Sialidase-Based Anti–Influenza Virus Therapy Protects against Secondary Pneumococcal Infection

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Background. DAS181 (Fludase) is a sialidase fusion protein in clinical development as a broad-spectrum anti–influenza virus (IFV) therapeutic agent. Previous reports by other investigators have raised the concern that desialylation of airway epithelium might increase susceptibility to Streptococcus pneumoniae infection.

Methods. To address whether DAS181 would lead to an increased risk of pneumococcal infection, we tested S. pneumoniae colonization after DAS181 treatment of human A549 cells, healthy mice, and mice challenged with a lethal dose of IFV A/PR/8/34 (H1N1) or A/Victoria/3/75 (H3N2), followed by cfu of S. pneumoniae (D39) on day 3 or day 7. DAS181 treatment was given 24–48 h after IFV challenge.

Results. DAS181 treatment did not increase S. pneumoniae colonization in vitro or in vivo in healthy animals. In IFV-infected mice, DAS181 prevented pneumonia and significantly prolonged survival and inhibited the IFV titer by \( \frac{1}{10^9} \) logs. None of the treated animals showed enhanced S. pneumoniae colonization of the lung. In addition, opportunistic infections with Citrobacter species or Klebsiella species occurred only in mice receiving vehicle, not in animals treated with DAS181.

Conclusions. These data indicate that DAS181 treatment does not exacerbate secondary bacterial infection in mice. DAS181 may reduce the risk of secondary bacterial infection by inhibiting IFV.

Secondary bacterial infection occurring after influenza virus (IFV) infection is a major health concern because of the increased risk of death. Historical records suggest that a large number of influenza-associated deaths occurring during the influenza pandemics of 1918–1919, 1957, and 1968 were the result of secondary bacterial pneumonia and not the initial influenza infection itself [1–4]. Of the bacterial pathogens causing secondary infections, Streptococcus pneumoniae (pneumococcus) is the most common cause of bacterial pneumonia [5]. Although vaccination against S. pneumoniae has shown great promise in preventing pneumonia associated with respiratory virus infection [6, 7], serotypes not covered by the vaccine are on the rise [8], and complications caused by secondary bacterial infections are still a significant cause of morbidity and mortality during seasonal influenza, and they represent an even more serious threat during pandemics.

DAS181 is a 46-kDa recombinant fusion protein consisting of a sialidase functional domain fused with an amphiregulin glycosaminoglycan-binding sequence that anchors the sialidase to the respiratory epithelium [9]. DAS181 is intended for therapeutic and prophylactic treatment of infections caused by IFV and parainfluenza virus (PIV). By cleaving sialic acids (SAs) from the host cell surface, DAS181 inactivates the host cell receptors recognized by both IFV and PIV [10] and thereby potentially renders the host cells resistant to IFV and PIV infection.

Many different virulence factors are thought to contribute to the secondary pneumococcal infection, including viral and/or bacterial sialidase [11–13]. Consequently, one concern regarding the safety of DAS181 is that desialylation of airway surface might increase the risk of pneumococcal infection by unmasking certain cryptic receptors for the bacteria. This concern was mainly derived from experimental observations made by McCullers et al [14, 15], who demonstrated that mice that were infected with IFV and then subsequently
In the present study, we performed experiments to directly assess whether DAS181 would increase pneumococcal colonization in healthy airway epithelium and whether DAS181 treatment would exacerbate secondary pneumococcal infection after influenza. Our data indicate that DAS181 does not increase pneumococcal colonization in human epithelial cells, nor does it increase *S. pneumoniae* colonization in naïve mice. DAS181 treatment of IFV infection did not exacerbate secondary pneumococcal infection in IFV-infected mice. Of interest, DAS181 treatment seems to prevent certain secondary opportunistic bacterial infections in mice.

