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Preventing and treating secondary bacterial infections with antiviral agents

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Summary

Bacterial super-infections contribute to the significant morbidity and mortality associated with influenza and other respiratory virus infections. There are robust animal model data but only limited clinical information on the effectiveness of licensed antiviral agents for the treatment of bacterial complications of influenza. The association of secondary bacterial pathogens with fatal pneumonia during the recent H1N1 influenza pandemic highlights the need for new development in this area. Basic and clinical research into viral-bacterial interactions over the last decade has revealed several mechanisms that underlie this synergism. By applying these insights to antiviral drug development, the potential exists to improve outcomes by means other than direct inhibition of the virus.

Influenza-associated mortality

Over the last decade, influenza and pneumonia have ranked as the seventh leading cause of death in the United States for all persons and the fifth for children [1]. In the developing world, respiratory tract infections are the leading cause of death in children past the neonatal period [2]. However, few of these deaths derive from direct viral damage alone. Instead, most influenza-associated mortality is "excess mortality," a term coined by William Farr in 1847 and first utilized by Selwyn Collins of the U.S. Public Health Service in the 1930s and 1940s to classify outcomes during influenza epidemics [3]. Because traditional records defining cause of death, such as death certificates, are unable to correctly account for the impact of influenza, deaths in excess of the seasonal baseline are attributed to influenza by statistical methods when they occur during an influenza epidemic [4]. A great deal of influenza-associated mortality is in people with co-morbidities and is due to either respiratory and circulatory causes or secondary bacterial pneumonia [5].

Influenza-associated mortality varies significantly from season to season, depending on the circulating virus strain (reviewed in [3]). In recent decades, greater excess mortality has been seen during seasons when H3N2 subtype viruses were the predominant strains than when H1N1 or influenza B viruses dominated [4]. The H1N1 1918 pandemic strain killed more than 40 million people worldwide [6], and more than 95% of fatal cases with detailed

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autopsy data were complicated by secondary bacterial pneumonia [4]. In contrast, the H3N2 pandemic killed only about 1 million people worldwide, with less excess mortality than many subsequent seasonal epidemics [4]. This subtype- and strain-specific dichotomy probably arises from differences in virulence between the strains. These differences may be expressed through interactions with host factors or secondary bacterial pathogens and may not be quantifiable through traditional measures of virulence such as replication efficiency and disease potential during primary infection in animal models. This scenario is further complicated by strain-specific differences in the bacterial co-pathogens through which influenza causes much of its morbidity and mortality. For example, after being a prominent secondary pathogen in the 1957 H2N2 pandemic and for several years thereafter [7], *Staphylococcus aureus* was an uncommon cause of secondary bacterial infections (SBI) for several decades. In regions where the USA300 clonotype of methicillin-resistant *S. aureus* (MRSA) has emerged, however, severe, necrotizing staphylococcal pneumonia is now one of the more common manifestations of severe SBI [8,9]. Thus, specific virulence factors expressed by the super-infecting bacteria must also contribute to the overall interaction with influenza viruses. The effect of such factors on disease implies that interventions such as antiviral therapies targeting them could reduce mortality.

The issue of treatment of SBI must be put into the larger context of acquisition, diagnosis, and management of influenza. Secondary pneumonia complicating influenza often has a fulminant presentation, particularly when *S. aureus* is the secondary pathogen [8-10]. Antibiotic therapy is often unsuccessful in these cases, and could even contribute to poor outcomes through enhanced inflammation during bacterial lysis [11]. Ideally, therefore, prevention of SBI by intervention prior to its development would be preferred. However, most cases of influenza are not brought to medical attention or are managed in outpatient settings. Antiviral treatment is currently recommended by the Centers for Disease Control (CDC) and the Infectious Diseases Society of America (IDSA) at presentation in persons with certain high-risk medical conditions, but for healthy persons only after hospitalization is required or complications develop [12,13]. Since the risk factors for development of SBI following influenza are not known, and less than half of persons who develop secondary bacterial pneumonia requiring hospitalization have medical conditions placing them at high-risk for hospitalization from influenza [9], this strategy is likely to miss most opportunities for preventing SBI with antiviral medications.

