Targeting Alpha Toxin and ClfA with a Multimechanistic Monoclonal-Antibody-Based Approach for Prophylaxis of Serious Staphylococcus aureus Disease

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ABSTRACT Staphylococcus aureus produces numerous virulence factors, each contributing different mechanisms to bacterial pathogenesis in a spectrum of diseases. Alpha toxin (AT), a cytolytic pore-forming toxin, plays a key role in skin and soft tissue infections and pneumonia, and a human anti-A T monoclonal antibody (MAb), MEDI4893*, has been shown to reduce disease severity in dermonecrosis and pneumonia infection models. However, interstrain diversity and the complex pathogenesis of S. aureus bloodstream infections suggests that MEDI4893* alone may not provide adequate protection against S. aureus sepsis. Clumping factor A (ClfA), a fibrinogen binding protein, is an important virulence factor facilitating S. aureus bloodstream infections. Herein, we report on the identification of a high-affinity anti-ClfA MAb, 11H10, that inhibits ClfA binding to fibrinogen, prevents bacterial agglutination in human plasma, and promotes opsonophagocytic bacterial killing (OPK). The need to target multiple virulence factors is supported by preclinical data demonstrating that vaccination with a multimechanistic MAb combination targeting alpha toxin and ClfA to effectively prevent S. aureus disease.

IMPORTANCE Alternative strategies to broad-spectrum antibiotics are required to combat the antibiotic resistance epidemic. Previous attempts at active or passive immunization against Staphylococcus aureus targeting single antigens have failed in clinical trials despite positive preclinical data. To provide broad disease and isolate coverage, an effective immunization strategy likely must target multiple virulence mechanisms of the pathogen. Herein, we tested a multimechanistic MAb combination targeting alpha toxin (AT) and clumping factor A (ClfA) that neutralizes AT-mediated cytotoxicity, blocks fibrinogen binding by ClfA, prevents bacterial agglutination, targets the bacteria for opsonophagocytic killing, and provides broad isolate coverage in a lethal-bacteremia model. Although each MAb alone was effective in bacteremia against some individual isolates, the MAB combination provided improved protection against other isolates. These results illustrate the importance of targeting multiple virulence mechanisms and highlight the potential for an MAB combination targeting AT and ClfA to effectively prevent S. aureus disease.

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S. aureus bacteremia, but treatment has yet to be tested with monoclonal antibodies (4, 5).

Alpha toxin (AT) is a key virulence factor in several S. aureus diseases, including pneumonia, skin and soft tissue infections (SSTI), and bacteremia (6–8). In fact, passive immunization with anti-AT MAbs reduced disease severity in pneumonia and dermatocnosis models (9–11), and vaccination with an AT toxoid with an H35L mutation (ATH35L) protected against death in mouse lethal bacteremia and pneumonia models (6, 8, 9, 11–13). AT contributes to multiple aspects of S. aureus pathogenesis during bacteremia and sepsis, including stimulating a hyperinflammatory response characteristic of sepsis and activating ADAM10-mediated cleavage of endothelial tight junctions, leading to a loss in vascular integrity (14–16). AT has also been demonstrated to target platelets, which prevents repair of the injured endothelial barrier and promotes organ dysfunction through platelet-neutrophil aggregate formation (17). The role of AT in various aspects of sepsis highlights the potential of an AT-neutralizing MAb to prevent or treat this serious disease.

Among the many S. aureus surface adhesins, clumping factor A (ClfA) has been demonstrated to play an important role in serious bloodstream infections (18, 19). ClfA binds fibrinogen and facilitates both bacterial adherence to fibrinogen and bacterial clumping, both of which are key attributes in the development of an S. aureus bloodstream infection (20–22). ClfA is known to bind fibrinogen or fibrinogen at a site of injury or coated on an indwelling device can facilitate bacterial colonization (18) and bacterial clumping, which is thought to enhance bacterial invasiveness (22–25). ClfA has also been reported to impair complement deposition required for OPK (26). Consistent with these observations, isogenic ΔclfA mutants exhibited reduced virulence in infection models (23, 27, 28) and passive immunization with human anti-ClfA-enriched intravenous (i.v.) immunoglobulin (Ig) (Veronate) or an MAb (12-9 or Aurexis) improved disease outcomes for patients with S. aureus bloodstream infections (29, 30). However, these antibody preparations failed to improve outcomes in clinical studies of prophylaxis or adjunctive therapy with vancomycin to prevent or treat S. aureus bacteremia in very-low-birth-weight infants (31–33). There are also conflicting reports of the value of targeting ClfA alone by active immunization (34, 35).

Given the distinct roles of AT and ClfA in S. aureus bloodstream infections, we hypothesized that neutralizing both virulence factors with high-affinity MAbs might provide benefit over prophylaxis with the individual MAbs in S. aureus bacteremia. Herein, we report on the identification of an anti-ClfA IgG (11H10) that prevents fibrinogen binding, inhibits bacterial agglutination, promotes OPK, and protects mice from a lethal S. aureus bacteremia. Additionally, passive immunization with 11H10 combined with an anti-AT MAb, MEDI4893*, provided enhanced efficacy and broader strain coverage than either MAb alone. These results suggest that immunoprophylaxis with an anti-ClfA–anti-AT MAb combination may provide a prophylactic approach superior to that using the individual MAbs for prevention of serious S. aureus bloodstream infections.

RESULTS

Alpha-toxin and ClfA are key virulence factors in a mouse lethal bacteremia model. To confirm a role for AT and ClfA in an S. aureus lethal bacteremia model, mice were i.v. infected with the wild-type (WT) community-acquired methicillin-resistant S. aureus (CA-MRSA) strain SF8300 or its isogenic Δhla, ΔclfA, or Δhla ΔclfA mutant. Consistently with published results, infection with the Δhla or ΔclfA mutant attenuated disease severity. The double (ΔclfA Δhla) mutant had a slight survival benefit over the individual mutants in this model in the same bacterial genetic background (Fig. 1A). Similarly, bacterial numbers were significantly reduced in the hearts of animals (P < 0.0001 versus WT SF8300) 14 h postinfection with each of the mutants compared to numbers in the hearts of animals infected with WT SF8300 (Fig. 1B). Numbers of bacterial CFU in the kidneys were also significantly reduced 48 h after infection with each of the mutants relative to numbers of CFU of WT SF8300 (P ≤ 0.0006) (Fig. 1C). These results indicate that both AT and ClfA play a role in this model and may be viable targets for immunoprophylaxis against S. aureus bacteremia and sepsis.

