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Targeting Alpha Toxin To Mitigate Its Lethal Toxicity in Ferret and Rabbit Models of *Staphylococcus aureus* Necrotizing Pneumonia

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ABSTRACT The role broad-spectrum antibiotics play in the spread of antimicrobial resistance, coupled with their effect on the healthy microbiome, has led to advances in pathogen-specific approaches for the prevention or treatment of serious bacterial infections. One approach in clinical testing is passive immunization with a monoclonal antibody (MAb) targeting alpha toxin for the prevention or treatment of Staphylococcus aureus pneumonia. Passive immunization with the human anti-alpha toxin MAb, MEDI4893*, has been shown to improve disease outcome in murine *S. aureus* pneumonia models. The species specificity of some *S. aureus* toxins necessitates testing anti-*S. aureus* therapeutics in alternate species. We developed a necrotizing pneumonia model in ferrets and utilized an existing rabbit pneumonia model to characterize MEDI4893* protective activity in species other than mice. MEDI4893* prophylaxis reduced disease severity in ferret and rabbit pneumonia models against both community-associated methicillin-resistant *S. aureus* (MRSA) and hospital-associated MRSA strains. In addition, adjunctive treatment of MEDI4893* with either vancomycin or linezolid provided enhanced protection in rabbits relative to the antibiotics alone. These results confirm that MEDI4893 is a promising candidate for immunotherapy against *S. aureus* pneumonia.

KEYWORDS *Staphylococcus aureus*, alpha toxin, antibacterial, antibiotic, hemolysins, monoclonal antibodies

The emergence and spread of multidrug-resistant methicillin-resistant *Staphylococcus aureus* (MRSA) strains within both hospital and community settings continue to make the prevention and treatment of these infections challenging (1). The situation is particularly urgent for community-associated pneumonia because the mortality rate is exceedingly high, ranging from 30 to 70%, despite appropriate antimicrobial therapy (2, 3). Alternative approaches are needed to prevent invasive diseases or to complement antimicrobial therapy to improve clinical outcomes. One such approach is the development of monoclonal antibodies (MAbs) targeting *S. aureus* or its secreted virulence determinants.

Alpha toxin (AT), a pore-forming cytolytic toxin, has been shown to be a key virulence determinant in mouse and rabbit *S. aureus* pneumonia models (4, 5). Consequently, it has been the focus of much research to better understand its role in pneumonia and its utility as a target for novel methods to treat or prevent *S. aureus*

pneumonia (6–10). In fact, two anti-AT MAbs (MEDI4893 and AR-301) are currently in clinical development for the treatment or prevention of *S. aureus* pneumonia (11). MEDI4893*, the precursor to clinical candidate MEDI4893, has demonstrated efficacy in both prophylaxis and therapy against *S. aureus* pneumonia in mice. Also, when administered in adjunctive therapy with antibiotics, MEDI4893* was shown to exhibit synergy over the use of antibiotics alone (7, 8). Although these results in mice are promising, it is unclear how they will translate into humans.

To better understand the role of AT in *S. aureus* pneumonia and protective efficacy of its neutralization beyond mouse models, we developed a ferret *S. aureus* pneumonia model and utilized an existing rabbit necrotizing pneumonia model for preclinical evaluation of MEDI4893*. The data presented here validate the critical role of AT in the pathogenesis of necrotizing pneumonia in ferrets. MEDI4893* prophylaxis increased survival from necrotizing pneumonia in both ferrets and rabbits. Also, when administered in adjunctive therapy with vancomycin or linezolid, two front-line antibiotics, MEDI4893* provided enhanced protection against death in rabbits compared to therapy with the antibiotics alone.

RESULTS

Critical role of alpha toxin in a ferret necrotizing pneumonia model. Although intraperitoneal intoxication with a high dose of purified AT was previously shown to be lethal in neonatal ferrets (12), it is not known whether this toxin plays a role in the pathogenesis of necrotizing pneumonia in this animal species. To establish and validate a ferret necrotizing pneumonia model, we compared the survival of animals challenged by endotracheal instillation with 4.9×10^9 (abbreviated as 4.9e9) CFU of SF8300 wild-type (WT) strain or 5.3e9 CFU of isogenic Δ*hla* mutant strain. Seventeen-week-old ferrets challenged with the SF8300 WT strain all succumbed to infection between 9 and 21 h postinfection (hpi), whereas ferrets challenged with Δ*hla* mutant all survived to 81 hpi ($P < 0.001$ as determined by log-rank test; Fig. 1A). The survival of ferrets infected with the Δ*hla* mutant was associated with bacterial clearance, as evidenced by 10^6-fold fewer CFU in the lungs and kidneys of animals infected with the Δ*hla* strain compared to the WT strain (Fig. 1B and C). The gross appearance of the WT-infected ferret lungs was markedly different from that of the Δ*hla* mutant-infected ferret lungs, with extensive areas of necrosis and hemorrhage (Fig. 1G and J). Histopathology analysis of WT-infected ferret lungs revealed severe inflammation with necrosis and hemorrhage of alveolar parenchyma (Fig. 1H and I), whereas Δ*hla* mutant-infected ferret lungs showed minimal perivascular cuffing by lymphocytes and few neutrophilic infiltrates in alveolar spaces (Fig. 1K and L). Taken together, these data strongly indicate a critical role for AT in the pathogenesis of necrotizing pneumonia in the ferret model.