**MATERIALS AND METHODS**

**Infectious agents and cells.** For viral challenge, mouse-adapted A/PR/8/34 (H1N1) was obtained from American Type Culture Collection (VR-95), and mouse-adapted A/Victoria/3/75 (H3N2) was obtained from Don Smee at Utah State University. Viral stocks were diluted in sterile phosphate-buffered saline (PBS) to 100 pfu and 5000 pfu per mouse for A/PR/8/34 and A/Victoria/3/75, respectively. Madin-Darby canine kidney cells (MDCKs) were used to propagate and to determine virus titer by plaque assay [9]. Type 2 *S. pneumoniae* strain D39 was kindly provided by Jonathan McCullers (St. Jude Children’s Research Hospital, Memphis, Tennessee). Bacteria were propagated in Todd-Hewitt broth with 0.5% yeast extract or on Trypticase soy agar with 5% sheep blood with 5% sheep erythrocyte plates (BD). To prepare bacteria for inoculation, a freshly inoculated culture was grown to an optical density (measured at 600 nm) of 0.6 and diluted in PBS to 10^4 cfu in 50 μL before use. Human lung carcinoma cell line A549 obtained from American Type Culture Collection was grown at 37°C in a humidified atmosphere of 5% carbon dioxide in Dulbecco’s modified Eagle medium nutrient mixture F-12 (DMEMF12) supplemented with 10% fetal bovine serum, 1 × Glutamax (Invitrogen), and 1 × antibiotic/antimycotic solution (Sigma).

**Quantitating the sialic acid level in A549 cells.** Use of the lectin-binding assay for quantitation of the sialic acid level has been described elsewhere [9]. In brief, confluent monolayers of A549 cells were treated with various doses of DAS181. Relative levels of either α(2,6)-linked sialic acid or α(2,3)-linked sialic acid were detected using lectins as follows. The cells were challenged with *S. pneumoniae* 3–7 days later had a higher rate of death due to secondary pneumococcal pneumonia if the IFV associated with primary infection was associated with higher neuraminidase (NA; a sialidase) activity. The authors hypothesized that the increased susceptibility to *S. pneumoniae* was due to desialylation of the airway epithelium by the viral NA activity. The role of sialidase function in pneumococcal infection also remains controversial. On the one hand, the pneumococcal sialidase gene *nanA* has been shown to enhance adhesion and colonization of pneumococci [16–21]. On the other hand, a recent report demonstrated that *nanA* deficiency did not compromise the in vivo fitness of pneumococci [22].

**In the present study, we performed experiments to directly assess whether DAS181 would increase pneumococcal colonization in healthy airway epithelium and whether DAS181 treatment would exacerbate secondary pneumococcal infection after influenza. Our data indicate that DAS181 does not increase pneumococcal colonization in human epithelial cells, nor does it increase *S. pneumoniae* colonization in naïve mice. DAS181 treatment of IFV infection did not exacerbate secondary pneumococcal infection in IFV-infected mice. Of interest, DAS181 treatment seems to prevent certain secondary opportunistic bacterial infections in mice.**

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Figure 2. *Streptococcus pneumoniae* colonization in vivo. Normal healthy mice were treated with phosphate-buffered saline (PBS) (A) or DAS181 (1 mg/kg) (B) intranasally for 3 days before intranasal inoculation with $10^4$ cfu of *S. pneumoniae*. At 0, 24, 48, 72, 96, and 168 h after challenge, 6 mice from each group were euthanized, and their lungs were harvested to determine bacterial counts. Statistical analysis was performed using Student’s t test.

**In vitro bacterial adhesion assays.** These assays were performed as described elsewhere [23]. In brief, confluent monolayers of A549 cells (~5 x 10⁵ cells/well) were incubated with streptavidin–horseradish peroxidase without biotinylated lectin. Absorbance (Abs) was measured at 450 nm, and the percentage of sialic acid that remained was calculated using 100%/(Abs of DAS181-treated cells/Abs of untreated cells).

**Animal infection and survival.** Animal studies were conducted according to the protocol approved by the NexBio animal care and use committee. Ten-week-old BALB/c female mice (Charles River) were used for the in vivo infection studies. The mice were assigned to groups on the basis of body weights (18–20 g), to achieve comparable mean group body weights. During the study, animals were observed daily, and clinical observations and body weights were recorded every 2 days. Before intranasal delivery of infectious agent or test article, the animals were anesthetized with 100 mg/kg ketamine. Subsequently, the animals were held in an upright position while a total volume of 50 μL was delivered by intranasal injection performed using a syringe with a 22G blunt-tipped needle. For *S. pneumoniae* colonization studies, normal healthy mice were treated with PBS or DAS181 (1 mg/kg) intranasally for 3 days before intranasal inoculation with $10^4$ cfu of *S. pneumoniae*. At 0, 24, 48, 72, 96, and 168 h after challenge, mice were euthanized and the lungs were harvested for determination of bacterial counts. For coinfection studies, mice were infected with 100 pfu of mouse-adapted A/PR/8/34 or 5000 pfu of mouse-adapted A/Victoria/3/75, and they received intranasal treatment at 24 or 48 h after infection (once daily for 3 days) with 1 mg/kg DAS181. *S. pneumoniae* challenge was performed intranasally on day 3 or day 7, respectively.