Isolation and characterization of the anti-ClfA MAb 11H10. We previously reported on a potent anti-AT MAb, MEDI4893*, capable of protecting animals against S. aureus pneumonia and skin infections (9, 10). This MAb is the precursor to clinical candidate MEDI4893, which is currently in a phase 2b trial for the prevention of S. aureus pneumonia in ventilated patients (http://www.clinicaltrialsregister.eu). In the present study, we wanted to
compare the protective activity of MEDI4893* to that of a highly potent antibody directed against ClfA in the *S. aureus* sepsis model. To this end, an anti-ClfA MAb was generated using hybridoma technology in VelocImmune mice immunized with the ClfA fibrinogen-binding domain ClfAN2N3 (9, 36–38). VelocImmune mice allow for easy conversion to a human IgG1. MAb 11H10 was selected from a panel of 15 anti-ClfA MAbs based on its superior performance in functional assays, which we hypothesized would translate into enhanced protection during infection (e.g., inhibition of fibrinogen binding and bacterial agglutination, binding to *S. aureus* ex vivo, and OPK activity). 11H10 kinetics of binding to ClfAN2N3 were measured to determine 11H10 affinity for its target antigen. Association and dissociation constants for ClfAN2N3 were determined to be 11.4×10^5 (1/M/s) and 4.8×10^3 (1/s), respectively, with an estimated KD (equilibrium dissociation constant) of 4.2 nM (Fig. 2A).

During a bloodstream infection, ClfA binding to fibrinogen facilitates *S. aureus* attachment to fibrinogen-coated surfaces (e.g., an indwelling catheter) and also promotes bacterial agglutulation in plasma, a key virulence mechanism reported to result in an invasive phenotype during bloodstream infections (39, 40). 11H10 inhibited ClfAN2N3 binding to fibrinogen in a dose-dependent manner (50% inhibitory concentration [IC_{50}] = 0.8 μg/ml) (Fig. 2B) and blocked *S. aureus* agglutination in human plasma at a MIC of ≈25 μg/ml (Fig. 2C).

11H10 anti-ClfA OPK and bacterial-clearance activities were evaluated by incubating the bacteria with the MAb in the presence of the human monocytic cell line HL-60 and human serum as a complement source. A collection of *S. aureus* clinical isolates representing diverse ClfA sequences was tested (19). 11H10 exhibited dose-dependent OPK activity against all tested isolates (Fig. 2D; see also Fig. S1 in the supplemental material).

For a therapeutic MAb to be effective, the target antigen must be expressed and the MAb’s epitope must be conserved and accessible to binding *in vivo*. To address this, 11H10 binding was assessed by flow cytometry on 24 different *S. aureus* clinical isolates recovered from the bloodstream of infected mice (see Table S1 in the supplemental material). These results confirmed that ClfA is differentially regulated among clinical isolates and that the 11H10 epitope is conserved and accessible following *in vivo* passage in mice. Taken together with results from the above-described functional assays, these data indicated that 11H10 was a promising candidate anti-ClfA MAb that binds *S. aureus* passaged *in vivo*, neutralizes fibrinogen binding, and mediates OPK.

**Anti-AT and anti-ClfA protection in CA-MRSA USA300-induced lethal bacteremia.** Mice were passively immunized with 11H10 or MEDI4893* 24 h prior to i.v. challenge with a lethal dose of SF8300 and monitored for survival for 14 days to evaluate rel-
ative protective activity in a lethal bacteremia model (Fig. 3A). Bacterial burden was measured in the hearts and kidneys. Both 11H10 and MEDI4893* prophylaxis resulted in a dose-dependent increase in survival and significantly reduced numbers of CFU in the hearts and kidneys of infected mice compared to those in c-IgG-administered mice (Fig. 3B and C), indicating that both MAbs were functionally active in this model.

**Anti-ClfA OPK is required for full 11H10 activity in vivo.** We previously showed that MEDI4893* protective activity in a mouse pneumonia model was Fc independent (9). Similarly, Fc function was not required for MEDI4893* protection from lethal bacteremia since MEDI4893*N297Q, containing an Fc mutation which abrogates binding to the Fc region of IgG (FcγR) and complement C3a (9, 41, 42), exhibited efficacy similar to what was observed with unmutated MEDI4893* (see Fig. S2 in the supplemental material). An 11H10N297Q Fc mutant was also generated and tested in a lethal-bacteremia model to determine if Fc function is necessary for 11H10 protection in this infection model. As expected, MAb 11H10N297Q exhibited no in vivo OPK activity but retained the ability to inhibit bacterial agglutination (Fig. S3). When administered to mice prior to i.v. challenge with different *S. aureus* strains, 11H10N297Q exhibited reduced protective capacity relative to that of 11H10 against 3 different strains (Fig. 4 and S3), indicating that anti-ClfA OPK activity is required for full 11H10-mediated protection in this model.

**An anti-ClfA and anti-AT MAb combination is beneficial in lethal bacteremia.** To determine whether there was benefit from combining anti-AT and anti-ClfA MAb activities, mice were passively immunized with a suboptimal MEDI4893* or 11H10 dose (2 mg/kg of body weight) or a combination of both MAbs (1 mg/kg each) 24 h prior to i.v. challenge with SF8300. Animals were monitored for survival, and bacterial burden was measured in hearts and kidneys. Although prophylaxis with the individual MAbs reduced bacterial burden in one (MEDI4893*) or both (11H10) organs, neither provided a survival benefit relative to that of c-IgG (Fig. 5). In contrast, the MAb combination resulted in significantly increased survival compared to that with c-IgG. In fact, the protective activity seen with a low dose of MAbs in combination exceeded the protection observed with either of the individual MAbs, even when they were administered at a much higher dose (compare Fig. 3A and 5A). Although the MAb combination provided a greater survival benefit, the reduction in organ burden observed with the combination was no greater than with 11H10 alone. These results support a previous finding that survival following i.v. challenge with *S. aureus* does not always correlate with bacterial burden at a snapshot in time (17). Overall, our data suggest that prophylaxis with a combination of MAbs having distinct mechanisms of action can provide benefit over individual MAbs in preventing *S. aureus* bloodstream infections.
An anti-AT–anti-ClfA MAb combination reduces *S. aureus* sepsis-associated markers of organ damage. Organ damage is a hallmark of bacterial sepsis and can be monitored by measuring serum levels of cardiac troponin (cTn1), creatine kinase myocardial band (CK-MB), alanine aminotransferase (ALT), and aspartate aminotransferase to assess heart and liver damage (43). Animals that received c-IgG prior to *S. aureus* challenge exhibited increased levels of all biomarkers relative to those of mock-infected animals, consistent with sepsis-mediated organ damage (Fig. 6). Treatment with anti-ClfA MAbs significantly reduced 3 of 4 (cTn1, CK-MB, ALT) markers, whereas the MAb combination significantly reduced all 4 biomarkers of organ damage compared to c-IgG. These results suggest that while anti-ClfA MAbs can reduce organ damage, the MAb combination provides the most complete protection against sepsis-associated organ damage.