MEDI4893* prophylaxis protected against lethal pneumonia in ferrets. To determine whether prophylaxis with an anti-AT MAb confers protection in ferrets, animals were randomized for intravenous injection with MEDI4893* (30 mg/kg) or c-IgG (30 mg/kg) 12 h before challenge with SF8300 WT strain. Compared to c-IgG-pretreated ferrets, which had survival rate of only 33% (4/12), all (12/12) of the animals pretreated with MEDI4893* survived the infection ($P < 0.001$ [log-rank test]; Fig. 1D). Mortality was associated with high bacterial titers in the lungs and kidneys, whereas survival was associated with bacterial clearance by 81 hpi (Fig. 1E and F, compare open versus closed symbols). Gross and histopathological analysis showed that MEDI4893* prophylaxis neutralized AT-induced acute lung injury and lung inflammation (Fig. 1M to R).

MEDI4893* prophylaxis against CA-MRSA and HA-MRSA strains in a rabbit model. Since both AT and Panton-Valentine leukocidin (PVL) have been shown to play critical roles in the pathogenesis of CA-MRSA USA300 necrotizing pneumonia in the rabbit model (5, 13), we next evaluated whether targeting AT alone with MEDI4893* would confer protection against the USA300 SF8300. The overall survival rates were 0% (0/12) for rabbits passively immunized with c-IgG (30 mg/kg) compared to 33% (4/12) for those that received MEDI4893* (30 mg/kg) 24 h before infection with SF8300 ($P < 0.001$ [log-rank test]), indicating that targeting of AT alone conferred protection against
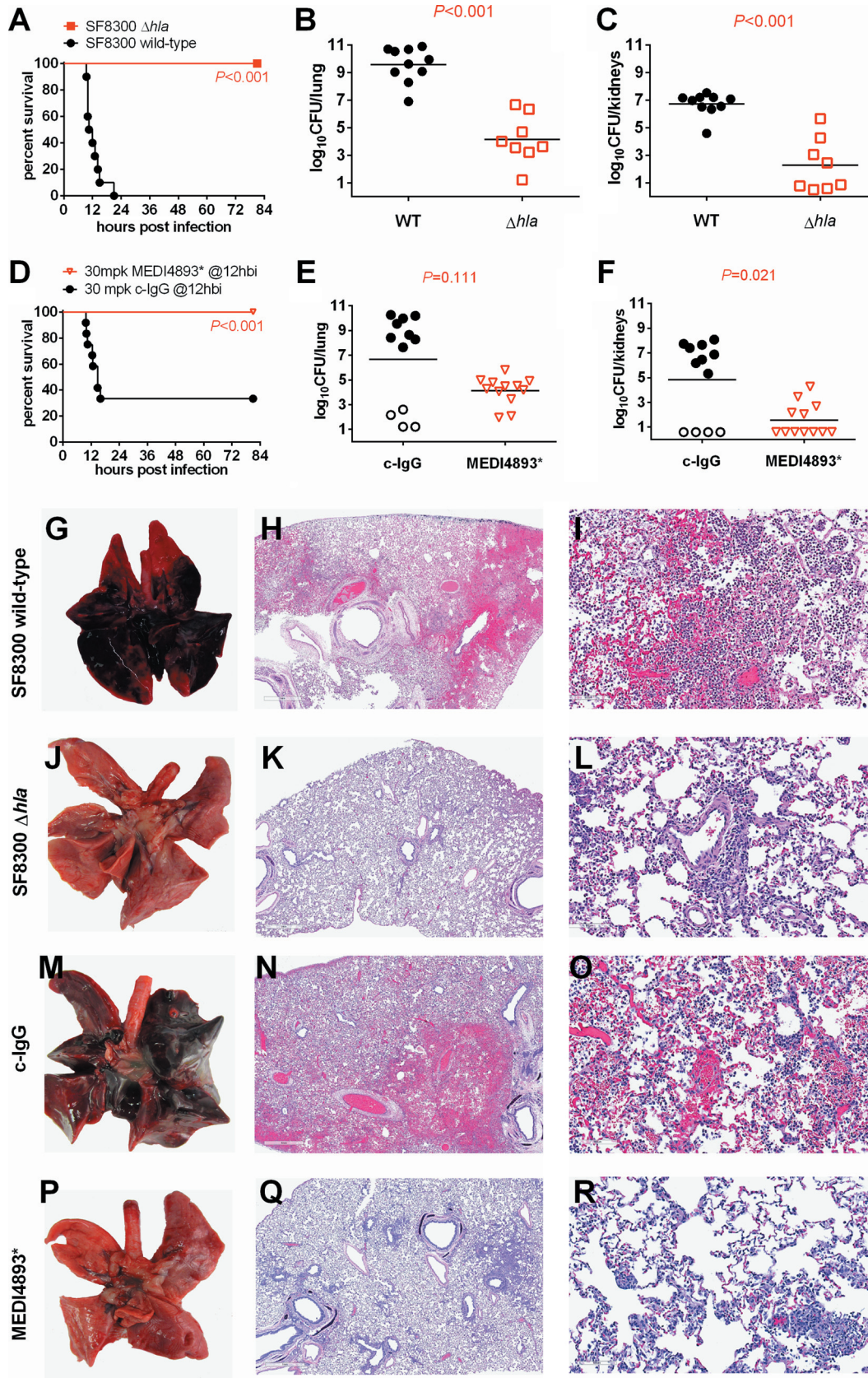
FIG 1 Alpha toxin-mediated pathogenesis and its neutralization by monoclonal antibody in a ferret necrotizing pneumonia model. (A to E) Comparison of Kaplan-Meier survival curves (A and D), $\log_{10}$(CFU/lung) (B and E), and $\log_{10}$(CFU/kidneys) (C and F) for two studies. (A to C) Ferrets challenged with USA300/SF8300 wild-type strain ($n = 10$ animals) and Δhla isogenic mutant

