How do viral infections predispose patients to bacterial infections?
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Purpose of review
Bacterial sepsis is a leading cause of death in the United States, accounting for over 200,000 fatalities annually. Approximately half of bacterial sepsis cases occur following acute respiratory infections, and the lungs are the most common organs to fail. Notably, outbreaks of respiratory viral infections are associated with an increased incidence or severity of bacterial co-infections, with normally innocuous infections often becoming fatal. Understanding the 'lethal synergism' associated with concomitant infections may point the way toward improved anti-sepsis treatments.

Recent findings
Murine models of viral and bacterial co-infection mimic the lethal synergism observed in humans and reveal at least two mechanisms of interaction. First, bacterial infiltration is heightened during acute viral infection. Secondly, the nature of responding cell populations is dramatically altered during concomitant infections. Although natural killer cells and macrophages are predominant cell populations responding to bacterial infection in a naïve host, there is also a large T cell component that is activated upon viral infection. Inflammatory cytokines produced by these cells contribute to lethal immunopathology, and therapeutic strategies need to target the initial causative microbes as well as subsequent inflammatory responses. Current therapies directed only at the host immune response have not been overly successful, owing largely to difficulties in reversing the severe immunopathology associated with sepsis.

Summary
Respiratory viral infections may facilitate secondary bacterial infections and increase host immunopathology through the overproduction of inflammatory cytokines. Preventive measures, including vaccination and aggressive antimicrobial therapy early in the course of infection, may significantly reduce the morbidity and mortality of sepsis.

Keywords
sepsis, influenza, RSV, bacteria, synergism

Introduction
Sepsis ranks among the top 10 causes of death in the United States, with an estimated 750,000 cases per year and 215,000 fatalities [1]. Sepsis is mainly due to secondary bacterial infections (52% Gram-positive, 38% Gram-negative bacteria) or fungal infections (approximately 5% of sepsis cases) [2]. The lungs represent the most common site of infection during sepsis (Fig. 1), and these organs are the most likely to fail [1,3]. Given the high frequency of respiratory infections precluding the development of sepsis, it is striking that influenza and respiratory syncytial virus (RSV) outbreaks are often associated with a heightened incidence of subsequent bacterial infection [4–8]. Moreover, the clinical symptoms associated with bacterial superinfection in the context of an ongoing viral infection are more severe and prolonged than with either single infection alone. Experimental models of co-infection are therefore critical for designing successful therapeutic strategies to combat sepsis.

Epidemiology of concomitant viral and bacterial infections
Influenza and RSV account for nearly 70,000 deaths annually in the United States alone, with the elderly being particularly at risk [9,10]. From 1990 to 1999, the annual influenza and RSV-associated mortality rate was 26.2 deaths per 100,000 person-years. Individuals over 65 years of age contribute disproportionately to this value, averaging 162.1 deaths per 100,000 (Fig. 2) [9]. Among nursing home residents aged over 65 years, 15% of hospitalizations and 14% of deaths could be attributed to influenza and RSV [11]. Among individuals aged over 85 years were 32 times more likely than those aged days 65–69 years to die of influenza-associated causes [9]. Thus, there is clearly increased risk of influenza and RSV-associated mortality in aging populations.

Often, mortality is not due directly to influenza or RSV infection per se, but is instead the result of associated...
complications with other underlying causes [12]. Numerous epidemiological studies of influenza or RSV epidemics have shown temporal correlations between these viral infections and increased incidence of bacterial pneumonia [7,13,14]. A seasonal correlation between the appearance of influenza virus and pneumococcus, Haemophilus influenzae, and Staphylococcus aureus infections (the most common bacterial co-infections) are marked with decreases in incidence during the interepidemic periods [4–8,13,14].

Influenza infection complicated by secondary bacterial infection typically leads to more prolonged and severe clinical symptoms than those observed following influenza alone [5]. In two out of three of cases, there is a biphasic illness with initial viral infection causing rapid onset of fever, followed by an improved condition over 3–5 days and then the onset of secondary bacterial pneumonia. In approximately one third of patients there is no intervening period of improved condition separating the clinical effects of the concomitant viral and bacterial infections [7]. Collectively, these observations indicate that influenza and RSV infections result in a predisposition to secondary bacterial infection and those individuals over age 65 years, as well as persons with underlying comorbid conditions, are especially susceptible.

Animal models of sepsis

Animal models have been established to elucidate the mechanisms of viral/bacterial sepsis in order to characterize both the nature of the bacterial infiltration and the attendant inflammatory response. Early models used lipopolysaccharide (LPS), a component of Gram-negative bacteria, to induce endotoxemia in mice. The observation that mice kept in germ-free conditions were approximately fivefold more resistant to LPS than conventional mice indicated that prior antigenic exposure heightens the response to subsequent bacterial infection [15]. Later models have expanded on this point, showing conclusively that concurrent viral infection greatly enhances sensitivity to subsequent exposure to microbial products. Doses of influenza or staphylococcal enterotoxin B (SEB) that alone are not lethal to mice will instead induce 100% mortality if SEB is administered at the peak of infection [16]. Similarly, naïve mice readily tolerate doses of LPS that cause rapid mortality when administered at the peak of a normally non-lethal infection with lymphocytic choriomeningitis virus (LCMV) [17,18].

Lethal synergism has also been demonstrated by respiratory co-infection with live bacteria at the peak of a viral infection. Mice infected with a non-lethal dose of influenza exhibit 90–100% mortality after challenge with Streptococcus pneumonia [19], Bacillus thuringiensis [20], or Streptococcus pyogenes [21], and 50% mortality after exposure to Neisseria meningitides [22]. In each instance, bacterial infections that are not lethal by themselves become fatal if they occur at the peak of an ongoing viral infection.