The most obvious way to prevent SBI is to prevent the antecedent viral infection entirely. Animal model data suggest that vaccination against influenza is an effective method to prevent subsequent secondary pneumonia [14]. Influenza vaccine studies in humans have typically not been designed or appropriately powered to assess effectiveness against SBI. It can be assumed that prevention of infection through vaccination would also prevent complications such as SBI, but does partial protection significantly impact bacterial super-infections? Is neutralization of virus as was shown in the animal model [14] necessary, or would cross-reactive T-cell based immunity also prevent SBI? These questions should be assessed in both pre-clinical and human vaccine trial settings. An alternate strategy would be to target the bacteria that most often complicate influenza. A clinical trial of a *Streptococcus pneumoniae* conjugate vaccine performed in South Africa showed that prevention of pneumococcal pneumonia had a significant impact on virus-associated pneumonias [15].

Pneumonia associated with influenza was reduced by 45%, that associated with parainfluenzaviruses by 44%, and with RSV by 22%. It is thus considered reasonable to prevent SBI by vaccinating against both influenza and the pneumococcus, and possibly against *Haemophilus influenzae* type B [16]. Since vaccines are not available against other common super-infecting bacteria such as *S. aureus* and group A *Streptococcus*, however, this strategy can impact only part of the problem.

The H1N1 Pandemic

In the spring of 2009, a new influenza virus strain of the H1N1 subtype emerged, causing the first pandemic in more than 40 years [17]. The epidemiology of this nascent pandemic differed from recent seasonal influenza. Although most hospitalized patients had chronic medical conditions known to predispose them to severe influenza, young people were disproportionately affected [18,19]. The relative sparing of the elderly population eliminated a major source of circulatory and respiratory deaths, resulting in less excess mortality than in some previous epidemics [20]. Bacterial super-infection as a contributor to hospitalization and death was not often recognized in early reports, but most of the early cases were treated empirically with antibiotics, and invasive assays to detect co-pathogens were not systematically done [17,21,22]. It was later shown that approximately 25-50% of severe or fatal cases were complicated by SBI [22-27]. The pathology of these severe cases has been characterized chiefly by diffuse alveolar damage, with superimposed findings in some cases typical of a necrotizing bacterial pneumonia. This is similar to the findings in autopsy series from the 1918 pandemic [24,28,29].

Co-infections in community-acquired pneumonia

The role of respiratory viruses other than influenza in the pathogenesis of SBI remains unclear [30]. There are numerous case reports and case series, mostly in children, documenting that serious viral-bacterial co-infections occur in patients with community-acquired pneumonia (CAP) [31-33]. No single virus predominates in studies of CAP in children (Table 1) [34-39]. Rhinovirus is most consistently first in incidence in studies where diagnostics capable of detecting it are used, but the incidence of rhinovirus as well as most other respiratory viruses varies widely by study and patient population (Table 1). Multiple other viruses, including respiratory syncytial virus (RSV), human bocavirus (hBoV), human metapneumovirus (hMPV), influenza A and B viruses, and parainfluenza viruses (PIV) 1, 2, and 3, are also commonly detected in these studies, with each occurring in around 7-15% of cases. Emerging viruses such as hMPV [40], hBoV [41], and several different human coronaviruses (hCoVs) [42] are filling in what was previously a gap in understanding of causes of CAP as new diagnostics become available. One problem caused by newer, more sensitive assays such as PCR-based techniques, however, is determining causality [43]. Some viruses, especially rhinoviruses and bocaviruses, are found very frequently in some case series but can also be found in asymptomatic subjects and in subjects co-infected with other viruses of known pathogenicity. For example, 64-83% of hBoV-infected patients are co-infected with other pathogens [41,44,45], and co-infections are found with rhinoviruses in 30-82% of children with CAP [44,45]. The questions of what contributions these viruses

make to the pathogenesis of CAP and whether they are bystanders or participants remain to be answered [46,47].

Animal models of secondary bacterial infection following influenza

Animals, particularly mice, ferrets, chinchillas, and non-human primates, have been utilized to model SBI following influenza since the time of the 1918 pandemic (reviewed in [3,48]). Most recent studies of SBI in mice using *S. pneumoniae* [49-51], *H. influenzae* [52], and *S. aureus* [53,54] are based on a co-infection model derived ultimately from work described early in the 1940s [55]. In this model, mice infected intranasally with sub-lethal doses of influenza virus followed some days later with sub-lethal doses of bacteria, develop severe pneumonia and succumb to illness 4-7 days after the secondary challenge [49,56]. The bacterial lung titer and the frequency with which bacteria access the bloodstream are both enhanced by antecedent viral infection. Interestingly, there are complementary effects on the virus as well, as viral lung load increases about 10-fold in the days immediately following bacterial super-infection. The virus must be given first, and at least 2-3 days must elapse for these synergistic effects to be observed [49,54].