(Continued on next page)

a CA-MRSA USA300 strain that produces both AT and PVL in rabbits (Fig. 2A). The lung weight/body weight (LW/BW) ratio, a quantitative measure of pulmonary edema that forms because of damage to the alveolar endothelial barrier (13), and bacterial counts in lungs, spleen, and kidneys were not statistically different between rabbits pretreated with MEDI4893* and c-IgG (Fig. 2B to E).

We next evaluated protective efficacy of MEDI4893* prophylaxis against NRS382, a HA-MRSA USA100 strain that produces AT but not PVL. Survival rates were 33% (3/9) for rabbits passively immunized with c-IgG (30 mg/kg) compared to 100% (9/9) for rabbits immunized with MEDI4893* (30 mg/kg) at 24 h before infection with NRS382 ($P < 0.001$ [log-rank test]), illustrating the enhanced protective efficacy of MEDI4893* against a non-PVL-producing strain (Fig. 2F). However, the LW/BW ratio and the bacterial counts in lungs, spleen, and kidneys were not statistically different between the two experimental groups (Fig. 2G to J).

Adjunctive therapy of MEDI4893* with vancomycin or linezolid in the rabbit model. MEDI4893* may have clinical benefit in adjunctive therapy with antibiotics for the postexposure treatment of severe *S. aureus* pneumonia. Because MEDI4893* prophylaxis showed partial protection in CA-MRSA USA300 lethal pneumonia compared to HA-MRSA USA100 in rabbits (Fig. 2), we next evaluated MEDI4893* adjunctive therapy with antibiotics against USA300, which may be more difficult to treat not only because of its potential to be multidrug resistant (1) but also because it produces PVL.

We first evaluated of MEDI4893* treatment efficacy either alone or in combination with vancomycin, which is recommended in clinical practice guidelines as a first-line agent for the treatment of MRSA pneumonia (14–16). Rabbits were randomized for treatment 1.5 hpi with (i) 30 mg/kg c-IgG; (ii) 30 mg/kg MEDI4893*; (iii) 30 mg/kg vancomycin twice daily, a dosing regimen that yields peak serum concentration of 36.1 ± 4.2 μg/ml at 1 h after dosing (17); or (iv) a vancomycin-MEDI4893* combination. The overall survival rates were 17% (2/12) for rabbits treated with c-IgG compared to 33% (4/12) for rabbits treated with vancomycin alone ($P = 0.08$ versus c-IgG [one-sided log-rank test]), 67% (8/12) for rabbits treated with MEDI4893* alone ($P = 0.008$ versus c-IgG), and 83% (10/12) for rabbits treated with the vancomycin-MEDI4893* combination ($P < 0.001$ versus c-IgG) (Fig. 3A). Rabbits treated with the vancomycin-MEDI4893* combination showed greater survival than those treated with vancomycin alone ($P = 0.005$). The LW/BW ratios were not significantly different between the various experimental groups (Fig. 3B). Significant reductions in bacterial count in the lungs, spleen, and kidneys were only observed for rabbits treated with the vancomycin-MEDI4893* combination (Fig. 3C to E).

Next, we evaluated linezolid, a protein synthesis inhibitor recommended in clinical practice guidelines as an acceptable alternative to vancomycin for treatment of MRSA pneumonia (14–16). Moreover, linezolid has been shown to be superior to vancomycin for the treatment of necrotizing pneumonia in the rabbit model (17), which is consistent with the improved clinical response seen with linezolid in a clinical trial comparing

FIG 1 Legend (Continued)