![Graphs showing survival rates and biomarker levels](image-url)
Anti-AT and anti-ClfA MAb combination provides improved strain coverage. The efficacy of 11H10 and MEDI4893* against a collection of clinical *S. aureus* isolates representing diverse clonal complexes was tested to evaluate strain coverage. Prophylaxis with 11H10 or MEDI4893* (15 mg/kg) alone significantly increased survival over that with c-IgG following challenge with 6/9 and 3/9 clinical isolates, respectively (Table 1; see also Fig. S4 in the supplemental material). No protection with either MAb alone was observed with two of the isolates (3049057 and NRS261). However, prophylaxis with the MAb combination (7.5 mg/kg each) resulted in a significant increase in survival relative to that with c-IgG following challenge with every isolate tested. These results indicate that although 11H10 and MEDI4893* can each provide protection in an *S. aureus* sepsis model, the protection is strain dependent and the MAb combination provides the greatest isolate coverage.

The MAb combination provides protection in dermonecrosis and pneumonia. MEDI4893* was previously reported to protect against pneumonia and dermonecrosis in murine infection models (9, 10). We next determined if 11H10 would improve MEDI4893* monotherapy in these two disease models where AT is the major virulence determinant. Passive immunization with MEDI4893* plus 11H10 resulted in lesion sizes similar to those observed in animals passively immunized with MEDI4893* in a mouse dermonecrosis model (Fig. 7A). Similarly, 11H10 combined with MEDI4893* did not improve survival relative to MEDI4893* monotherapy in a pneumonia model (Fig. 7C). Addition of 11H10 also did not potentiate bacterial clearance seen in these models with MEDI4893*, indicating either that ClfA is not expressed or that 11H10 does not effectively pro-

### TABLE 1 The anti-AT–anti-ClfA MAb combination provides broad strain coverage in i.v. lethal sepsisa

| Clinical isolate | AT MAb (15 mg/kg) | ClfA MAb (15 mg/kg) | MAb combination (7.5 mg/kg each) |
|------------------|-------------------|---------------------|---------------------------------|
| 2784 (CC1)       | +                 | +                   | +                               |
| NRS382 (CC5)     | –                 | –                   | –                               |
| 3049043 (CC3)    | –                 | –                   | +                               |
| 4211 (CC5)       | +                 | +                   | +                               |
| SF8300 (CC8)     | –                 | –                   | +                               |
| 3049057 (CC2)    | –                 | –                   | +                               |
| NRS261 (CC30)    | –                 | –                   | +                               |
| 3049157 (CC30)   | –                 | –                   | +                               |
| 3049048 (CC45)   | –                 | –                   | +                               |

a BALB/c mice (n = 10) were injected i.p. with MEDI4893* (15 mg/kg), 11H10 (15 mg/kg), MEDI4893* plus 11H10 (7.5 mg/kg each), or c-IgG (15 mg/kg). Twenty-four hours later, animals were infected i.v. in the tail vein with an LD<sub>90</sub> of different *S. aureus* clinical isolates from diverse clonal complexes (CC). Survival was monitored for 2 weeks. Results were analyzed with a log rank (Mantel Cox) test. + indicates a P value of <0.05, and — indicates a P value of >0.05. Each strain was tested at least three times.
multe OPK in this infection context (Fig. 7B and D). These data support previous findings showing that AT is a key virulence determinant in skin and lung infections and indicate that the addition of 11H10 is neither beneficial nor detrimental to the protection observed with MEDI4893* in these models.

DISCUSSION

Despite positive preclinical data, previous active or passive immunization attempts targeting single S. aureus virulence factors (i.e., ClfA, IsdB, or CP5/CP8) failed to prevent S. aureus disease in clinical trials (44–46). S. aureus expresses a diverse array of virulence factors which contribute to disease pathogenesis (47, 48). Many of these antigens are not highly conserved and can be differentially regulated depending on the site of infection (49–52). Current efforts are shifting to a multitarget immunization approach to neutralize multiple virulence factors and to provide broad strain coverage (4, 5, 53–55). AT has been reported to be essential for S. aureus skin and soft tissue infections (SSTI) and pneumonia (6, 7, 14), and immunization strategies targeting AT reduced disease severity in both models (6, 9–11, 56, 57). Because of its major role in these diseases, AT is currently under development as a stand-alone MAb target for the prevention or treatment of S. aureus pneumonia (http://www.clinicaltrialsregister.eu).

AT plays an important role in S. aureus bacteremia and sepsis, in addition to SSTI and pneumonia. It contributes to many of the pathogenic effects seen during S. aureus bacteremia by disrupting endothelial barriers, altering the coagulation cascade, modifying immune cell function, and contributing to the hyperinflammatory response in sepsis (17, 58–60). ClfA, another virulence determinant shown to play a role in S. aureus bloodstream infections, binds fibrinogen and promotes bacterial agglutination and complement evasion. Active immunization strategies with ClfA, despite generating functional polyclonal opsonic antibodies that also inhibit bacterial binding to fibrinogen in vitro, resulted in various levels of protection against multiple strains in bloodstream infection models that may be dependent on the adjuvant and mouse strain (34, 35). Such variability in active immunization strategies highlights the potential benefit of MAb therapy in providing a reliable and potent functional antibody response.

