Treatment with Protein Synthesis Inhibitors Improves Outcomes of Secondary Bacterial Pneumonia after Influenza

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Pneumonia occurring as a secondary infection after influenza is a major cause of excess morbidity and mortality, despite the availability and use of antibiotics active against Streptococcus pneumoniae. We hypothesized that the use of a bacteriostatic protein synthesis inhibitor would improve outcomes by reducing the inflammatory response. BALB/cJ mice infected with influenza virus and superinfected with S. pneumoniae were treated with either the cell-wall–active antibiotic ampicillin or the protein synthesis inhibitor clindamycin or azithromycin. In the model, ampicillin therapy performed significantly worse (survival rate, 56%) than (1) clindamycin therapy used either alone (82%) or in combination with ampicillin (80%) and (2) azithromycin (92%). Improved survival appeared to be mediated by decreased inflammation manifested as lower levels of inflammatory cells and proinflammatory cytokines in the lungs and by observation of less-severe histopathologic findings. These data suggest that β-lactam therapy may not be optimal as a first-line treatment for community-acquired pneumonia when it follows influenza.

Over the past decade, influenza and pneumonia have ranked as the seventh leading cause of death in the United States for all persons and as the fifth leading cause of death for children [1]. Two bacteria are most commonly associated with influenza virus: Streptococcus pneumoniae and Staphylococcus aureus. The pneumococcus primarily affects young children, in whom it causes otitis media, sinusitis, and pneumonia, and elderly individuals, in whom it causes pneumonia [2]. During annual epidemics, pneumococcal disease that occurs after influenza is a particularly common cause of death among elderly persons with comorbidities, such as lung or heart disease. A bacterial etiology was found more commonly during the influenza pandemics of 1918, 1957, and 1968 than during seasonal epidemics, with as many as 50%–95% of patients developing fatal or life-threatening pneumonia [3–10]. Thus, it can be predicted that the highly pathogenic influenza virus strains that are most likely to cause the next pandemic or to be used as agents of bioterrorism would achieve much of their influence through secondary bacterial infections.

Secondary bacterial pneumonia occurring as a complication of influenza has historically been viewed by clinicians as being more difficult to treat than primary pneumonia [7, 8, 11–13]. Several reasons for this difficulty have been inferred from case series. In many instances, the infection may occur while the host is still dealing with the viral infection itself or its aftermath, and the additive effects of the 2 diseases may predispose to a worse outcome [5, 7]. It has also been suggested that the occurrence of bacterial pneumonia after influenza is more likely to involve complex characteristics, such as pleural effusions and bacteremia, and to present at a more advanced stage of disease, with multiple lobes of the lung involved [4, 11]. Host factors are undoubtedly also involved, because frail elderly individuals, the population usually afflicted with influenza and pneumococcal pneumonia during seasonal influenza epidemics,
have little reserve and would be expected to more easily develop and succumb to severe infections.

We have developed relevant animal models of secondary bacterial pneumonia to facilitate a study of the mechanisms that underlie this synergistic interaction [3, 14, 15]. In mice, preinfection with influenza virus increases the incidence of pneumococcal pneumonia, promotes bacteremia and systemic spread, hastens the tempo of the progression of pneumonia, and predisposes to multilobar disease [4, 14, 16]. In addition, viral titers from the lungs are enhanced, and the character of the pneumonia is altered, featuring more airway necrosis, fibrin deposition, and inflammatory changes [14, 17, 18]. To date, 2 specific mechanisms have been implicated by these studies: improved adherence through the sialidase activity of the viral neuraminidase [4, 17, 19, 20] and enhanced inflammation mediated by expression of the newly discovered viral accessory protein PB1-F2 [18]. This latter mechanism is of particular interest, because it may help to explain the striking pathogenicity of the 1918 “Spanish flu” pandemic strain. A laboratory virus engineered to express the PB1-F2 protein from the 1918 strain A/Brevig Mission/1/18 (H1N1) was more virulent in mice than the wild-type parent, more strongly stimulated an inflammatory response in the lung, and more efficiently supported secondary bacterial pneumonia [18].