($n = 8$ animals); (D to F) ferrets were pretreated with either 30 mg/kg (mpk) MEDI4893* or 30 mpk c-IgG at 12 h before infection with USA300/SF8300 wild-type strain. A one-sided log-rank (Mantel-Cox) test was used to test the hypothesis that the survival of animals challenged with SF8300 wild type is shorter than the survival of animals challenged with Δ*hla* mutant or that the survival of animals pretreated with c-IgG is shorter than survival of those pretreated with MEDI4893*. A nonparametric Mann-Whitney U test was used to evaluate between-group differences in bacterial counts. Filled symbols represent data from dead animals, and open symbols represent data from surviving animals that were euthanized 81 h after infection. (G, J, M, and P) Photographs depict the gross pathology of representative ferret lungs. (H and I) Hematoxylin-eosin (H&E)-stained lungs from ferrets challenged with SF8300 wild type showing areas of alveolar hemorrhage (H, ×2 magnification) and severe inflammation with necrosis and hemorrhage of alveolar parenchyma (I, ×20 magnification). (K and L) H&E-stained lungs from ferrets challenged with SF8300 Δ*hla* mutant infection showing little to no inflammation (K, ×2 magnification) and minimal perivascular cuffing by lymphocytes and few neutrophilic infiltrates in alveolar spaces (L, ×20 magnification). (N and O) H&E-stained lungs from ferrets challenged with SF8300 wild type after pretreatment with c-IgG showing coalescing areas of marked alveolar hemorrhage (N, ×2 magnification) and alveolar spaces