**Lung homogenization.** After dissection, each lung/trachea was weighed and placed in 2 mL of DMEMF12 medium (HyClone Labs) and placed on ice. Homogenization of tissues was performed using a handheld homogenizer (Tissue Tearor; Biospec Product) on ice (at speed 15 for ~20 s). Before each homogenization, the small probe tip was rinsed in 5 successive PBS washes in 50-mL conicals, sterilized once in 70% ethanol, and rinsed one final time in PBS. Tissue homogenates were centrifuged at 4°C in a microfuge for 10 min at 2000 g. Supernatants from these cleared homogenates were stored at −80°C until further analysis of viral load by quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was performed.

**Viral copy number analysis.** The cleared homogenate was used for quantitation of viral load by qRT-PCR assay. Viral RNA was purified from 50 μL of cleared homogenate sample by use of the MagMAX-96 Viral RNA isolation kit (Applied
Figure 3. Bacterial counts and viral copy numbers of mice coinfected with influenza virus (IFV) and Streptococcus pneumoniae. Mice (n = 3–6 per group) were infected with 100 pfu of mouse-adapted A/PR/8/34 or 5000 pfu of mouse-adapted A/Victoria/3/75; 24 h later, they received intranasal treatment (once daily for 3 days) with 1 mg/kg DAS181. S. pneumoniae challenge was performed intranasally on day 3. At 24 h after bacterial challenge, lungs were harvested from 3 mice per group and homogenized for analysis of viral copy numbers (A) and bacteria counts (B). Statistical analysis was performed using Student’s t test. * P < .05; ** P < .01. PBS, phosphate-buffered saline.

Biosystem). The RNA was purified in accordance with the manufacturer’s instructions and eluted in 50 μL of elution buffer. Complementary DNA (cDNA) was synthesized with 10 μL of the purified viral RNA by use of the cDNA Synthesis kit (Applied Biosystems). For the standard curve, 10-fold serial dilutions of the A/PR/8/34 or A/Victoria/3/75 M-gene were performed starting from 10⁷ to 10⁴ copies per quantitative reaction. A no-template control was included to control for cross-contamination. The qRT-PCR reaction was performed with 4 μL of cDNA, standard-curve DNA, or water for the no template control. 2X Fast Universal Master Mix (Applied Biosystems), 900 nm of forward and reverse primers, and 225 nm of Taqman probe were added to the templates. PCR reactions were run under the conditions of 1 cycle at 95°C for 20 s and then 45 cycles at 95°C for 3 s and at 60°C for 30 s, on an ABI 7500 Fast Real-Time PCR System. Data were collected at 60°C for 30 s. The cycle threshold was determined automatically, and the cycle threshold values were plotted to form a standard curve by use of the Applied Biosystem software. Typically, amplification with good linearity was observed in 10⁷ to 10⁴ copies. Copy numbers for the samples were interpolated with a fresh standard curve in each run.

Viable bacterial counts and microbiological characterization. Whole lungs were aseptically harvested and placed in 2 mL of sterile PBS on ice. The lungs were homogenized on ice with a handheld homogenizer (Biospec Products). Viable plate count assay was performed by plating 0.5 mL of undiluted lung homogenate and 0.1 mL of serial 10-fold dilutions of each sample in sterile PBS onto tryptic soy agar with 5% sheep erythrocytes (BD). The plates were grown overnight, and the colony-forming units were counted and adjusted for dilution to determine the number of bacteria in each sample. Colonies with a green zone of α-hemolysis were denoted as S. pneumoniae and included in viable plate counts. All colonies with other morphologic findings were also noted and sent to IDEXX Laboratories for identification.

Histologic analysis and immunostaining. Lungs for histologic evaluation were perfused with PBS and fixed in 10% buffered formalin for 24 h. Paraffin embedding and preparation of hematoxylin-eosin–stained slides were performed at Pacific Pathology. Sections were stained with hematoxylin-eosin for histologic review.