Since Austrian and Gold [21] first demonstrated the efficacy of penicillin in the treatment of adults with pneumococcal pneumonia >40 years ago, penicillin or other β-lactam agents have been considered to be the treatment of choice for most patients with pneumococcal pneumonia. The general goal of treatment has been to eliminate pathogens as rapidly as possible, so bactericidal agents have been preferred. The Infectious Diseases Society of America (IDSA) and the American Thoracic Society (ATS) specifically recommend β-lactam agents as the first line of therapy for bacterial pneumonia occurring after influenza [22]. Because *Mycoplasma* and *Chlamydia* organisms are unlikely to be copathogens with influenza, macrolides generally are not recommended for this situation. For patients suspected to have infections with highly pathogenic H5N1 strains, the IDSA/ATS guidelines suggest the use of both antiviral therapy and antimicrobials targeting *S. pneumoniae* and *S. aureus*. In adults in whom influenza is not suspected, macrolides, fluoroquinolones, or a combination of a β-lactam and a macrolide can be used for outpatient therapy; however, for inpatients, a fluoroquinolone or combination β-lactam/macrolide therapy is recommended. The more ill the patient, the greater the emphasis on the β-lactam component of therapy to target *S. pneumoniae* [22]. In children, however, macrolides and fluoroquinolones are not commonly used, so β-lactams are the mainstay of therapy unless *Mycoplasma* infection is documented or strongly suspected [23].

In the present report, we examine the question of whether β-lactams are the most appropriate therapy for secondary bacterial pneumonia when it occurs after influenza. In a previous study of pneumococcal coinfection with influenza virus, treatment with ampicillin cleared bacteria from the lungs but did not improve mortality [4]. The use of antiviral therapy to remove the contribution of the virus to the interaction was necessary to effect a cure with a β-lactam agent. We hypothesize that the robust inflammatory response that occurs during severe influenza virus infections and is magnified by subsequent bacterial superinfections [16] is further exacerbated by β-lactam–mediated lysis of bacteria. If this hypothesis is correct, the use of alternate antimicrobials with different mechanisms of action may treat the infection without producing adverse effects. We present data suggesting that the treatment of secondary pneumococcal pneumonia with protein synthesis inhibitor antibiotics, either alone or in combination with a β-lactam, may result in better outcomes by lessening the inflammatory response engendered by lysis of the bacteria.

**MATERIALS AND METHODS**

**Infectious agents.** The St. Jude strain of mouse-adapted influenza virus A/Puerto Rico/8/34 (H1N1; PR8), generated by reverse genetics [18], was grown in Madin-Darby canine kidney (MDCK) cells. *S. pneumoniae* strain A66.1 (type 3) was transformed with the lux operon (Xenogen) [17]. The MICs for this strain were 0.023 μg/mL for ampicillin, 0.047 for clindamycin, and 0.064 for azithromycin.

**Measurement of tumor necrosis factor (TNF)–α levels expressed by macrophages.** After growth to an OD 

\[ \text{OD}_{400} \] of 0.6 in Todd–Hewitt broth, pneumococci were exposed to ampicillin or clindamycin for 2 h at 37°C. The resulting cultures were centrifuged for 5 min at 10,000 g, and 100 μL of supernatant was added to wells containing 100 μL of infection media (Dulbecco’s modified Eagle medium supplemented with 3% bovine serum albumin) overlaying 95% confluent J774 macrophages. Supernatants from exposed macrophages were assayed for TNF-α production by use of the BD optEIA TNF ELISA Kit (BD Biosciences) according to the manufacturer’s instructions.

**Mice.** In these studies, 7- to 8-week-old female BALB/cJ mice were used in biosafety level 2 facilities in a manner in accordance with the guidelines of the Animal Care and Use Committee at St. Jude Children’s Research Hospital. All experimental procedures were performed with the mice under general anesthesia, with the use of inhaled isoflurane 2.5% (Baxter Healthcare).

**Infectious model.** Anesthetized mice received intranasal administration of Infectious agents in a volume of 100 μL (50 μL per nostril). Influenza virus was administered at a dose of 37 TCID₅₀, which is equivalent to 0.03 doses lethal for 50% of mice (MLD₅₀) for this age and strain of mice. *S. pneumoniae* was given 7 days later at a dose of 200 cfu (0.02 MLD₅₀). After secondary bacterial infection, bioluminescent imaging was used to follow the development and progression of pneumonia as described,
with the use of defined parameters to ensure that experimental groups were balanced, with all mice at an early stage of pneumonia [17, 18]. Bacterial counts and cytokine levels in the lungs were determined by evaluation of lung homogenates as described elsewhere [4, 17, 18]. Cell counts and differential counts for bronchoalveolar lavage fluid (BAL) samples were determined using flow cytometry as described elsewhere [18].