Further exploration of the timing of exposure in both mice and ferrets demonstrated that mucosal infections, such as otitis media or sinusitis, occurred in animals colonized with pneumococcus which later developed influenza [57,58]. However, invasive disease including pneumonia, bacteremia, and meningitis, were only seen when the influenza infection occurred first [59], suggesting that once systemic immunity to the bacterial pathogen is established, virus-induced effects on transitions to sterile compartments are blunted. This has significant implications for the epidemiology and prevention of SBI because it suggests that the strains that commonly colonize the nasopharynx of susceptible humans during influenza seasons are not necessarily the ones that need to be targeted. Since viral infections increase susceptibility to acquisition of new strains [59-61], vaccination coverage for bacteria should include those strains most capable of infecting and invading a host during their viral illness. Comprehensive, longitudinal studies in humans to confirm these findings should be undertaken.

Mechanisms underlying viral-bacterial synergism

Numerous studies in the 1920s and 1930s of mortality during the 1918 pandemic concluded that bacteria were secondary invaders and not the primary agents of disease [3,28,62,63]. The prevailing dogma since that time to explain this phenomenon has been that airway damage provides a foothold for adherence of bacteria to damaged epithelium and exposed extracellular matrix, facilitating the development of pneumonia [64]. This concept is supported by studies in both mice [65] and humans [66,67] that showed a physical association of bacteria with damaged airway epithelium. Tracheae removed from mice previously infected with a pathogenic influenza virus better supported adherence of bacteria than did similar tissues from uninfected mice [65]. In humans, bacteria were found on autopsy to be adherent to the areas of the tracheo-bronchial tree denuded by viral infection [66,67] If this mechanism is indeed a major factor in priming the host for development of

bacterial super-infections, then any virulence factors that enhance the ability of the virus to cause lung damage should contribute to SBI.

Virulence in influenza viruses is a multi-factorial trait with all gene products capable of contributing, either independently or through interaction with other viral proteins. Thus, antiviral strategies targeting any function of the virus that results in decreased viral fitness or diminished virus-associated lung injury should also decrease SBI. The chief mechanism of the greater virulence of the novel pandemic H1N1 strain relative to seasonal influenza viruses appears to be its ability to cause infections deep within the lung, similar to the 1918 pandemic strain [24,25,29,68-70]. This is likely due to the lack of glycosylation of the hemagglutinin (HA) protein expressed by the novel H1N1 [18,71], which facilitates escape from collagenous lectins, preventing clearance [72]. Differences in receptor specificity of the HA have been implicated in differences in tropism within respiratory tract of H5N1 subtype avian viruses in animals [73], but the novel H1N1 strain shares the human-like pattern of receptor specificity common to recently circulating seasonal H1N1 strains [74]. The observed differences in respiratory tract localization between different strains may help explain differences in support for SBI between viruses.

While enhancement of adherence due to acute lung injury is an appealing mechanism for pathogenic pandemic strains, it does not explain the association of SBI with less virulent, seasonal strains. Indeed, several other mechanisms have been proposed (Table 2). The sialidase activity of viral neuraminidase (NA) proteins has been found to correlate with support for SBI in mice [30,56,57,75-77]. Viral sialidase activity is thought to facilitate access to the lower respiratory tract by exposing bacterial receptors or destroying sialylated mucins [30]. The pneumococcal NA has been shown to support the transition from nasopharynx to lung in a mouse model utilizing mutants deficient in sialidase activity [78]. While this is an appealing model for the effects of viral NAs on SBI, direct proof is lacking, and enzymatic cleavage of other substrates, including latent transforming growth factor-beta (TGF-β) and cellular sialidases, may provide “off-target” effects involved in pathogenesis [79,80]. In addition, the recognition that bacterial sialidases cleave sialic acids in part to avail themselves of a carbohydrate source and assist in biofilm formation has implications for pathogenesis [81]. Viral NA-mediated release of free sialic acids into the respiratory tract may facilitate the transition from nasopharynx to lung by this mechanism.