The results presented herein indicate that a combination of anti-AT and anti-CIFA MAbs provides both improved coverage against a representative strain panel and enhanced protective activity. Passive immunization with either the anti-AT or the anti-CIFA MAB protected against challenge with 3 or 6 of 9 S. aureus clinical isolates, respectively, and neither MAB provided protection against 2 of the 9 isolates tested. However, the MAB combination provided protection against all strains tested, even with equivalent total MAB doses. Furthermore, passive immunization with subprotective doses of 11H10 (1 mg/kg) plus MEDI4893* (1 mg/kg) provided strong protection following challenge with CA-MRSA SF8300 (Fig. 5), a strain for which single MAB prophylaxis at a much higher dose (15 mg/kg) was required to provide increased survival in this model (Fig. 3). Taken together, these results suggest that a lack of protection against some isolates by the individual MAbs is not due to a complete lack of antigen expression because the anti-CIFA–anti-AT combination increases survival against multiple strains with apparent synergy against some strains against which neither MAB alone protects even when tested at higher doses (Table 1). These data indicate that neutralizing both AT and ClfA virulence activities with functional MAbs can provide benefit or even synergy relative to that provided by the individual MAbs.

Another multimechanistic MAB approach was shown to provide benefit against Pseudomonas aeruginosa in preclinical disease models with a bispecific antibody targeting the cell surface polysaccharide PsI (OPK and anti-cell attachment) and PcrV (anticytotoxic activity) of the type 3 secretion system (61). Combination vaccine approaches to prophylaxis against S. aureus disease have been reported; however, the exact mechanisms of action of antibodies generated against each antigen are not clear (4, 5, 62). In our MAB combination, 11H10 blocks ClfA binding to fibrinogen, inhibits bacterial agglutination, and targets the bacteria for OPK (Fig. 2), while MEDI4893* neutralizes the toxic effects of AT (17, 59).

Bacterial antigens targeted by passive or active immunization must be conserved and expressed during infection on circulating clinical isolates. Both of the genes encoding AT (hla) and ClfA (clfA) are reported to be highly conserved among clinical isolates and are likely expressed in humans, since most individuals tested have IgG titers against these antigens (19, 63–65). These observations are supported by data from mice indicating that both clfA and hla expression levels increase during a bloodstream infection (49) and ClfA is present on bacteria harvested from the bloodstream of infected mice (Fig. 2E; see Table S1 in the supplemental material) (50). Although humans mount a response against these antigens during natural exposure, the antibodies are generally not thought to be of sufficient quality, activity, and/or quantity to protect from disease (63, 65). Therefore, providing a patient with sufficient quantities of potent, highly functional MAbs may provide benefit over a natural mixed-polyclonal response.

Previous failed attempts at prophylaxis against S. aureus have all targeted a single antigen, and none were aimed at neutralizing a secreted toxin (31, 46, 66, 67). Herein, we present data indicating that prophylaxis against S. aureus bacteremia with a multimechanistic MAB combination targetingClfA and AT provides benefit over the individual MAbs by neutralizing multiple virulence mechanisms and targeting the bacteria for OPK. Such an MAB combination may not only extend isolate coverage against S. aureus bacteremia but also extend disease coverage beyond SSTI and pneumonia, where AT plays a dominant role. Future clinical studies will be required to further test this hypothesis in human disease.

MATERIALS AND METHODS

Bacterial strains. CA-MRSA SF8300 (USA300) and its isogenic knockout strains for AT (Δhla) and CIFA (ΔclfA) were previously described (49). The Δhla ΔclfA double knockout was constructed in the Δhla background as described using the pKOR1 allelic-replacement mutagenesis system and the primers in Table S2 in the supplemental material (49). Reynolds and Wright strains were obtained from the ATCC (Manassas, VA). NRS3382 (CC5, MRSA) and NRS261 (CC30, methicillin-susceptible S. aureus [MSSA]) were obtained from the Network on Antimicrobial Resistance in Staphylococcus aureus (NARSA), 3049043 (CC5, MSSA), 3049057 (CC8, MRSA), 3049157 (CC30, MSSA), and 3049048 (CC45, MSSA) were obtained from Eurofins. Strains ARC4211 (CC5) and ARC2784 (CC1) were obtained from AstraZeneca’s Research Collection. Clonal complex (CC) identifications were determined by protein A typing as previously described (68). Bacteria were grown to mid-log phase (optical density at 600 nm [OD600] of 0.8) in tryptic soy broth (TSB; VWR International), washed twice in ice-cold phosphate-buffered saline (PBS) (Invitrogen), and frozen in 10% glycerol-TSB. Challenge inocula were prepared from
one frozen vial diluted in ice-cold PBS to 3 × 10^9/ml and placed on ice until injection.

**ClfA MAb generation.** Anti-ClfA MAbs were generated by following the repetitive immunization at multiple sites (RIMMS) protocol (69), with slight modifications. Eight-week-old VeloMice mice were immunized with ClfA containing residues 221 to 559 (ClfA221–559) and TiterMax gold adjuvant (Sigma) every 2 to 3 days for 13 days. Lymph nodes were collected and B-cell hybridomas were generated after fusion with a P3X myeloma partner. Hybridoma supernatants were first screened for anti-ClfA reactivity by ClfA enzyme-linked immunosorbent assay (ELISA) and an anti-human Fc biosensor plate (hydrated in kinetic buffer) with anti-ClfA MAb or c-IgG. The most-active hybridomas were then cloned by limiting dilution and tested for fibrinogen binding inhibition, OPK, and ex vivo binding to strain SF8300. 11H10 was selected and converted to fully human antibody by grafting a human Fc to the human variable region (37).

**Agglutination inhibition in human plasma.** Six different *S. aureus* clinical isolates were cultured overnight in TSB, washed in PBS, and suspended to 1/10 of the original volume in ice-cold PBS. Anti-ClfA MAb was 2-fold serially diluted starting at 200 μg/ml and mixed with 20 μl of citrated human plasma in a 96-well U-bottom plate (Thermo, Fisher Scientific). Bacteria were added (20 μl) and incubated for 5 min at 37°C. Each well was evaluated visually, and the lowest MAb concentration at which bacteria agglutinated was recorded. R347, a human anti-gp120 MAb (10), was utilized as an isotype control human IgG1 (c-IgG).