Statistical analysis. Statistical analysis of survival data was performed using the Kaplan-Meier log-rank test. Any mice that
Survival and body weight change in mice coinfected with influenza virus (IFV) and Streptococcus pneumoniae. MICE (n = 6–7 per group) were infected with 100 pfu of mouse-adapted A/PR/8/34 or 5000 pfu of mouse-adapted A/Victoria/3/75; 24 h later, they received intranasal treatment (once daily for 3 days) with 1 mg/kg DAS181. S. pneumoniae challenge was performed intranasally on day 3. A, Survival was monitored daily, and Prism software (version 4.2; GraphPad) was used to assess statistical differences in survival curves by the Kaplan-Meier log-rank test. B, Body weights were recorded every 2 days. Differences in body weights changes were compared by 2-way analysis of variance. PBS, phosphate-buffered saline.

**RESULTS**

Desialylation of human epithelial cells in vitro. We previously reported that DAS181 treatment did not increase colonization of well-differentiated human airway epithelium by 3 different strains of S. pneumoniae [24]. In the present study, we further tested pneumococcal colonization of DAS181-treated A549 cells, which are derived from human type II pneumocytes and contain high levels of both α(2,6)-linked and α(2,3)-linked SAs. DAS181 dose-response curves show that, at 1 U/mL DAS181, desialylation of A549 cells approached a plateau after 30 min, with ~90% of the α(2,6)-linked SA and 70% of the α(2,3)-linked SA removed based on SNA and MAL-II lectin binding (Figure 1A). To test the effect of desialylation on S. pneumoniae cell adhesion, confluent layers of A549 cells were treated with DAS181 (1 U/mL) for 30 min before different amounts of S. pneumoniae were added to the cells. The level of adherent bacteria after washing was determined by viable count analysis. As shown in Figure 1B, DAS181 treatment did not increase adhesion of S. pneumoniae to A549 cells overall. Similar to adhesion by S. pneumoniae, adhesion by Haemophilus influenzae and Pseudomonas aeruginosa to A549 cells over a broad range of bacterial input was not augmented by DAS181 treatment (data not shown).

Colonization of S. pneumoniae after DAS181 treatment in vivo. To evaluate whether DAS181 would predispose healthy animals to pneumococcal colonization, healthy mice were treated intranasally with DAS181 or PBS for 3 days and subsequently were infected intranasally with cfu of S. pneumoniae/mouse. This infectious dose of S. pneumoniae was chosen because it was the highest dose used by previous investigators (100–10,000 cfu/mouse) [23, 25, 26]. After DAS181 treatment, we harvested mouse lungs at various time points and determined bacterial counts in the lung homogenates. We found essentially identical bacterial counts in the lungs of the healthy mice treated with either PBS or DAS181 during the 96 h after infection, with peak counts observed immediately after infection approximating the total counts inoculated (Figure 2). At 7 days after infection, 4 of 6 mice in the DAS181-treated groups had completely cleared the bacteria, whereas only 2 of 6 animals survived the observation period were assigned a survival value of 21 days. Data on the mean day to death were found to follow normal distribution, according to the D’Agostino & Pearson omnibus normality test; hence, they were analyzed using a parametric test (ie, Student’s t test). Changes in body weight data were analyzed using 2-way analysis of variance. All statistical tests were performed using Prism software (version 4.02 for Windows; GraphPad).
Figure 5. Histopathologic analysis of lungs from infected mice infected with influenza virus and Streptococcus pneumoniae. Lungs from infected control mice (A) and from mice infected with either A/PR/8/34 or A/Victoria/3/75, with or without 10^7 cfu of S. pneumoniae (B), were harvested at 24 h after bacterial challenge, processed for paraffin sections, and stained with hematoxylin-eosin for histologic review. The photomicrographs shown here are representative of 3 of 3 mice from each respective group. Original magnification, ×100. PBS, phosphate-buffered saline.

in the PBS group had cleared the bacteria from their lungs. In all of the animals, pneumococcal inoculation only caused slightly ruffled coats and mild lethargy and no mortality.

**DAS181 treatment in mice with secondary pneumococcal infection.** To evaluate whether DAS181 treatment of IFV would predispose animals to secondary bacterial infection, mice were infected intranasally with 2 mouse-adapted IFV strains at lethal doses: A/PR/8/34 (H1N1) at 100 pfu/mouse or A/Victoria/3/75 (H3N2) at 5000 pfu/mouse. These lethal infectious doses of the respective virus were determined in preliminary studies (data not shown). After viral infection, mice were treated with DAS181 (1 mg/kg) once daily for 3 days, with the first dose given 24 h after viral infection. The intranasal *S. pneumoniae* challenge (10^7 cfu/mouse) was administered 6 h after the final DAS181 treatment dose was given on day 3. The timing of the pneumococcal inoculation was selected to coincide with the timing of the peak lung viral titer in the untreated animals.