Another receptor-mediated mechanism involving upregulation of the platelet-activating factor (PAF) receptor had been proposed [49]. Since pneumococcus can utilize PAF receptor as a cellular receptor for attachment, and inflammatory stimuli upregulate PAF receptor expression, virus-mediated changes in PAF receptor distribution were hypothesized to facilitate adherence in the lower respiratory tract. However, studies in knock-out animals later revealed that this receptor was not needed for the synergistic enhancement of bacterial super-infections by the virus [82]. Previous studies attributing a role to PAF receptor [83,84] were likely confounded by use of strains that can cause bacteremia, as it was later shown that the main role of PAF receptor in pneumococcal pathogenesis is in enhancing transitions across endothelial cells from the lungs to the blood and from the blood to the cerebrospinal fluid [85,86].

Recent investigations into mechanisms of viral-bacterial synergism have centered around modulation of host immune responses by the virus. Influenza is commonly associated with leukocytosis but can also cause lymphopenia and neutropenia in humans [22,87,88]. Both cytokine storm and general leukopenia are commonly associated with humans infections by highly pathogenic avian influenza viruses of the H5N1 subtype [88]. The interplay of viral and host factors in these immune responses is poorly understood at present. Are there viral strain-specific factors or host variations that account for the marked differences seen between individuals and studies? Some of this puzzle is now being unraveled. Early after infection, the influenza virus cytotoxin PB1-F2 drives inflammatory responses, causing infiltration of neutrophils and macrophages into the lungs and resulting in acute lung injury in mouse models [89,90]. These responding cells may be functionally impaired by the virus, diminishing the capacity of the host to clear bacterial co-pathogens while simultaneously causing ALI. Functional deficits in neutrophils, macrophages, and natural killer cells have all been linked to worse outcomes from SBI in animal models [50,91,92]. Numerous pro-inflammatory cytokines and chemokines are upregulated in the lung during secondary bacterial pneumonia as a result of interactions with multiple viral and bacterial virulence factors including PB1-F2 [89,93]. PB1-F2 mediated effects seem to be particularly important for pandemic and other highly virulent viruses, but have not been shown to contribute to SBI associated with recent seasonal strains which do not express functional forms of the cytotoxin [54,89,94].

Late in infection or after resolution of the primary viral infection, desensitization of innate responses can increase the incidence of SBI in mice [51]. The effect of negative regulators of inflammation such as CD200 during resolution of influenza may prevent adequate host responses to secondary pathogens [95,96]. The release of interferon-gamma from T-cells may also contribute by diminishing the capacity of alveolar macrophages to clear bacteria from the lungs [50]. This biphasic response, with inflammation prominent early and anti-inflammatory processes dominating late, implies that the timing of the onset of the SBI may have a major influence on what mechanisms are operative. The prominent role of host processes implies that cellular targets for pharmaceutical interventions should exist. Both specific anti-inflammatory agents to target pathways utilized by the viral and bacterial cytotoxins and agonists to reverse the effects of influenza virus on innate response should be investigated.

The species and strain of super-infecting bacteria also be affected by these bi-phasic host responses to influenza viruses. In animal models it is clear that different bacterial strains can contribute to SBI with different patterns of disease [54,59,93]. In addition, significant differences between the mouse models of *S. pneumoniae* and *S. aureus* suggest that their pathogenesis and the mechanisms each uses to interact with influenza virus differ [54]. It was proposed during the 1957-1958 pandemic that three distinct sets of outcomes could be recognized among those who developed bacterial pneumonia. This classification scheme divided patients into those with primary influenza virus pneumonia, those with late bacterial pneumonia presenting after resolution of primary influenza, and those with concomitant viral-bacterial pneumonia [67]. Cases in the concomitant viral-bacterial pneumonia group tended to be more fulminant and more often fatal than those with late bacterial pneumonia, and *S. aureus* was more commonly found in the combined group when mechanisms

involving inflammation predominate. The ability of *S. aureus*, particularly the USA300 clonotypes [97], to express multiple cytoxins likely contributes to this phenotype. The pneumococcus was the predominant cause of SBI in patients who developed SBI during the late phase, when presumably an inability to clear the secondary pathogen due to the anti-inflammatory milieu was responsible [50,51,95].