containing moderate inflammatory infiltrates and hemorrhage (O, ×20 magnification). (Q and R) H&E-stained lungs from ferrets challenged with SF8300 wild-type strain showing multifocal areas of mild to moderate inflammation (Q, ×2 magnification) and mild to minimal scattered alveolar infiltrates and perivascular cuffing by lymphocytes in alveolar spaces (R, ×20 magnification).
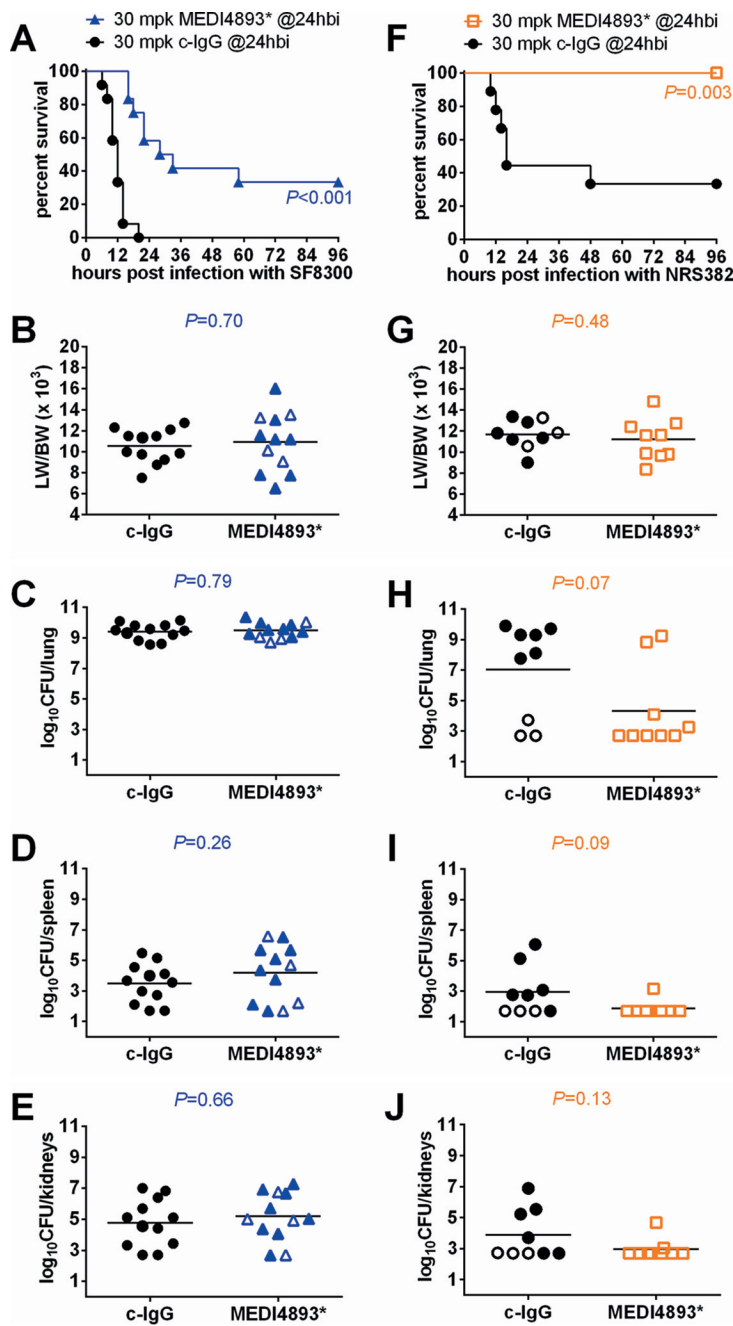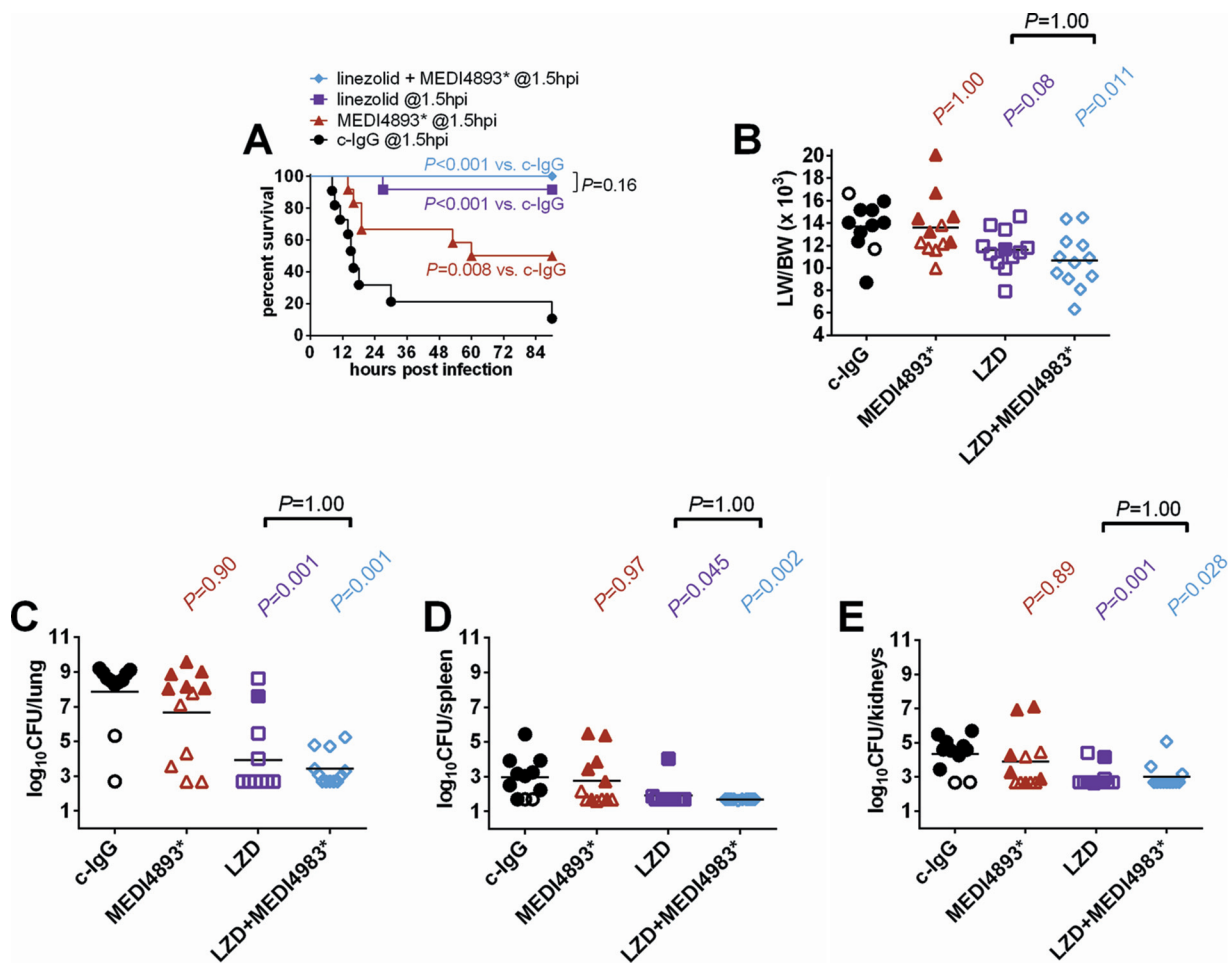
FIG 2 MEDI4893* prophylaxis protected against AT-induced mortality in a rabbit necrotizing pneumonia model. (A to J) Comparison of Kaplan-Meier survival curves (A and F), lung weight to body weight ratios (LW/BW $\times 10^3$) (B and G), $\log_{10}$(CFU/lung) (C and H), $\log_{10}$(CFU/spleen) (D and I), and $\log_{10}$(CFU/kidneys) (E and J) for rabbits ($n = 12$ animals per experimental group) that were randomized to receive either 30 mg/kg (mpk) MEDI4893* or 30 mpk c-IgG at 24 h before infection with USA300/SF8300 wild-type strain (A to E) or with USA100/NRS382 wild-type strain (F to J). A one-sided log-rank (Mantel-Cox) test was used to evaluate the hypothesis that survival of animals pretreated with c-IgG is shorter than survival of animals pretreated with MEDI4893*, with $P < 0.05$ considered statistically significant. Nonparametric Mann-Whitney U test was used to evaluate between-group differences in LW/BW and bacterial counts. Filled symbols represent data from dead animals, and open symbols represent data from surviving animals that were euthanized 96 h after infection.