**ClfA/fibrinogen binding inhibition assay.** Nunc Maxisorp plates (Thermo, Fisher Scientific) were coated with 1 μg/ml human fibrinogen (Sigma) overnight at 4°C, washed 3 times with PBS containing 0.1% Tween 20 (wash buffer), and blocked for 1 h at room temperature (RT) with 200 μl/well PBS–1% bovine serum albumin (BSA). Following 3 washes, the plates were incubated for 1 h at RT with a mix of 50 μg/ml ClfA221–559 (2 μg/ml) and serial dilutions of anti-ClfA MAb in a 100-μl final volume of PBS. After the washes, bound ClfA was detected using purified rabbit anti-ClfA IgG (5 μg/ml) for 1 h at RT, followed by detection with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG Fcγ (1:10,000; Jackson ImmunoResearch Lab) and the 3′,3′,5′,5′-tetramethylbenzidine (TMB) substrate (KPL). The reaction was stopped after 10 min with 100 μl 0.2 M H₂SO₄. Plates were read on a spectrophotometer at an OD₅₅₀. The percentage inhibition of ClfA binding to fibrinogen was calculated with the following formula: 100 – (100 × [ODClfA, no MAb] / [ODClfA, no MAb]).

**Ex vivo binding assay by flow cytometry.** Six- to eight-week-old female CD1 mice (Harlan) were injected intraperitoneal (i.p.) with 5×8 CFU of *S. aureus*. After 1 or 4 h, cardiac blood was pooled from 4 mice and mixed with ice-cold sodium citrate at a 0.35% final concentration. Eu karyotic cells were lysed with 1% NP-40 (Thermo, Fisher Scientific), and bacteria were recovered after a 5-min centrifugation at 7,000 rpm. The pellet was sonicated in 2 ml ice-cold PBS and washed once in PBS. Bacteria were transferred to a 96-well U-bottom plate (Thermo, Fisher Scientific) and incubated for 30 min at 4°C with rabbit anti-protein A immune sera (1:1,000). The bacteria were then incubated with anti-ClfA MAb or c-IgG (30 μg/ml) for 1 h at 4°C, washed in PBS, and incubated with Alexa 633-conjugated goat anti-human IgG for 30 min at 4°C (Jackson ImmunoResearch Laboratories). Following one wash, live bacteria were stained for 15 min at RT with BODIPY FL vancomycin, and MAB binding was quantified by flow cytometry with an LSR II cell analyzer (BD). As controls, in vitro-grown bacteria were stained by following the same protocol with anti-ClfA MAB or c-IgG.

**Octet affinity measurement.** 11H10 binding kinetics were analyzed using the FortéBio Octet 384 instrument with 384 slanted-well plates (FortéBio). An anti-human Fc biosensor plate (hydrated in kinetic buffer) was first loaded with 1 μg/ml of 11H10 (300 s). The association of purified ClfA in concentrations ranging from 100 to 6,400 nM was measured for 30 s, followed by dissociation into kinetic buffer (FortéBio) (200 s). All steps were performed using a 3-mm sensor offset with 0.6-Hz sensitivity. Data were exported to Prism (GraphPad) for global association/dissociation affinity curve fitting.

**OPK assay** HL-60 cells (ATCC) were differentiated as described previously (70). Cells were washed in saline and adjusted to 1×10⁶ cells/ml in high-glucose Hanks balance salt solution (HG-HBSS) (Invitrogen)—0.1% gelatin (Sigma). Human serum collected from a healthy volunteer was adsorbed against *S. aureus* Reynolds capsule type 5 and *S. aureus* Wright capsule type 8 to deplete endogenous *S. aureus*-specific IgG and used as a complement source (1:100). Clinical isolates were grown overnight in TSB, washed in cold saline, and diluted to 1×10⁶ CFU/ml in saline. Ten microliters of bacteria was incubated on ice for 30 min with 10 μl of serial MAb dilution in 60 μl of HG-HBSS 0.1% gelatin. Ten microliters of sera and 10 μl of HL-60 were then added to the opsonized bacteria. Ten-microliter samples of each well were serially diluted in water—0.1% saponin and dropped on a TSA plate (VWR International) before and after incubation for 1 h at 37°C with 100-rpm orbital shaking. Bacterial colonies were counted after a 16-h incubation of TSA plates at 37°C. The percentage of OPK was calculated as follows: 100 × (100 – [CFUat 1 h] / [CFUat time zero]).

**Mouse survival and organ burden in sepsis.** Groups of 10 6- to 8-week-old female BALB/c mice (Harlan) were passively immunized i.p. injection of c-IgG, 11H10, MEDI4893*, or 11H10 plus MEDI4893* and then challenged 24 h later by intravenous (i.v.) injection of the 90% lethal dose (LD₉₀) of each *S. aureus* isolate. Survival was monitored over 2 weeks. Statistical analysis of MEDI4893* or 11H10 versus c-IgG was performed with a log rank (Mantel Cox) test. For bacterial enumeration in the hearts and kidneys, animals were euthanized with CO₂, 14 or 48 h postinfection, respectively. The organs were homogenized in lysis matrix A tubes (VWR International), diluted, and plated for CFU enumeration. Statistical differences between two MAb-treated groups were analyzed with a Mann-Whitney U test. Data were considered statistically different if P was <0.05, and this is indicated with an asterisk in the figures.

All experiments were performed in accordance with institutional guidelines following experimental protocol review and approval by the Institutional Biosafety Committee (IBC) and the Institutional Animal Care and Use Committee (IACUC) at MedImmune.

**Circulating markers of organ damage.** Cardiac troponin levels were determined by ELISA using a high-density mouse cardiac troponin 1 kit (Life Diagnostics, Inc.). Albumin, alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), and creatine kinase myocardial band (CK-MB) were determined using an AU400 automated clinical chemist analyzer equipped with an ion-selective electrode (Beckman Coulter, Indianapolis, IN). Associated analysis software was operated through a Microsoft Windows NT operating system. Internal quality control materials were analyzed to ensure the precision of the equipment.

**Mouse dermonecrosis and pneumonia models.** Female BALB/c mice (Harlan) were passively immunized i.p. with MEDI4893*, 11H10, or an MAb combination. Dermonecrosis was induced 24 h later with intradermal challenge of SF8300 (3×7 CFU). Lesion sizes and numbers of CFU were measured as previously described (10). Female C57/86 mice (Jackson) were injected i.p. with a single MAb or a combination of both, and pneumonia was induced by intranasal infection with SF8300 (1×8 CFU) as described previously (9).