The mechanisms underlying potential interactions between other respiratory viruses and bacteria are less clear but show some parallels to mechanisms proposed for influenza virus [30]. Several medically important viruses have been shown to increase bacterial adherence in both in vitro and ex vivo models [98,99]. RSV and PIVs can upregulate receptors for common respiratory bacteria [98]. Sialidase activity of the Sendai virus and PIV hemagglutinin-neuraminidase (HN) proteins enhance SBI in a mouse model [76]. Alterations to innate immunity, including both disruption of mucosal barriers and dysregulation of defensins, have been implicated in animal models of co-infection between respiratory viruses and bacteria [48,100,101].

Use of antivirals to prevent or treat secondary bacterial infections

Given the strong association of bacterial disease with antecedent viral infections, there has been understandable interest in determining whether prevention or treatment of the virus can eliminate or ameliorate SBI. Two classes of antiviral drugs are currently approved for use in patients with influenza [102]. In addition, numerous antiviral drugs directed against multiple targets of the influenza virus life cycle have progressed to preclinical or early clinical stages (reviewed in [103,104]). Fewer candidates exist for other respiratory viruses [105], and none so far has reached the approval stage [106]. Unlike antibiotics, which can eliminate or greatly reduce pathogen burden, existing influenza antiviral drugs serve only to halt progression of disease by preventing new host cells from being infected. If this intervention is administered early enough in the clinical course, it may alter the tempo of infection, allowing normal immune clearance mechanisms to gain the upper hand. Earlier treatment works better in most cases because the infection is not yet widespread [107,108]. Thus, the major effects of treatment are symptom reduction and a more rapid recovery, not immediate clinical cure. In the context of preventing SBI, the continued presence of the virus and the ongoing host response suggests that many of the mechanisms discussed above may remain operative despite treatment. However, it might also be expected that a continuum of treatment effects exists, whereby decreases in virus replication, the resulting decreases in ALI and thus the presumably diminished host response, can provide some clinical benefit.

Preclinical work in a mouse model demonstrated that prophylaxis or early treatment with an NAI decreased viral load and weight loss and significantly reduced SBI [75]. Antiviral use decreased the incidence of SBI, lengthened the interval between exposure to bacteria and development of disease, slowed progression of pneumonia when it developed, and facilitated antibiotic treatment of the super-infection. Similar effects were seen in paramyxovirus models utilizing a specific inhibitor of the PIV HN [76]. However, influenza virus NA-specific effects independent of the effect on viral replication were also seen, as delayed treatment up to 5 days after the initial viral infection also significantly decreased SBI [75]. Since viral lung load and virus-induced clinical illness were not different between groups

with delayed therarpy, off-target effects of the drug or inhibition of secondary effects of the viral NA on substrates unrelated to the viral life cycle must be involved. To strengthen this association, a second study in mice revealed that the level of NA activity of historical and recombinant viruses correlated with their support for SBI [77]. This implies that NAI treatment of influenza might have different effects on SBI depending on the viral strain being targeted.

Although there are convincing animal data suggesting that NAIs are effective against SBI, the mechanism remains unclear. Does inhibition of NA-mediated desialylation of potential receptors for bacterial adherence contribute to the effect as hypothesized [30,75]? To date, this has been shown only indirectly through inhibition of adherence in vitro and prevention of pneumonia in vivo. The use of recombinant NA in mice did not have effects similar [109] to those of NA in the context of the full virus. Is this an issue of specificity of the enzyme? Or does NA act on other targets? The ability of influenza virus NA to cleave and activate latent TGF-β suggests pleiotropic effects of influenza viruses on immunity and wound healing are possible [79]. Inhibition of this effect or prevention of cleavage of other potential substrates of the enzyme could influence multiple pathways important for immune responses. On the other hand, NAIs may have off-target effects as well, through inhibition of cellular sialidases [80]. One of these cellular enzymes is involved in toll-like receptor (TLR) 4 signaling and activation [110]. Since TLR4 is involved in host responses to bacterial pathogens including *S. pneumoniae* [111], acute lung injury from influenza viruses [112], and induction of SBI [51], interactions such as this with NAIs could have significant downstream effects.