linezolid with vancomycin for the treatment of MRSA nosocomial pneumonia (18). Here, the protective efficacies of MEDI4893* alone or in combination with linezolid were also evaluated. Rabbits were randomized for treatment with (i) 30 mg/kg c-IgG, (ii) 30 mg/kg MEDI4893*, (iii) 50 mg/kg linezolid three times daily, or (iv) the linezolid-MEDI4893*
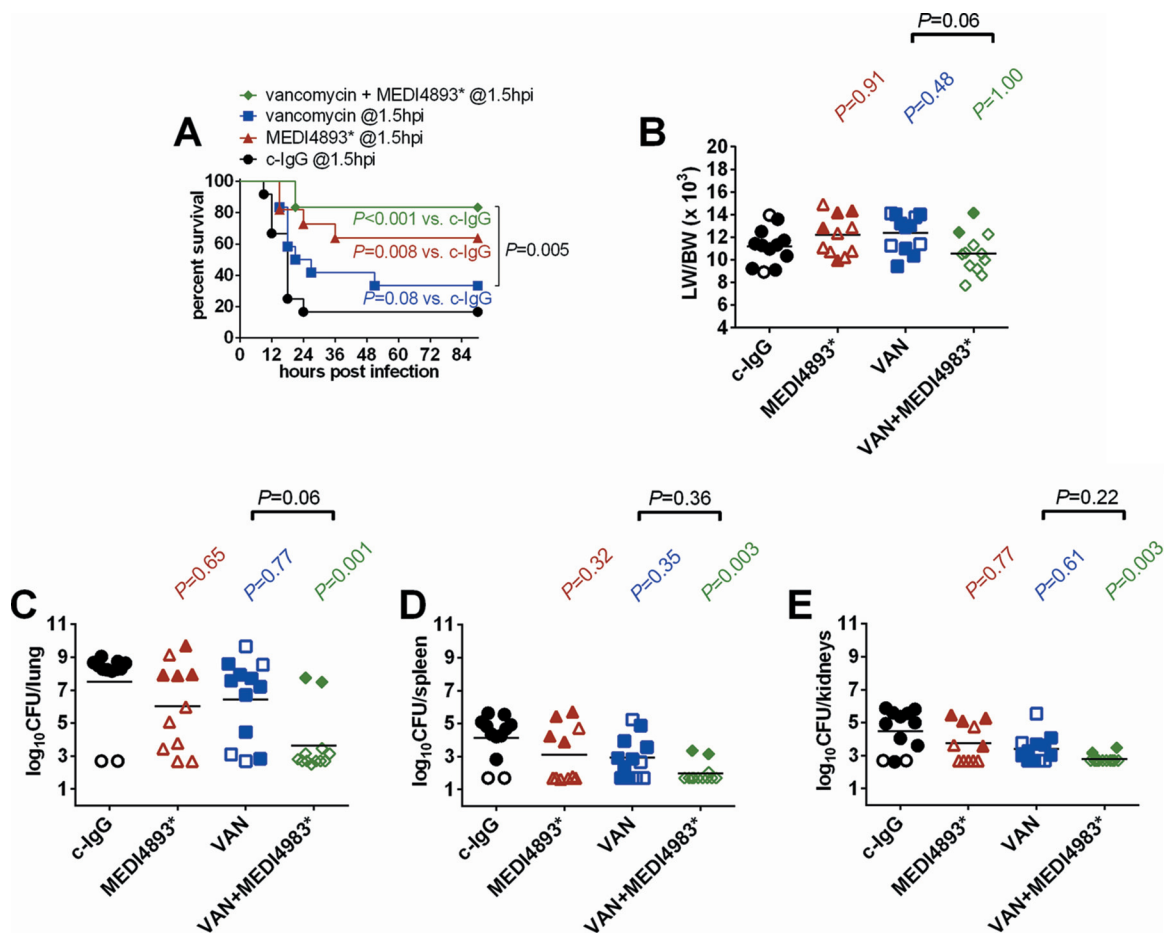
FIG 3 Postexposure treatment with MEDI4893*, either alone or in combination with vancomycin, protected against death in a rabbit necrotizing pneumonia model. (A to E) Comparison of Kaplan-Meier survival curves (A), lung weight to body weight ratio (LW/BW × 10³) (B), log_{10}(CFU/lung) (C), log_{10}(CFU/spleen) (D), and log_{10}(CFU/kidneys) (E) for rabbits ($n = 12$ animals per experimental group) that were randomized to receive (i) 30 mpk c-IgG at 1.5 h postinfection (hpi); (ii) 30 mpk MEDI4893* at 1.5 hpi; (iii) 30 mpk vancomycin at 1.5, 13, 25, and 37 hpi; and (iv) 30 mpk MEDI4893* at 1.5 hpi and 30 mpk vancomycin at 1.5, 13, 25, and 37 hpi with USA300/SF8300 wild-type strain. A one-sided log-rank (Mantel-Cox) test was used to test the hypothesis that the survival of animals treated with c-IgG is shorter than survival of animals treated with MEDI4893*, vancomycin, or a combination of vancomycin+MEDI4893, as well as the hypothesis that the survival of animals treated with vancomycin alone is shorter than for animals treated with the combination of vancomycin+MEDI4893, with $P < 0.0125$ (significance level of 0.05 divided by four different comparisons) being considered statistically significant to account for multiple comparisons using the Bonferroni method. Nonparametric one-way ANOVA with the Kruskal-Wallis test, followed by Dunn's multiple-comparison test, was used to compare between-group differences in LW/BW ratio and bacterial count. Filled symbols represent data from dead animals, and open symbols represent data from surviving animals that were euthanized 96 h after infection.