**Antibiotic treatment.** Treatment was started after identification of pneumonia by bioluminescent imaging. Mice were given antibiotics (Sigma-Aldrich) intraperitoneally twice daily in divided doses (ampicillin, 200 mg/kg/day; clindamycin, 30 mg/kg/day or 120 mg/kg/day) or once daily (azithromycin, 10 mg/kg/day for the first dose and 5 mg/kg/day thereafter) for a total of 7 days, or they were mock treated with the diluent (PBS).

**Histopathologic examination.** Microscopic evaluation of the lungs was performed by an experienced veterinary pathologist (K.L.B.) who was blinded to the study purpose and design and to group composition. A semiquantitative grading scheme was used to score 2 parameters, the overall character of the pneumonic process and the pathology specifically observed in the interstitium and terminal airways, as described elsewhere [18].

**Statistical analyses.** Comparison of the survival rate between groups of mice was done using the log-rank $\chi^2$ test on the Kaplan-Meier survival data. Comparisons of weight loss, bacterial lung titers, cell counts in BAL fluid samples, flux of light through the lung, and cytokine levels between groups were done using analysis of variance (ANOVA). $P < .05$ was considered to denote statistical significance for these comparisons. SigmaStat software for Windows (version 3.11; SysStat Software) was used for all statistical analyses.

**RESULTS**

Elicitation of a higher TNF-\(\alpha\) response by ampicillin-mediated killing than by clindamycin-mediated killing.** We hypothesized that the poor clinical outcomes observed with secondary pneumococcal pneumonia after influenza could result from the immunopathology engendered by the lysis of bacteria caused by this \(\beta\)-lactam antibiotic. We elected to first test this possibility in vitro by killing pneumococci with either the cell-wall-active agent ampicillin or clindamycin, a protein synthesis inhibitor. Production of TNF-\(\alpha\) by macrophages was used as a surrogate for the inflammatory response. Killing of bacteria with clindamycin resulted in significantly less ($P < .001$, by ANOVA) TNF-\(\alpha\) production than did either killing of bacteria with ampicillin (figure 1) or exposure to live pneumococci (controls). Neither of the antibiotics had an effect on TNF-\(\alpha\) production by macrophages when they were exposed in the absence of bacteria (mean, 25.3 and 27.0 pg/mL for ampicillin and clindamycin, respectively, compared with 51.4 pg/mL for media alone). The 6-fold increase in the level of this proinflammatory cytokine as a result of ampicillin treatment relative to clindamycin treatment prompted us to compare these 2 antibiotics in our mouse model of secondary bacterial pneumonia.

**Figure 1.** Tumor necrosis factor (TNF)-\(\alpha\) released due to antibiotic-mediated killing of *Streptococcus pneumoniae*. J774 macrophages were exposed to supernatants from bacteria that either were treated for 2 h with ampicillin or clindamycin or were mock treated. TNF-\(\alpha\) production by the macrophages (expressed as the mean value $\pm$ SD for 6 wells from 4 consecutive, independent experiments) was determined by ELISA. *Significant difference ($P < .05$), as determined by analysis of variance, compared with the other groups. There were no statistical differences between the data for animals treated with ampicillin and the data for control animals.

**Protein synthesis inhibitors and improvement in the outcomes of secondary bacterial pneumonia.** We studied the effect of antibiotic treatment on outcomes by infecting mice with influenza virus and, 7 days later, challenging them with a type 3 strain of *S. pneumoniae* [16]. Mice were monitored twice daily for the development of pneumonia by bioluminescent imaging, and treatment was initiated immediately on identification of disease. In a preliminary study, all mice treated with ampicillin (8 of 8 mice) cleared the bacteria from their lungs by bioluminescent imaging, but 3 (38%) of the 8 mice died at a mean of 63 h after clearance. This result was significantly better than that achieved with the untreated control group, in which no mice (i.e., 0 of 7 mice) cleared the infections or survived for $>72$ h. When treated with clindamycin at a dose of 30 mg/kg/day, only 10 of 16 mice cleared infection, but all of these mice (i.e., 10 of 10 mice) survived. Thus, administration of clindamycin at this dose was poorly effective in eliminating bacteria from the lungs of mice coinfected with influenza virus and *S. pneumoniae*, but the survival rate after bacterial clearance was significantly improved ($P < .05$, by log-rank test), compared with the group treated with ampicillin. In addition, mice treated with clindamycin had significantly less weight loss on days 3–9 after secondary infection than did mice treated with ampicillin ($P < .05$, by repeated-measures ANOVA) (data not shown).