It is likely, based on vaccine effects and the improved efficacy of early vs. late treatment with oseltamivir [14,107], that direct inhibition of viral replication of viral-induced lung damage will prevent or ameliorate SBI. Complications of influenza such as SBI should be systematically assessed during preclinical development of novel antiviral drugs. Human data on the efficacy of existing antiviral agents against SBI are limited at present. Because the incidence is low in the general population in developed countries, a study designed specifically to assess this outcome would have to be extremely large to be well-powered. The use of high-risk groups, which could reduce the sample size, also presents challenges because the study of subjects likely to see high rates of SBI, such as the frail elderly, would be confounded by use of influenza and pneumococcal vaccines, which could not be ethically withheld from study participants. Nonetheless, some data are available from existing clinical studies.

In a randomized, double-blind, placebo-controlled trial of oseltamivir use in children, treatment of influenza resulted in a 44% reduction in diagnoses of otitis media and lower prescription rates for antibiotics than for the placebo group [113]. In a re-analysis of pooled data from several prospective clinical trials of NAIs, antiviral use in adults reduced the incidence of lower respiratory tract infections by 55% and antibiotic use by 27% [114]. A meta-analysis of similar data also concluded that antivirals have an effect in preventing SBI but cautioned that the groups most at risk for these outcomes had typically been excluded from participation [115]. Lack of a coordinated study design and the unavailability of some of the data from these trials have led some to criticize the conclusions drawn in these

analyses [116], pointing to the need for randomized clinical trials specifically designed with these endpoints in mind. Retrospective review of outcomes derived from an insurance claims database found that prescription of oseltamivir to adults and children with influenza resulted in reductions in the risk of pneumonia by 7% (27% in children under 18), otitis media by 15%, and hospitalization by 30% [117]. A similar analysis of children with high-risk medical conditions, primarily chronic lung disease, showed that treatment with oseltamivir was associated with reductions in otitis media by 43-62%, respiratory illnesses other than pneumonia by 6-17%, and hospitalization by 44-54%, but did not impact pneumonia [118]. Analysis of a specific risk group, adult diabetics, showed similar outcomes, with a 17% reduction in the risk of respiratory illness, a 30% reduction in the risk of hospitalization, but no significant effect on otitis media or pneumonia [119]. Because the diagnoses in these trials were derived from ICD-9 codes, specific attribution of bacterial disease as distinct from severe viral illness could not be made. Since widespread prophylaxis and treatment with NAIs were undertaken during the recent H1N1 pandemic [120], it may be possible to analyze these treated populations for an effect on SBI. Without a gold-standard clinical trial, however, we may need to continue to rely on pre-clinical data from animal models and expert opinion for some time.

Other approaches for prevention and treatment of secondary bacterial infections

The ability of NAIs to prevent SBI independent of effects on viral load suggest that interference with other viral targets by vaccination, small molecule therapies, monoclonal antibodies, or siRNA approaches might have effects that would not be anticipated based on direct viral suppression. Specific blockade of the pro-inflammatory effects of PB1-F2 [89] might diminish the severity of highly pathogenic influenza viruses and reduce SBI. However, knowledge of PB1-F2s binding partner(s) and mechanism of action is needed before this can become a realistic goal. Complementary strategies targeting bacterial toxins that are shown to synergistically interact with influenza viruses to enhance disease could also be explored. Inhibition of the interferon antagonist non-structural protein 1 (NS1) [121] should allow enhanced clearance of the virus, which could prevent several downstream effects of the virus on host immunity [122] that likely increase susceptibility to bacteria. Use of collectin-like molecules that recognize the HA itself or glycans on the HA [72,123] might diminish the diffuse alveolar damage caused by viruses that can access the lower respiratory tract, with secondary effects on SBI. Exogenous surfactant therapy containing these proteins has not been successful in adult patients with acute respiratory distress syndrome (ARDS) [124], but has not been evaluated as a specific treatment for influenza or SBI. Targeting host pathways important for either the virulence of influenza viruses [125] or their interactions with secondary bacterial pathogens [3,126] should be considered as alternate or potentially complementary strategies to targeting the virus itself. It is clear that further basic research into both influenza virus pathogenesis and interactions with bacteria are needed.