combination. The linezolid dosing regimen yields a C_{max} of 22.0 ± 3.3 mg/ml after 1 h with a $t_{1/2}$ of 0.78 ± 0.09 h and an $AUC_{0-\infty}$ of 50.9 ± 4.7 μg·h/ml. The overall survival rates were 25% (3/12) for animals treated with c-IgG compared to 50% (6/12) for animals treated with MEDI4893* ($P = 0.008$ versus c-IgG), 92% (11/12) for animals treated with linezolid alone ($P < 0.001$ versus c-IgG), and 100% (12/12) for animals treated with the linezolid-MEDI4893* combination ($P < 0.001$ versus c-IgG) (Fig. 4A). Animals treated with the linezolid-MEDI4893* combination showed greater survival than those treated with linezolid alone ($P = 0.16$), although this small difference was not statistically significant. The LW/BW ratio was significantly reduced for rabbits treated with linezolid alone or with the linezolid-MEDI4893* combination, but not for animals treated with MEDI4893* alone (Fig. 4B). Bacterial counts in the lungs, spleen, and kidneys were significantly reduced for rabbits treated with linezolid alone or the linezolid-MEDI4893* combination but not for rabbits treated with MEDI4893* alone (Fig. 4C, D, and E).

FIG 4 Postexposure treatment with MEDI4893*, either alone or in combination with linezolid, protected against death in a rabbit necrotizing pneumonia model. (A to E) Comparison of Kaplan-Meier survival curves (A), lung weight/body weight ratio (LW/BW × 10^3) (B), $\log_{10}$(CFU/lung) (C), $\log_{10}$(CFU/spleen) (D), and $\log_{10}$(CFU/kidneys) (E) for rabbits ($n = 12$ animals per experimental group) that were randomized to receive (i) 30 mpk c-IgG at 1.5 hpi; (ii) 30 mpk MEDI4893* at 1.5 hpi; (iii) 50 mg/kg linezolid at 1.5, 10, 18, and 26 hpi; and (iv) 30 mpk MEDI4893* at 1.5 hpi and 50 mg/kg linezolid at 1.5, 10, 18, and 26 hpi with USA300/SF8300 wild-type strain. A one-sided log-rank (Mantel-Cox) test was used to test the hypothesis that survival of animals treated with c-IgG is shorter than the survival of animals treated with MEDI4893*, linezolid, or a combination of linezolid+MEDI4893, as well as the hypothesis that survival of animals treated with linezolid alone is shorter than for animals treated with a combination of linezolid+MEDI4893, with $P < 0.0125$ (significance level of 0.05 divided by four different comparisons) being considered statistically significant to account for multiple comparisons using the Bonferroni method. A nonparametric one-way ANOVA with the Kruskal-Wallis test, followed by Dunn's multiple-comparison test, was used to compare between-group differences in LW/BW ratio and bacterial count. Filled symbols represent data from dead animals, and open symbols represent data from surviving animals that were euthanized 96 h after infection.

DISCUSSION

S. aureus is a leading cause of respiratory infections in hospitalized patients. These infections are severe and can be difficult to treat regardless of antibiotic susceptibility. Coupled with an increasing incidence of antibiotic resistance and a greater understanding of the detrimental effects broad-spectrum antibiotic therapy can have on the beneficial microbiome, efforts have begun to explore pathogen-specific methods to prevent or treat serious *S. aureus* infections. One approach currently in clinical development is the prevention or treatment of *S. aureus* pneumonia with a MAb targeting AT, MEDI4893*, and AR-301 (11). AT is a cytolytic pore-forming toxin reported to have a number of effects in *S. aureus* pneumonia models such as cell death, inducing ADAM10-mediated cleavage of epithelial tight cell junctions, stimulating a damaging proinflammatory cytokine response, and altering bacterial processing within alveolar macrophages (19–22). Although a definitive role for AT in human pneumonia has yet to be defined, Stulik et al. recently reported that AT expression levels by colonizing

methicillin-susceptible *S. aureus* is a marker for progression to ventilator associated pneumonia (VAP), implicating alpha toxin in VAP (23).

The multiple activities of AT mentioned above indicate that toxin neutralization may inhibit multiple aspects of *S. aureus* pathogenesis. In fact, AT neutralization with MAbs does effectively prevent pneumonia caused by diverse *S. aureus* isolates in murine *S. aureus* mono- and coinfection models (8–10, 19, 24). Although murine disease models have been used to successfully select drug candidates for human testing, there are instances where they have not been predictive, and there is debate over how well mouse results translate to a human population, particularly when the immune response is involved (25–28).

In the present study, we utilized two different mammalian species to better under-stand the role of AT in *S. aureus* pneumonia and evaluate MEDI4893* protective capacity. Infection with a *S. aureus* mutant defective for AT expression (SF8300 Δ*hla*) rendered *S. aureus* grossly attenuated in ferrets (Fig. 1 and 2), thus supporting results in mice and rabbits indicating that alpha toxin is a key virulence determinant in *S. aureus* pneumonia. Likewise, prophylaxis with the AT-neutralizing MAb, MEDI4893*, improved disease outcome in acute pneumonia models in ferrets and rabbits. MEDI4893* prophylaxis conferred greater protection against lethal pneumonia due to HA-MRSA USA100 compared to CA-MRSA USA300 (Fig. 3) in rabbits, likely because MEDI4893* does not neutralize PVL, which was also shown to play a role in the pathogenesis of USA300 necrotizing pneumonia (5, 13). This hypothesis is supported by a recent publication demonstrating a MAb specific for alpha toxin, PVL, LukED, and gamma hemolysin provided greater protection in USA300 necrotizing pneumonia in rabbits than an anti-AT MAb alone (6). Whether this differential protection translates into differences in clinical efficacy is unknown, particularly given the acute nature of *S. aureus* pneumonia in rabbits. Also, the burden of pneumonia caused by PVL-producing strains (i.e., CA-MRSA USA300, USA400, and USA1100) remains significantly less than those caused by the non-PVL-producing HA-MRSA USA100 strain in the United States (29). Infection caused by PVL-producing strains accounted for an even lower proportion of the total *S. aureus* disease burden elsewhere in the world (30–33). In addition, a recent publication by Rose et al. (34) raises questions about the role of leukocidins in human pneumonia since patients were more likely to die from an infection with an *S. aureus* strain expressing low levels of cytotoxic activity against leukocytes, a measure of leukocidin and not AT activity. It is noteworthy that infection caused by hospital-associated MRSA strain USA200, which does not produce much alpha toxin (35–37) but does produce numerous superantigens (e.g., toxic shock syndrome toxin 1) (38, 39), may not benefit from MEDI4893* treatment or prophylaxis, although the incidence of USA200 has declined dramatically in the United States (29).