We next tested several alternate strategies involving a protein synthesis inhibitor, including a higher dose of clindamycin (120 mg/kg/day) and use of the macrolide azithromycin or combination therapy with clindamycin and ampicillin. Increasing the dose of clindamycin used for treatment improved outcomes by improving clearance of pneumonia; 17 (100%) of 17 mice that were treated with the higher dose cleared pneumococci from...
their lungs within 36 h, and 14 (82%) of 17 of these mice survived (figure 2). In this group, the 3 mice that died had severe diarrhea, which may have contributed to their deaths. Combination therapy that consisted of 24 h of clindamycin therapy begun at the time of identification of pneumonia, followed by combined therapy with both ampicillin and clindamycin, resulted in a similar outcome; all mice cleared pneumonia, and 12 (80%) of 15 mice survived. Azithromycin, which shares a mechanism of action with clindamycin but possesses anti-inflammatory qualities as well, performed best in the model, clearing pneumonia from all 12 mice and promoting survival in 11 (92%) of 12 mice. Although ampicillin therapy was superior

Figure 2. Survival of mice treated for secondary bacterial pneumonia with a cell-wall–active agent or with protein synthesis inhibitors. Groups of mice were infected with influenza virus PR8; they then were challenged 7 days later with *Streptococcus pneumoniae* and were monitored for the development of pneumonia by means of bioluminescent imaging (defined as a flux of light through the thorax that was >22,000 rlu/min). After the development of pneumonia, treatment with azithromycin (n = 12); clindamycin (n = 17); a combination of clindamycin for 24 h, followed by clindamycin plus ampicillin (n = 15); ampicillin alone (n = 9); or mock treatment (n = 6) was initiated. Weight loss (A) and survival (B) are plotted. Error bars denote the SD of the mean weights. *Significant difference (P < .05), as determined by the use of the log-rank test on the Kaplan-Meier survival data, compared with all other groups. C, Representative images from bioluminescent imaging of mice with pneumonia before (Pre) development of pneumonia, at the initiation of treatment (0 h), and at 12, 24 and 36 h after treatment initiation. The scale (right) denotes the no. of relative light units per pixel. A down-pointing arrow denotes death of the animal before that imaging time point.
Increased inflammatory response associated with ampicillin. The underlying hypothesis for the present study was that the inflammatory response accompanying lysis by influenza virus PR8. They then were challenged 7 days later with Streptococcus pneumoniae and were monitored for the development of pneumonia by means of bioluminescent imaging. Treatment with either clindamycin (gray-shaded bars; dose, 120 mg/kg/day) or ampicillin (black bars) or mock treatment (white bars) was initiated after the development of pneumonia. Four h after administration of the first antibiotic dose, lungs were homogenized and assayed for cytokines. Error bars denote the SD of the mean cytokine levels. *Significant difference (P < .05), as determined by analysis of variance, compared with the other groups. IL, interleukin; MIP, macrophage inflammatory protein; TNF, tumor necrosis factor.

Figure 3. Cytokine levels in lung homogenates after treatment for secondary bacterial pneumonia. Groups of mice (n = 9) were infected with influenza virus PR8. They then were challenged 7 days later with Streptococcus pneumoniae and were monitored for the development of pneumonia by means of bioluminescent imaging. Treatment with either clindamycin (gray-shaded bars; dose, 120 mg/kg/day) or ampicillin (black bars) or mock treatment (white bars) was initiated after the development of pneumonia. Four h after administration of the first antibiotic dose, lungs were homogenized and assayed for cytokines. Error bars denote the SD of the mean cytokine levels. *Significant difference (P < .05), as determined by log-rank test for each comparison.