The broader strategy of immunomodulation to reduce the inflammatory response during SBI has been proposed (Table 3) [127]. Although this argument currently rests on biologic plausibility and is not yet supported by either animal model or clinical data in humans, some

inferences from studies designed to target sepsis, ARDS, or severe influenza can be made [124,128]. Systemic steroids were used quite frequently during the 2009 H1N1 pandemic [129]; in some published studies more than 50% of severely ill patients were treated with corticosteroids [21]. However, no clinical benefit of steroids has been shown for ARDS or specifically for influenza [124,130]. Antibody therapies, including currently available intravenous immune globulin (IVIG) preparations, hyperimmune sera from recovered or vaccinated individuals, and specific monoclonal antibody therapies have all been proposed as potential treatments for severe influenza [131-133]. While specific monoclonal approaches would be expected only to have an antiviral effect, IVIG and hyper-immune sera might also have immunomodulatory effects. Data from mouse models support the efficacy of all three approaches for primary influenza [134-137], although no data are available in models of SBI. Limited clinical data in humans support this approach conceptually, primarily from uncontrolled studies of treatment of pandemic influenza or H5N1 [138-140].

Data from animal models suggest that targeting specific pathways involved in inflammation might have better success. Proposed drugs for immunomodulation of severe influenza including statins, which inhibit a cholesterol biosynthesis pathway enzyme [141], agonists of peroxisome proliferator-activated receptors including fibrates and thiozolidinediones [142,143], cyclooxygenase pathway inhibitors [144], and antioxidants such as N-acetyl-L-cysteine [145] have shown some benefit against influenza in mice. The thialidinedione ciglitazone has also been shown to reduce inflammatory responses to *S. pneumoniae* in mice [146], suggesting it might be of use in dual infections. Of these potential candidates, only statins have been studied in humans thus far, with disappointing results. Cohort studies of persons prescribed statins for their cholesterol-lowering properties have shown no obvious clinical benefit against influenza morbidity [147,148]. Alternative medicines derived from plant or animal sources have also been proposed as potential therapeutics, based on animal model data and their traditional uses as anti-inflammatory agents (reviewed in [149]). If any of these agents reduce the inflammatory response to influenza and its consequences, without impairing immune clearance of the virus, then it is possible that SBI would be impacted as well.

A complementary approach to treatment of SBI might be to alter the antibiotic(s) used for treatment of the bacterial super-infection. The standard cell-wall active agents currently in clinical use rapidly lyse bacteria releasing pro-inflammatory components containing pathogen-associated molecular patterns (PAMPs) which are recognized by the innate immune system, triggering an inflammatory burst [150,151]. Use of alternative antibiotics which do not immediately lyse bacteria might provide a clinical cure without contributing to the inflammatory milieu. This theory was tested in a mouse model of secondary bacterial pneumonia. Mice with pneumococcal pneumonia following influenza were clinically cured using a protein synthesis inhibitor such as clindamycin or a macrolide such as azithromycin, but did not survive if treated with the cell wall active agent ampicillin [11]. Azithromycin, which has anti-inflammatory activity independent of its mechanism of antibacterial action, performed best in the model. Although this strategy has not been tested in combined viral-bacterial pneumonia in humans, it has been (indirectly) assessed in complicated pneumococcal pneumonia. Two retrospective studies [152,153] and one prospective, multicenter trial [154] demonstrated that combined therapy with a macrolide and a beta-

lactam resulted in a significant reduction in mortality (compared with beta-lactam therapy alone) in adults with bacteremic pneumonia from *S. pneumoniae*. The recent demonstration that a macrolide can have immunomodulatory effects in children with influenza suggests beneficial effects beyond antibacterial activity may be possible [155].

Concluding remarks

Antiviral drug development for respiratory viruses has been a surprisingly difficult task. Only a very few agents have made it to market, and there has been a paucity of candidates in the pipeline since the initial licensing of the NAIs [156]. However, promising new agents are in development [104]. Much of the recent progress has been spurred by 2 factors: the need to prepare for a severe influenza pandemic and our expanding understanding of viral pathogenesis. Accompanying this has been an increasing recognition that much of the morbidity and mortality associated with respiratory virus infections is mediated through SBI [3,16,63]. An improved understanding of the pathogenesis of these interactions should lead to measures to prevent or interrupt this synergism. Specific factors from the virus, the super-infecting bacteria, and the host could all be targets alone or in combination with other approaches. Finally, there is a need for improved clinical epidemiology data on secondary infections, and clinical trials of viral vaccines and antiviral drugs should include bacterial complications as endpoints to allow true data-driven guidance on treatment of SBI to be developed.