Of interest, without DAS181 treatment, the pneumococci grew to higher titers in the IFV-infected animals than in the healthy animals, in spite of identical inoculation dose (compare Figure 3B with Figure 2). Animals coinfected with A/Victoria/3/75 and *S. pneumoniae* also had a shorter duration of survival than did animals infected with A/Victoria/3/75 alone (Figure 4A). These observations demonstrate that a previous episode of IFV infection predisposes animals to an exacerbated bacterial infection. DAS181 significantly improved survival and prevented body weight loss and clinical signs in the animals coinfected with IFV and pneumococcus (*P* < .001, for A/PR/8/34-infected mice; *P* < .01, for A/Victoria/3/75-infected mice) (Figure 4). The DAS181-treated animals had a significantly extended mean day to death (± standard deviation [SD]), which was 21 ±0 days (for A/PR/8/34) and 18.6 ± 6.4 days (for A/Victoria/3/75), compared with that of their respective PBS-treated IFV-infected control groups, which was 8.8 ± 0.7 days and 7.2 ± 0.7 days, respectively. As expected, DAS181 treatment inhibited the IFV titer by ∼3 logs in all of the treated animals (Figure 3A). Of importance, DAS181 treatment resulted in significantly lower bacterial counts in the lungs of all animals infected with either A/PR/8/34 or A/Victoria/3/75 (*P* < .01) (Figure 3B).

Lung histologic findings further confirmed that DAS181 treatment prevented secondary pneumonia (Figure 5). The lungs harvested from DAS181-treated animals coinfected with IFV and *S. pneumoniae* showed near normal histologic findings; however, in control animals infected with either A/PR/8/34 or A/Victoria/3/75 virus alone or coinfected with bacteria, obvious signs of pneumonia with multifocal infection were observed throughout the bronchiolar tree and lung parenchyma, and displaced alveolar structures, hemorrhage, and infiltration of inflammatory cells were also noted. Consistent with H3N2 being more virulent than H1N1 strains, lungs from the A/Victoria/3/75-infected mice appeared to have more severe pathologic changes, compared with animals infected with A/PR/8/34.

**Effect of delayed DAS181 treatment and bacterial challenge.** In a previous study, the time of bacterial challenge subsequent to IFV infection was found to affect the rate of mortality in the mouse coinfection model, with the time point of bacterial
Figure 6. Survival, body weight change, viral copy numbers, and bacterial counts of mice infected with influenza virus (IFV) and challenged with *Streptococcus pneumoniae* on day 7. Mice (*n* = 6–7 per group) were infected with 100 pfu of mouse-adapted A/PR/8/34 or 5000 pfu of mouse-adapted A/Victoria/3/75; 48 h later, they received intranasal treatment (once daily for 5 days) with 1 mg/kg DAS181. *S. pneumoniae* challenge was performed on day 7. At 24 h after bacterial challenge, lungs were harvested from 3 mice per group and homogenized for analysis of bacteria counts and viral copy numbers. Prism software (version 4.2; GraphPad) was used to assess statistical differences in survival curves (A) by the Kaplan-Meier log-rank test. B, Body weights were recorded every 2 days, and differences in body weight loss were analyzed by 2-way analysis of variance. Analyses of viral copy numbers (C) and bacterial counts (D) were performed using Student’s *t* test. E, Endogenous bacterial colonization was determined by counting the colonies not denoted as *S. pneumoniae*. These colonies were subsequently isolated and sent to IDEXX Laboratories for identification. Titers of *Citrobacter* species are shown for the H1N1-infected animals, and titers of *Klebsiella* species are shown for H3N2-infected animals. PBS, phosphate-buffered saline.
challenge associated with the greatest mortality rate combined with the most rapid death at 7 days after viral challenge [23]. Hence, in the next study, we selected this time frame to evaluate the effect of DAS181 on secondary bacterial infection. Mice were infected with either A/PR/8/34 or A/Victoria/3/75 on day 0. DAS181 treatment was initiated 48 h after infection and was continued for 5 days until day 6. On day 7, we inoculated mice with S. pneumoniae and monitored the animals until day 21. All groups receiving DAS181 treatment showed increased survival and improved clinical signs, compared with the PBS-treated control mice (Figure 6A and 6B). In animals coinfected with bacteria and IFV A/PR/8/34 or A/Victoria/3/75, DAS181 significantly extended the mean day to death (± SD) to 21 ± 0 days and 17 ± 6.8 days, respectively; this finding is in contrast to the mean day to death (± SD) for the respective PBS-treated control groups: 7.6 ± 0.5 days and 8.5 ± 3 days (P < .001 and P < .05, respectively). As expected, DAS181 treatment reduced viral titer by >4 logs in all groups (Figure 6C). In addition, DAS181 treatment did not enhance S. pneumoniae colonization and growth in all animals (Figure 6D).