**SUPPLEMENTAL MATERIAL**

Supplemental material for this article may be found at http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.00528-16/-/DSupplemental.
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REFERENCES
1. Laupland KB, Lyrilikäinen O, Segard M, Kennedy KJ, Knudsen JD, Ostergaard C, Galbraith JC, Valiquette L, Jacobsson G, Collignon P, Schonheyder HC. International Bacteremia Surveillance Collaborative. 2013. The changing epidemiology of Staphylococcus aureus bloodstream infection: a multinational population-based surveillance study. Clin Microbiol Infect 19:465–471. http://dx.doi.org/10.1111/j.1469-0691.2012.03903.x.
2. DiGiandomenico A, Sellman BR. 2015. Antibacterial monoclonal antibodies: the next generation? Curr Opin Microbiol 27:78–85. http://dx.doi.org/10.1016/j.mib.2015.07.014.
3. Morrison C. 2015. Antibacterial antibodies gain traction. Nat Rev Drug Discov 14:737–738. http://dx.doi.org/10.1038/nrd4770.
4. Bagnoli F, Fontana MR, Soldaini E, Mishra RP, Fiaschi L, Cartocci E, DiGiandomenico A, Sellman BR. 2014. Available from surface proteins of Staphylococcus aureus. Proc Natl Acad Sci U S A 103:16942–16947. http://dx.doi.org/10.1073/pnas.1408683103.
5. Bubeck Wardenburg J, Schneewind O. 2008. Vaccine protection against Staphylococcus aureus pneumonia. J Exp Med 205:287–294. http://dx.doi.org/10.1084/jem.20072208.
6. Inoshima N, Wang Y, Bubeck Wardenburg J. 2012. Genetic requirement for ADAM10 in severe Staphylococcus aureus skin infection. J Invest Dermatol 132:1513–1516. http://dx.doi.org/10.1038/jid.2011.462 http://dx.doi.org/10.1038/jid.2011.462.
7. Foletti D, Strop P, Shaugnessy L, Hasa-Moreno A, Casas MG, Russell MJ, Bagnoli F, Fontana MR, Soldaini E, Trouillet JL, Zhou Y, Cardona P, Nosari S. 2014. The interplay between the Staphylococcus aureus clumping factor (ClfA) and fibrinogen. Eur J Immunol 43:377–385. http://dx.doi.org/10.1002/eji.201330616.
8. Hua L, Hair PS, Echague CG, Sholl AM, Watkins JA, Foster TJ. 2014. Assessment of an anti-alpha-toxin monoclonal antibody for prevention and treatment of Staphylococcus aureus a-hemolysin-mediated cellular injury. Proc Natl Acad Sci U S A 111:3400-3406. 2013. The changing epidemiology of Staphylococcus aureus bloodstream infection: a multinational population-based surveillance study. Clin Microbiol Infect 19:465–471. http://dx.doi.org/10.1111/j.1469-0691.2012.03903.x.
9. Inoshima N, Wang Y, Bubeck Wardenburg J. 2012. Genetic requirement for ADAM10 in severe Staphylococcus aureus skin infection. J Invest Dermatol 132:1513–1516. http://dx.doi.org/10.1038/jid.2011.462 http://dx.doi.org/10.1038/jid.2011.462.
10. Foletti D, Strop P, Shaugnessy L, Hasa-Moreno A, Casas MG, Russell MJ, Bagnoli F, Fontana MR, Soldaini E, Trouillet JL, Zhou Y, Cardona P, Nosari S. 2014. The interplay between the Staphylococcus aureus clumping factor (ClfA) and fibrinogen. Eur J Immunol 43:377–385. http://dx.doi.org/10.1002/eji.201330616.
11. Hua L, Hair PS, Echague CG, Sholl AM, Watkins JA, Foster TJ. 2014. Assessment of an anti-alpha-toxin monoclonal antibody for prevention and treatment of Staphylococcus aureus a-hemolysin-mediated cellular injury. Proc Natl Acad Sci U S A 111:3400-3406. 2013. The changing epidemiology of Staphylococcus aureus bloodstream infection: a multinational population-based surveillance study. Clin Microbiol Infect 19:465–471. http://dx.doi.org/10.1111/j.1469-0691.2012.03903.x.
12. Inoshima N, Wang Y, Bubeck Wardenburg J. 2012. Genetic requirement for ADAM10 in severe Staphylococcus aureus skin infection. J Invest Dermatol 132:1513–1516. http://dx.doi.org/10.1038/jid.2011.462 http://dx.doi.org/10.1038/jid.2011.462.
13. Foletti D, Strop P, Shaugnessy L, Hasa-Moreno A, Casas MG, Russell MJ, Bagnoli F, Fontana MR, Soldaini E, Trouillet JL, Zhou Y, Cardona P, Nosari S. 2014. The interplay between the Staphylococcus aureus clumping factor (ClfA) and fibrinogen. Eur J Immunol 43:377–385. http://dx.doi.org/10.1002/eji.201330616.
14. Hua L, Hair PS, Echague CG, Sholl AM, Watkins JA, Foster TJ. 2014. Assessment of an anti-alpha-toxin monoclonal antibody for prevention and treatment of Staphylococcus aureus a-hemolysin-mediated cellular injury. Proc Natl Acad Sci U S A 111:3400-3406. 2013. The changing epidemiology of Staphylococcus aureus bloodstream infection: a multinational population-based surveillance study. Clin Microbiol Infect 19:465–471. http://dx.doi.org/10.1111/j.1469-0691.2012.03903.x.
15. Inoshima N, Wang Y, Bubeck Wardenburg J. 2012. Genetic requirement for ADAM10 in severe Staphylococcus aureus skin infection. J Invest Dermatol 132:1513–1516. http://dx.doi.org/10.1038/jid.2011.462 http://dx.doi.org/10.1038/jid.2011.462.
16. Foletti D, Strop P, Shaugnessy L, Hasa-Moreno A, Casas MG, Russell MJ, Bagnoli F, Fontana MR, Soldaini E, Trouillet JL, Zhou Y, Cardona P, Nosari S. 2014. The interplay between the Staphylococcus aureus clumping factor (ClfA) and fibrinogen. Eur J Immunol 43:377–385. http://dx.doi.org/10.1002/eji.201330616.
30. Vernachio JH, Bayer AS, Ames B, Bryant D, Prater BD, Syrbybes PJ, Gorovits EL, Patti JM. 2006. Human immunoglobulin G recognizing fibrinogen-binding surface proteins is protective against both Staphylococcus aureus and Staphylococcus epidermidis infections in vivo. Antimicrob Agents Chemother 50:511–518. http://dx.doi.org/10.1128/AAC.50.2.511-518.2006.

