive S. aureus disease; however, large-scale investigations of preformed anti-Hla responses in subjects without such disease have not been reported (10, 11, 18). To evaluate the human antibody response to epitopes within the first 50 amino acids of Hla, we examined sera from 25 healthy volunteers using an ELISA. While all individuals had detectable antibodies to Hla1-50, the coefficient of variation within the population was calculated to be 50.3% when a 1:1 dilution of serum was analyzed (Fig. 6B). This observation has two important implications. First, it suggests that there is a defined population of humans with relatively lower levels of antibody to at least one epitope of Hla shown in this work to be pro-
ective. These individuals may benefit from vaccine strategies targeting Hla, perhaps especially in clinical situations such as critical illness, major surgical procedures, and influenza infection that confer risk for the development of S. aureus pneumonia. Second, the data highlight the importance of understanding the human immune response to S. aureus disease and the immunologic correlates of protection from invasive disease. In order to fully appreciate the role of preexisting antibodies to Hla in conferring protection from pneumonia, it will be necessary to conduct prospective clinical studies in which the anti-Hla antibody profile of “at-risk” subjects can be correlated with the development of disease. A definitive link between anti-Hla antibody levels in general or levels of an antibody to a specific protective epitope and risk for pneumonia would make it possible to stratify risk for the development of disease. The capacity to prospectively identify at-risk individuals would facilitate highly focused application of developing immunotherapies.

The clinical complexity of S. aureus infection and the fact that there is no single virulence factor central to the progression of all disease manifestations pose a challenge for development of a universal vaccine. The essential role of Hla in pneumonia, coupled with the clinical burden of this disease, highlights the potential of the observations described here to have a significant impact upon ongoing and future efforts to design novel immunotherapies to specifically combat lung infection. Further, these observations shed light on a new strategy with which to conceptualize the targeting of Hla in a multifaceted universal S. aureus vaccine.

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