Of interest, we also found that untreated animals were infected with other bacteria in addition to pneumococci. On day 8, after analyzing the lungs of the IFV-infected mice with or without the pneumococcal challenge, we found that all of the mice in the PBS group were also infected with Citrobacter species (H1N1-infected animals) or Klebsiella species (H3N2-infected animals) (Figure 6E), which was not observed at earlier time points after IFV infection. These bacteria are known opportunistic pathogens in mice and humans [27, 28]. Strikingly, none of the DAS181-treated animals exhibited any secondary opportunistic bacterial infection. This observation suggests that DAS181 may prevent secondary infection by opportunistic bacterial pathogens.

**DISCUSSION**

Our studies differ from previous research on the synergy between IFV and pneumococci in that we used a higher infectious dose of IFV than what was previously used [14, 15, 23, 29]. However, we observed a similar synergy between IFV and pneumococci, as reported elsewhere [14, 15, 23, 29]. Specifically, S. pneumoniae given to mice infected with IFV resulted in an increased bacterial lung burden and increased mortality. Although previous reports attributed the increased severity of secondary bacterial infection to desialylation by IFV NA [14, 15, 23, 29], our results contradict this hypothesis and indicate that desialylation per se does not increase susceptibility to secondary bacterial infection. Quite the contrary, desialylation by DAS181 seemed to protect animals by inhibiting IFV infection and subsequently reducing bacterial colonization. Because whole IFV virions, rather than a purified NA protein (a sialidase), were used in the previous reports [14, 15, 23, 30], the previous experimental system cannot distinguish the effects of IFV virions from those of the NA function alone. An IFV strain with higher NA activity, such as that used in the previous reports, is likely more virulent and thus causes more severe primary viral infection, which may lead to more severe secondary bacterial infection. To our knowledge, before this report, there had not been any publication that directly addressed the effect of a purified sialidase on secondary bacterial infection.

A positive correlation between viral virulence and increased severity of secondary bacterial infection has been well known [31–33]. IFV infection causes cellular and tissue damages, cytokine changes, and impaired immune function that all contribute to increased susceptibility to S. pneumoniae [11, 26, 34–37]. Extensive airway epithelium denudation happens during severe influenza [38, 39]. It was reported that S. pneumoniae only adheres to the denuded basal cells and basement membrane but not to the intact tracheal epithelium [39]. By inhibiting IFV infection as the primary cause of the epithelial damage, DAS181 treatment may protect the airway epithelium from inflammation and denudation, which may be the mechanism for the prevention of secondary bacterial infection.

Consistent with the lack of effects of DAS181 on bacterial colonization in healthy airway epithelium, we and other investigators have observed that the subterminal polysaccharide structures exposed after sialidase treatment—that is, Galβ1-4GlcNAc and Galβ1-3GalNAc—are abundantly present on the native human airway epithelium and normal lung tissue unexposed to a sialidase [24, 40]. Thus, sialidase treatment does not seem to introduce novel polysaccharide structures on the human airway surface.

In summary, results reported in the present study indicate that DAS181 treatment does not increase S. pneumoniae colonization in either human lung epithelial cells or the lungs of healthy mice. In mice coinfected with IFV and S. pneumoniae, DAS181 treatment inhibited IFV replication as well as secondary pneumococcal infection. However, it is noted that the mouse influenza model in this study rapidly progressed to pneumonia, which does not exactly mimic the common path of disease progression in humans, in whom influenza is often associated with tracheobronchitis rather than pneumonia. Ultimately, the effect of DAS181 on secondary bacterial infection in human will be elucidated by clinical trials.

**References**

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