Histopathologic findings are worse with ampicillin. We next performed microscopic examination of the lungs of mice treated for pneumonia, at both 4 and 24 h after the development of pneumonia and the start of treatment. In mice infected only with virus, minimal to mild changes in the airways and interstitium were noted in all animals with mild airway hyperplasia and an inflammatory infiltrate consisting of lymphocytes and plasma cells (table 1). All mice that were secondarily infected with pneumococcus had extensive broncho-interstitial pneumonia (table 1) (values for airway and interstitial involvement between groups that were infected with bacteria were similar). However, there was significant variation in the character and severity of the pneumonic process comparing mice treated with clindamycin with those treated with ampicillin or left untreated (figure 6). Findings were similar in untreated mice and mice treated with ampicillin or left untreated (figure 6A). These findings included (1) extensive epithelial necrosis throughout the bronchial tree; (2) lobar fibrinonecrosis, which was characterized by acute coagulative necrosis of mice treated with clindamycin. The amount of bacteria in the lungs, as quantitated by bioluminescence, decreased significantly (by >23-fold) (P < .05, by ANOVA) in the 24 h after treatment with ampicillin, increased more than 2-fold in untreated mice (P < .05), and then remained stable in mice treated with clindamycin (figure 5). Bacterial counts in homogenized lungs 4 h after the detection of pneumonia and the onset of treatment (at the time that cytokine levels were determined) were not different between the ampicillin and clindamycin groups (mean, 5.3 × 10^6 cfu/mL lung homogenate, for mice treated with ampicillin; 3.0 × 10^6 cfu/mL, for mice treated with clindamycin; and 2.8 × 10^7 cfu/mL, for mice who were not treated). Thus, increased killing and more-rapid clearance of bacteria from the lungs resulted in worse cytokinemia, more infiltration of inflammatory cells, and poorer outcomes.

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of the alveolar walls, filling of the alveoli with viable and degenerate neutrophils, fibrin, edema, and hemorrhage; (3) and pleuritis consisting of mats of fibrinocellular exudates along the pleura. Unique findings in the group treated with clindamycin were limited to the character of the interstitial component of the disease; the extent of lung involvement was similar between the groups (figure 6B). A combination of necrosis and hyperplastic airway epithelium similar to that seen in other groups was present. However, fibrinonecrosis and acute coagulative necrosis were not the predominant findings. When present, these findings were limited to small, localized areas, and entire lobes were never affected.

**DISCUSSION**

Significant mortality rates are associated with severe lung infections, despite the availability of effective antibiotics for treatment of the most common causes of pneumonia. Even with appropriate therapy, pneumococcal pneumonia causes death in 4%–5% of uncomplicated cases and in 13% of cases associated with bacteremia [24–27]. These rates have essentially remained unchanged since the introduction of penicillin [21]. On the basis of findings from limited case series and accumulated clinical experience, bacterial pneumonia that occurs after influenza is generally considered to be more difficult to treat and to be associated with a high case-fatality rate [3, 4, 12]. We have suggested that this may stem from the robust inflammatory response that occurs in response to combined infections [16, 18]. Use of β-lactam agents, such as ampicillin, may exacerbate the problem, because these agents act by (1) lysing the bacteria and (2) releasing proinflammatory substances, such as cell-wall compo-

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**Figure 4.** Cell counts in bronchoalveolar lavage (BAL) fluid samples after treatment for secondary bacterial pneumonia. Groups of mice were infected with influenza virus PR8. They then were either challenged 7 days later with *Streptococcus pneumoniae* or mock challenged (*n* = 6) and were monitored for the development of pneumonia by means of bioluminescent imaging. Treatment with clindamycin (*n* = 6) or ampicillin (*n* = 9) or mock treatment (*n* = 6) was initiated after the development of pneumonia. At 24 h after administration of the first antibiotic dose, lungs were underwent lavage and were assayed for cell counts by flow cytometry. Error bars denote the SD of the mean counts. The differences are not statistically significant. WBCs, white blood cells.

**Figure 5.** Bacterial load in the lungs during treatment of secondary bacterial pneumonia. Groups of mice were infected with influenza virus PR8. They then were challenged 7 days later with *Streptococcus pneumoniae* and were monitored for the development of pneumonia by bioluminescent imaging. Treatment with clindamycin (*n* = 6) or ampicillin (*n* = 9) or mock treatment (*n* = 6) was initiated after the development of pneumonia. The bacterial lung load was assessed in live, anesthetized animals at 0, 12, and 24 h after the start of treatment, by means of bioluminescent imaging, and it is reported as the mean ± SD of the flux of light from the thorax (expressed as relative light units per minute). *Significant difference (*P* < .05), as determined by analysis of variance, compared with the 0-h time point for that group.
nents, cytotoxins, and bacterial DNA, which are recognized by the innate immune system and which trigger the inflammatory response [28, 29]. In support of this model, mice with severe pneumococcal pneumonia and bacteremia occurring after influenza did not survive when treated with ampicillin, despite effective killing and clearance of bacteria [4]. In the present study, we extended this finding by using a less virulent strain of S. pneumoniae that remains confined to the lung [16]. Use of ampicillin therapy only resulted in a survival rate of 56%–62% in mice that were superinfected with this strain after developing influenza (figure 1) (data not shown). In these mice, treatment was associated with (1) increases in levels of proinflammatory cytokines and chemokines, (2) an increased influx of inflammatory effector cells into the lungs, and (3) observation of more severe histopathologic findings, despite rapid and complete clearance of bacteria.