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Table 1

Detection of bacterial and viral pathogens from respiratory specimens of children with community-acquired pneumonia[1]

Bacterial Causes	Frequency (%)[2] (range)	Viral Causes	Frequency (%) (range)
Streptococcus pneumoniae	36 (7-46)	Rhinoviruses	18 (3-45)
Mycoplasma pneumoniae	12 (3-35)	Respiratory syncytial virus (RSV)	15 (3-29)
Haemophilus influenzae	8 (0-29)	Parainfluenza viruses (PIV) [3]	13 (1-19)
Moraxella catarrhalis	7 (0-28)	Bocavirus	10 (5-18)
Chlamydia pneumoniae	5 (3-9)	Influenza viruses [4]	8 (3-21)
Staphylococcus aureus	2 (0-12)	Human metapneumovirus (hMPV)	7 (1-13)
Other	1 (0-3)	Adenoviruses	6 (0-12)
		Coronaviruses [5]	4 (3-7)
		Other enteroviruses	1 (0-3)
No viral or bacterial pathogen identified [6]	16 (3-23)		

[1] Cumulative data from references [34-39,41,42,44,45]

[2] Denominator varies by pathogen and ranges from 483-1831 subjects

[3] PIV3 > PIV1 > PIV2

[4] Influenza A virus > influenza B virus

[5] Human coronavirus OC32 > 229E = NL63 > HKU1

[6] Data from studies where comprehensive attempts were made to identify both viruses and bacteria

Table 2
Proposed mechanisms of viral-bacterial synergism

Factors enhancing bacterial adherence
Epithelial damage enhancing bacterial adherence [65,67]
Alteration of epithelium through sialidase activity [30,56,105]
Upregulation of receptors for bacterial adherence [49,82,98]
Factors facilitating bacterial access to normally sterile sites
Mechanical alterations to airway or Eustachian tube function [3]
Changes in tropism of virus (ability to access the lower lung) [18,71,73,157]
Factors altering innate immune responses
Increased inflammation through expression of PB1-F2 [54,89,94]
Anergy of pattern recognition receptors to bacteria during resolution of inflammation [51,96]
Dysregulation of protective immune pathways (RIG-I, PKR, 2′-5′ OAS, PI3K) by NS-1 [122]
Alteration of bacterial clearance by viral effects on specific immune cells (macrophages, neutrophils, NK cells) [50,91,92,158]
Complementation of viral virulence by factors expressed by bacteria
Cleavage of influenza virus hemagglutinin by bacterial proteases [159]
Synergistic effects on inflammation and cell death of bacterial cytotoxins with the viral cytotoxin PB1-F2 [54]

Table 3

Immunomodulatory agents that may be useful as adjunctive treatment for secondary bacterial infections

Type / class of agent	Example(s)	Proposed target / mechanism of action
Corticosteroids	Dexamethasone, methylprednisolone	Pleiotropic anti-inflammatory effects [124]
Antibody-based therapies	IVIG, hyperimmune serum monoclonal antibodies	Specific neutralization of virus and non-specific binding of inflammatory intermediates [132,136]
Statins	Simvastatin, pravastatin, atorvastatin	Co-enzyme A reductase inhibitors, broad anti-inflammatory effects [160]
PPAR agonists	Gemfibrozil, piaglitazone, ciglitazone	Prevent excessive release of pro-inflammatory cytokines [143,146]
COX inhibitors	Celecoxib, mesalazine	Decrease pro-inflammatory cytokines and eicosanoids [144]
Antioxidants	N-acetyl-L-cysteine	Decrease production of pro-inflammatory mediators [161]
Herbs, extracts	Glycyrrhizin, *Angelica sinensis*, *Salvia miltiorrhiza*	Decrease production of pro-inflammatory cytokines [149,162]
Anti-inflammatory antibiotics	Azithromycin, clarithromycin	Eliminate super-infecting bacteria without lysis, potentially additional anti-inflammatory effects by unknown mechanism [11]