Importantly, in the context of postexposure treatment, MEDI4893*, either alone or in combination with vancomycin or linezolid, improved survival outcomes in the rabbits infected with a PVL-expressing isolate (Fig. 3 and 4). "Antitoxin" combina-tion therapy with linezolid and MEDI4893*, which dually targeted both bacterial toxin synthesis and preformed AT, yielded the best survival outcomes (Fig. 4A). Taken together, these results strengthen the hypothesis that alpha toxin plays a key role in *S. aureus* pneumonia and is a valid target for methods to treat or prevent *S. aureus* pneumonia.

In summary, we demonstrated a role for AT in *S. aureus* necrotizing pneumonia in ferrets, and that toxin neutralization with MEDI4893* significantly reduced disease severity in ferrets and rabbits. These data indicate MEDI4893* protective efficacy previously reported in mice translates to these species, as does the adjunctive activity with antibiotics. The interpretation of these data is, however, limited by the fact that the infection in all of these models is acute and rapidly fatal, which differs from a typical human course of infection. This likely results from the high challenge doses (2×10^9 to 5×10^9 CFU) required to overcome the innate immune response to allow for a productive infection. Nonetheless, these data increase confidence regarding a role for AT and its potential as a target to reduce severity of *S. aureus* pneumonia. The true

value of targeting AT will come from clinical trials under way that are testing anti-AT MAb efficacy in the treatment or prevention of *S. aureus* pneumonia.

MATERIALS AND METHODS

Bacterial strains. SF8300 wild-type strain, a minimally passaged clinical strain representative of the epidemic clone USA300-0114, was used to establish necrotizing pneumonia in the ferret and rabbit models for evaluating efficacy of various prophylaxis and treatment modalities. An isogenic mutant of SF8300 containing in-frame deletions of the gene encoding AT (Δ*hla*) was used to validate the role of this toxin in disease pathogenesis in the ferret model. Bacterial strains were prepared for infection as previously described (13).

***In vivo* models.** All animal studies were approved by either the MedImmune (ferret studies) or UCSF (rabbit studies) Institutional Animal Care and Usage Committees and were conducted in an Association for Accreditation and Assessment Laboratory Animal Care (AAALAC)-accredited facilities in compliance with U.S. regulations governing the housing and use of animals.

Ferret model of necrotizing pneumonia. To establish necrotizing pneumonia in ferrets, a 1.0-ml suspension of wild-type strain SF8300 or its isogenic Δ*hla* mutant was delivered endotracheally via a 18-gauge flexible feeding needle into the lungs of anesthetized ferrets (*Mustela putorius*; Simonsen Labs, Gilroy, CA). We used 17-week-old ferrets, weighing 0.8 to 1.2 kg. Ferrets were anesthetized using 4 to 5% isoflurane under 4 liter/min oxygen in an induction chamber. Ferrets were monitored every 2 to 3 hpi for the first 36 h and then three times daily thereafter. Survivors were euthanized at 81 hpi.

Histopathology. Lungs were fixed in 10% neutral buffered formalin (VWR, Radnor, PA) for a minimum of 48 h and then transferred to 100% ethanol. Fixed tissues were then processed according to standard methods as described previously (8) and stained with Gill's hematoxylin (Mercedes Medical, Sarasota, FL) and eosin (Surgipath, Richmond, IL) for histologic evaluation by a pathologist blinded to the experimental conditions.

Rabbit model of necrotizing pneumonia. To establish necrotizing pneumonia in rabbits, a 1.5-ml portion of instillate containing SF8300 wild-type strain was delivered directly into the lungs of anesthetized New Zealand White outbred rabbits via a 2.5-mm pediatric endotracheal tube as previously described (13). We used 8- to 12-week-old rabbits weighing 2.0 to 2.8 kg. Rabbits were monitored every 2 hpi for the first 30 h and then three times daily thereafter. The survivors were euthanized at 96 hpi. Lungs, spleen, and kidneys were removed aseptically from euthanized rabbits or those that were found dead and processed as described above for the ferret organs.

The following therapeutic regimens were administered either for preexposure prophylaxis (24 h before induction of experimental pneumonia) or postexposure treatment (1.5 h after induction of experimental pneumonia): 30 mg/kg c-IgG intravenously, 30 mg/kg MEDI4893* intravenously, 30 mg/kg vancomycin twice daily (BID) intravenously, the combination of 30 mg/kg MEDI4893* plus 30 mg/kg vancomycin BID intravenously, 50 mg/kg linezolid three times per day (TID) subcutaneously, or a combination of 30 mg/kg MEDI4893* intravenously and 50 mg/kg linezolid TID subcutaneously.

Serum concentrations of linezolid. Three rabbits were subcutaneously administered 50 mg/kg linezolid (LGM Pharma, Boca Raton, FL), which was dissolved to 12 mg/ml in a solution of 5% (2-hydroxypropyl)-β-cyclodextrin (Sigma-Aldrich, St. Louis, MO). Blood was drawn for serum preparation at 0, 0.5, 1, 2, 3, 4, 6, and 8 h postdosing. Linezolid serum concentrations were determined as previously described using liquid chromatography-tandem mass spectrometry.

Statistical analyses. Survival curves were generated using the Kaplan-Meier method, and significance was assessed by means of the log-rank (Mantel-Cox) test, with Bonferroni correction for multiple comparisons where appropriate (GraphPad version 6.0). Normal distribution was not assumed, so the LW/BW ratio and the $\log_{10}$ CFU were compared using a nonparametric two-sided Mann-Whitney U test or one-way analysis of variance (ANOVA) with Kruskal-Wallis test, followed by Dunn's multiple-comparison post hoc test where appropriate.

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