31. DeJonge M, Fairbridge D, Bloom B, Duenas M, Walker W, Polak M, Jung E, Millard D, Schelonka R, Eyal F, Morris A, Kapik B, Roberson D, Kesler P, Hetherington S. 2007. Clinical trial of safety and efficacy of INH-A21 for the prevention of nosocomial staphylococcal bloodstream infection in premature infants. J Pediatr 151:260–265, 265.e1. http://dx.doi.org/10.1016/j.jpeds.2007.04.060.

32. Capparelli EV, Bloom BT, Kueser TJ, Oelberg DG, Bifano EM, White RD, Warchol SA, Parentea SV, Patti JM, Hetherington S. 2005. Multicenter study to determine antibody concentrations and assess the safety of administration of INH-A21, a donor-selected human staphylococcal immunoglobulin, in low-birth-weight infants. Antimicrob Agents Chemother 49:4121–4127. http://dx.doi.org/10.1128/AAC.49.10.4121-4127.2005.

33. Bloom B, Schelonka R, Kueser T, Walker W, Jung E, Kaufman D, Kesler K, Robinson D, Patti J, Hetherington S, INH-A21 Phase II Study Team. 2005. Multicenter study to assess safety and efficacy of INH-A21, a donor-selected human staphylococcal immunoglobulin, for prevention of nosocomial infections in very low birth weight infants. Pediatr Infect Dis J 24:858–866. 00006454-200510000-00003.

34. Narita K, Hu DL, Mori F, Wakahayashi K, Iwakura Y, Nakano A. 2010. Role of interleukin-17A in cell-mediated protection against Staphylococcus aureus infection in mice immunized with the fibrinogen-binding domain of clumping factor A. Infect Immun 78:4234–4242. http://dx.doi.org/10.1128/IAI.00447-10.

35. Li X, Wang X, Thompson CD, Park S, Park WB, Lee JC. 2016. Preclinical efficacy of clumping factor A in prevention of Staphylococcus aureus infection. mBio 7(e02232–e02215. http://dx.doi.org/10.1128/mBio.02232-15.

36. Murphy AJ, Macdonald LE, Stevens S, Karow M, Dore AT, Pohursky K, Huxham LT, Thompson CD, Watson M, Sall S, Krueger P, Fairhurst J, Valenzuela DM, Papadopoulos N, Yancopoulos GD. 2014. Mice with megabase humanization of their immunoglobulin genes generate antibodies as efficiently as normally human. Proc Natl Acad Sci U S A 111:5153–5158. http://dx.doi.org/10.1073/pnas.1324022111.

37. Boulianne GL, Hozumi N, Shulman MJ. 1984. Production of functional chimaeric mouse/human antibody. Nature 312:643–646. http://dx.doi.org/10.1038/312643a0.

38. Deivanayagam CC, Wann ER, Chen W, Carson M, Rajashankar KR, Hoök M, Narayana SV. 2002. A novel variant of the immunoglobulin fold in surface adhesins of Staphylococcus aureus: crystal structure of the fibrinogen-binding MS1CRAMM, clumping factor A. EMBO J 21:6660–6672. http://dx.doi.org/10.1093/emboj/cdf619.

39. Rauch S, DeDent AC, Kim HK, Bubeck Wardenburg J, Missiakas DM, Schneewind O. 2012. Abscess formation and alpha-hemolysin induced toxicity in a mouse model of Staphylococcus aureus peritonal infection. Infect Immun 80:3721–3732. http://dx.doi.org/10.1128/IAI.00442-12.

40. Gresham HD, Lowrance JH, Caver TE, Wilson BS, Cheung AL, Lindblade SK, Tkaczyk et al. 2013. At the crossroads of bacterial metabolism and virulence factor synthesis in staphylococci. Microbiol Mol Biol Rev 73:233–248. http://dx.doi.org/10.1128/MMBR.00005-09.

41. Arya R, Princy SA. 2013. An insight into pleiotropic regulators Agr and Sar: molecular probes paving the new way for antiviral therapy. Future Microbiol 8:1339–1353. http://dx.doi.org/10.2112/fmb.13.92.

42. Jenkins A, Diep BA, Mai TT, Vo NH, Warrender P, Suzich J, Stover CK, Sellman BR. 2015. Differential expression and roles of Staphylococcus aureus virulence determinants during colonization and disease. mBio 6:e02272–e02214. http://dx.doi.org/10.1128/mBio.02272-14.

43. Nanja NS, Timofeyeva Y, Buitrago SM, Sellman BR, Dilts DA, Fink P, Nunez I, Hagen M, Matsuka Y, Mininni T, Zhu D, Pavlik V, Green BA, Jansen KU, Andersson AS. 2009. Heterogeneous in vivo expression of clumping factor A and capsular polysaccharide by Staphylococcus aureus: implications for vaccine design. Vaccine 27:3276–3280. http://dx.doi.org/10.1016/j.vaccine.2009.06.002.

44. Date SV, Modrusan Z, Lawrence M, Morisaki JH, Toy K, Shah IM, Kim J, Park S, Xu M, Basuino L, Chan L, Zeitschel D, Chambers HF, Tan MW, Brown EJ, Diep BA, Hazenbos WL. 2014. Global gene expression of methicillin-resistant Staphylococcus aureus USA300 during human and mouse infection. J Infect Dis 209:1542–1550. http://dx.doi.org/10.1093/infdis/jit066.

45. Somervaille N, Whitney AR, Kobayashi SD, Sturdevant DE, Kennedy AD, Braughton KR, Shabb DW, Diep BA, Chambers HF, Otto M, DeLeo FR. 2011. Global changes in Staphylococcus aureus gene expression in human blood. PLoS One 6:e18617. http://dx.doi.org/10.1371/journal.pone.0018617.

46. Lattar SM, Noto Llana M, Denoël P, Germain S, Buzzola FR, Lee JC, Sordelli DO. 2014. Protein antigens increase the protective efficacy of a capsule-based vaccine against Staphylococcus aureus in a rat model of osteomyelitis. Infect Immun 82:83–91. http://dx.doi.org/10.1128/IAI.01050-13.

47. Maira-Litrán T, Bentancor LV, Bozkurt-Guzel C, O’Malley JM, Cywes-Bentley C, Pier GB. 2012. Synthesis and evaluation of a conjugate vaccine composed of Staphylococcus aureus poly-N-acetyl-glucosamine and clumping factor A. PLoS One 7:e3813. http://dx.doi.org/10.1371/journal.pone.003813.