On the basis of this model of the pathogenesis of these severe lung infections, we have suggested that treatment with nonlytic antibiotics may result in improved outcomes [30]. Clindamycin is a lincosamide antibiotic that targets the 50S ribosomal subunit, inhibiting protein synthesis, and is bacteriostatic when used at typical treatment doses. In our model, low-dose therapy with clindamycin was poorly effective, clearing bacteria from the lungs in only 10 of 16 mice, but all mice that demonstrated a response to the antibiotic survived. Increasing the dose of clindamycin improved clearance but also resulted in toxicity. Nonetheless, overall survival was improved with clindamycin, compared with the cell-wall–active agent. The improved outcomes appear to be the result of a decreased inflammatory response, made manifest by lower levels of proinflammatory cytokines and chemokines, the presence of fewer inflammatory cells in the lungs, and improved histopathologic findings, despite slower clearance. The use of clindamycin therapy before ampicillin therapy was equally efficacious. We hypothesize that a shutdown of protein synthesis before lysis by ampicillin decreased the release of proinflammatory toxins. Because of the incomplete response but favorable outcomes achieved with the use of clindamycin alone, we also studied a second antibiotic with a similar mechanism of action, azithromycin. Azithromycin is a 15-member ring macrolide that also targets the 50S ribosome but exhibits anti-inflammatory activities independent of its antimicrobial properties [31]. This antibiotic resulted in clinical cure in 11 (92%) of 12 mice, although it is unclear whether the im-

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<td>Interstitial acute coagulative necrosis</td>
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**NOTE.** Data are the mean ± SD of semiqualitative scores on a scale of 0–4, as determined by a veterinary pathologist (K.L.B.) blinded to the study purpose and design and to the composition of the groups.

![Figure 6](https://academic.oup.com/jid/article-abstract/199/3/311/821855)
proved outcomes are solely the result of the mechanism of action or whether they are the result of this factor in addition to the anti-inflammatory properties of the drug.

Similar approaches to therapy have previously been considered for severe bacterial infections. It is now well appreciated that the use of steroids in children with *Haemophilus influenzae* meningitis [32, 33] or adults with pneumococcal meningitis [34] improves clinical outcomes. However, the effects of steroids on severe lung infections, including acute respiratory distress syndrome (ARDS), have been disappointing, suggesting that other options are needed. In a rabbit model of pneumococcal meningitis, Nau et al. [35–37] demonstrated that treatment with a non-lytic antibiotic, such as rifampin, results in improved outcomes. Limited data from these investigators suggest that the benefits of rifampin therapy in this model are preserved when used in combination with ceftriaxone [35]. In the present study, we also showed that combination therapy was successful when the protein synthesis inhibitor preceded the cell-wall–active agent by 24 h; further work on the relative timing of administration may be helpful. In humans, 2 retrospective studies [38, 39] and 1 prospective, multicenter trial [40] have concluded that the addition of a macrolide to a β-lactam results in a significant reduction in mortality (compared with β-lactam therapy alone) in adults with bacteremic pneumococcal pneumonia. The mechanism(s) responsible for this effect were not apparent but were unrelated to β-lactam resistance. The results of the present study suggest that the improved outcomes are related to suppression of the inflammatory response.

Secondary bacterial infections are more common during pandemic years, when highly virulent virus strains circulate. Accumulating evidence suggests that this is in part due to the robust inflammatory response induced in the lung by these pandemic strains [18, 41]. Over the past 10 years, highly pathogenic avian influenza viruses of the H5N1 subtype have emerged as a cause of significant mortality and a pandemic threat in Southeast Asia, parts of the Middle East, Europe, and Africa [42]. A hallmark of these infections is severe lung inflammation similar to that seen with ARDS [43–46]. The only criterion missing for one these viruses to become the next pandemic strain is ease of transmissibility [47]. It can be predicted, based on history, that when these viruses adapt to humans and begin to cause widespread disease, secondary bacterial infections will emerge as a leading cause of morbidity and mortality. Antiviral agents, if available, may help to mitigate the severity of complications as suggested by our earlier work [4]. However, alternate strategies, including non-lytic antibiotics and immunomodulation need to be considered [30, 48].

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**References**