48. Tuchscherr LP, Buzzola FR, Alvarez LP, Lee JC, Sordelli DO. 2008. Antibodies to capsular polysaccharide and clumping factor A prevent mastitis and the emergence of unencapsulated and small-colony variants of Staphylococcus aureus in mice. Infect Immun 76:5738–5744. http://dx.doi.org/10.1128/IAI.00874-08.

49. Kennedy AD, Bubeck Wardenburg J, Gardner DJ, Long D, Whitney AR, Braughton KR, Schneewind O, DeLeo FR. 2010. Targeting of alpha-hemolysin by active or passive immunization decreases severity of USA300 skin infection in a mouse model. J Infect Dis 202:1050–1058. http://dx.doi.org/10.1086/656043.

50. Rouha H, Badarau A, Visram ZG, Battles MB, Prinz B, Magyarcis Z, Nagy G, Mirinka I, Stulik L, Zerbs M, Jägerhofer M, Maierhofer B, Teubenbacher A, Doležilikova I, Gross K, Banerjee S, Zauner G, Malafa S, Zmajkovic J, Maier S, Mabry R, Krauland W, Wittrup KD, Gerngross TU, Nagy E. 2015. Five birds, one stone: neutralization of alpha-hemolysin and 4 bi-component leukocidins of Staphylococcus aureus with a single human monoclonal antibody. Mabs 7:243–254. http://dx.doi.org/10.1080/19420682.2014.985132.

51. Berube BJ, Bubeck Wardenburg J. 2013. Staphylococcus aureus alpha-toxin: nearly a century of intrigue. Toxins (Basel) 5:1140–1166. http://dx.doi.org/10.3390/toxins5061140.

52. Powers ME, Wardenburg JB. 2014. Igniting the fire: Staphylococcus au-
reus virulence factors in the pathogenesis of sepsis. PLoS Pathog 10: e1003871. http://dx.doi.org/10.1371/journal.ppat.1003871.

60. Bhakdi S, Muhly M, Korom S, Hugo F. 1989. Release of interleukin-1beta associated with potent cytoidal action of staphylococcal alpha-toxin on human monocytes. Infect Immun 57:3512–3519.

61. DiGiandomenico A, Keller AE, Gao C, Rainey GJ, Warren P, Camara MM, Bonnell J, Fleming R, Bezabeh B, Dimasi N, Sellman BR, Hilliard J, Guenther CM, Datta V, Zhao W, Gao C, Yu XQ, Suzich JA, Stover CK. 2014. A multifunctional bispecific antibody protects against Pseudomonas aeruginosa. Sci Transl Med 6:262ra155. http://dx.doi.org/10.1126/scitranslmed.3009655.

62. Rauch S, Gough P, Kim HK, Schneewind O, Missiakas D. 2014. Vaccine protection of leukopenic mice against Staphylococcus aureus bloodstream infection. Infect Immun 82:4889–4898. http://dx.doi.org/10.1128/I AI.02328-14.

63. Hawkins J, Kodali S, Matsuka YV, McNeil BK, Mininni T, Scully IL, Vernachio JH, Severina E, Girgenti D, Jansen KA, Anderson AS, Donald RG. 2012. A recombinant clumping factor A-containing vaccine induces functional antibodies to Staphylococcus aureus that are not observed after natural exposure. Clin Vaccine Immunol 19:1641–1650. http://dx.doi.org/10.1128/CVI.00354-12.

64. Fritz SA, Tiemann KM, Hogan PG, Epplin EK, Rodriguez M, Al-Zubeidi DN, Bubeck Wardenburg J, Hunstad DA. 2013. A serologic correlate of protective immunity against community-onset Staphylococcus aureus infection. Clin Infect Dis 56:1554–1561. http://dx.doi.org/10.1093/cid/cit123.

65. Sharma-Kuinkel BK, Wu Y, Tabor DE, Mok H, Sellman BR, Jenkins A, Yu I, Jafari HS, Rude TH, Ruffin F, Schell WA, Park LP, Yan Q, Thaden JT, Messina JA, Fowler VG, Jr, Esse MT. 2015. Characterization of alpha-toxin hla gene variants, alpha-toxin expression levels, and levels of antibody to alpha-toxin in hemodialysis and postsurgical patients with Staphylococcus aureus bacteremia. J Clin Microbiol 53:227–236. http://dx.doi.org/10.1128/JCM.02023-14.

66. Weisman LE, Thackray HM, Steinhorn RH, Walsh WF, Lassiter HA, Dhanireddy R, Brozanski BS, Palmer KG, Trautman MS, Escobedo M, Meissner HC, Sasidharan P, Fretz J, Kokai-Kun JF, Kramer WG, Fischer GW, Mond JJ. 2011. A randomized study of a monoclonal antibody (pagibaximab) to prevent staphylococcal sepsis. Pediatrics 128: 271–279. http://dx.doi.org/10.1542/peds.2010-3081.

67. Rupp ME, Holley HP, Jr, Lutz J, DiCipignaivts PV, Woods CW, Levine DP, Veney N, Fowler VG, Jr. 2007. Phase II, randomized, multicenter, double-blind, placebo-controlled trial of a polyclonal anti-Staphylococcus aureus capsular polysaccharide immune globulin in treatment of Staphylococcus aureus bacteremia. Antimicrob Agents Chemother 51:4249–4254. http://dx.doi.org/10.1128/AAC.00570-07.

68. Mathema B, Mediavilla J, Kreiswirth BN. 2008. Sequence analysis of the variable number tandem repeat in Staphylococcus aureus protein A gene: spa typing. Methods Mol Biol 431:285–305.

69. Kilpatrick KE, Wring SA, Walker DH, Macklin MD, Payne JA, Su JL, Champion BR, Caterson B, McIntyre GD. 1997. Rapid development of affinity matured monoclonal antibodies using RIMMS. Hybridoma 16: 381–389. http://dx.doi.org/10.1089/hyb.1997.16.381.

70. Romero-Steiner S, Libutti D, Pais LB, Dykes J, Anderson P, Whitin JC, Keyserling HL, Carlone GM. 1997. Standardization of an opsonophagocytic assay for the measurement of functional antibody activity against Streptococcus pneumoniae using differentiated HL-60 cells. Clin Diagn Lab Immunol 4:415–422.