Mechanisms of lethal synergy

One of the most readily observed differences between bacterial challenge of a naïve animal and a virally-infected animal is the much greater bacterial infiltration observed in the latter. Influenza-infected mice subse-
quently exposed to *S. pneumoniae* [19,23**], *N. meningitides* [22], or *S. pyogenes* [21**] exhibit high bacterial titers in the lungs, and the bacteria rapidly spread systemically. In contrast, mice that are not challenged with bacteria until after fully resolving the viral infection are able to completely clear bacteria from the lungs, without spread to the bloodstream [22]. Notably, while there was a transient increase in influenza titers in the lungs of mice co-infected with *S. pyogenes* compared with virus alone at 24 h post-infection, by 72 h there was no difference between these two groups [21**]. Therefore, the primary effect of dual infection appears to be heightened levels of bacterial infiltration/replication, with less effect on virus titers.

Certain viral infections may result in increased bacteremia by impairing the ability of the host to mount a productive immune response against subsequent bacterial infection. For example, experimental measles infection inhibits both innate and adaptive immune responses and allows a concurrent *Listeria monocytogenes* infection to persist [24*]. The accumulation of macrophages and neutrophils in the spleen, as well as T cell cytokine production and proliferation, are impaired by measles infection [24*,25*]. Thus, by crippling the host immune response, viral infection may create an environment that is more permissive to subsequent bacterial infection.

Another mechanism for virus-mediated augmentation of bacterial infiltration involves increased adherence of bacteria to host cells [13,14]. This may be triggered by a viral neuraminidase, which cleaves sialic acid residues on host cell surface carbohydrates, generating more bacterial binding sites. The neuraminidase inhibitor, oseltamivir, greatly reduces pneumococcal infiltration of lungs in a dual influenza/pneumococcus infection [26**].

Viral proteins on the surface of infected cells may also act as receptors for bacteria. Influenza infection of alveolar cells results in expression of viral hemagglutinin on the cell surface and increases adherence of *S. pyogenes*, but anti-hemagglutinin antibody inhibits bacterial infection *in vitro*, and *in vivo* [21**]. The results of these studies demonstrate how viral infections may increase bacterial adherence and infiltration, thereby allowing an otherwise innocuous bacterial infection to traverse epithelial barriers and become systemic.

An overactive host immune response often contributes to the pathology of viral/bacterial dual infection. In order to mount a productive immune response, effector cells must migrate to the site of infection and produce sufficient numbers of cytokines to orchestrate clearance of the invading organisms. There is a fine line, however, between an effective inflammatory response and excessive cytokine production that will result in immunopathology [27,28]. Viral/bacterial co-infection may push the host immune response to immunopathological levels (Fig. 3). In a murine model of influenza infection, subsequent exposure to either *S. pneumoniae* [23**,26**], *N. meningitides* [22], or *S. pyogenes* [21**] causes inflammation and enlargement of the lungs, due to infiltration of bacteria, as well as lymphocytes, neutrophils and macrophages. The immune cell infiltration that occurs after dual infection is much greater than with either viral or bacterial infection alone, and is likely a major contributing factor in the severe disruption of alveolar architecture [21**,22,23**,26**].

The timing of bacterial superinfection relative to the initial viral infection generally determines the nature of the antibacterial response and the innate and adaptive stages of antiviral immunity correlate with different degrees of sensitization to bacterial toxin and distinct patterns of inflammatory cytokine production. In the first 1–2 days after LCMV or vesicular stomatitis virus infection of mice, the innate immune response

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**Figure 3. Development of sepsis due to the combined effects of viral and bacterial infections and the consequent host immune response**

- **Mild to moderate disease**
- **Moderate to severe disease**
- **Severe, life-threatening disease or death**

Disease symptoms associated with either viral infections or bacterial infections alone are usually less severe than the symptoms observed during concomitant infection. Likewise, antiviral or antibacterial host immune responses may result in a certain level of disease severity, but the combination of viral infection, secondary bacterial infection, and an overly hyperactive host immune response may together result in severe and potentially lethal disease with the clinical features of sepsis.
predominates (i.e. mainly macrophages and natural killer cells), and there is heightened sensitivity to LPS-induced endotoxemia [29,30]. At these early stages of infection, virus-induced interferon-αβ and tumor necrosis factor (TNF)-α production by macrophages contribute substantially to endotoxemia [29,30]. However, in cases of human sepsis, the onset of septicemia rarely occurs at these early stages of infection, indicating that the innate immune response is not likely to be the major contributor to immunopathology in the clinical setting.

Adaptive immunity to viral infection typically peaks at 1–2 weeks after exposure, and correlates temporally with human susceptibility to secondary infection. Sensitivity to bacterial toxin-induced systemic shock is even greater at this later stage than during the innate response, and virus-activated T cells are significant contributors to the ensuing immunopathology [17,18,29,30]. When mice infected with influenza are challenged 7 days later with SEB, high levels of TNF-α and interferon-γ are found in bronchoalveolar lavage fluid and cytokine levels are higher, and persist longer than after exposure to either agent alone [16]. Similar observations have been made in mice infected with LCMV, followed 7–8 days later by challenge with LPS [17,18]. Interferon-γ gene-deficient mice are protected from endotoxemia, indicating a central role of this cytokine in virus-induced immunopathology [16–18]. At late stages of viral infection, LPS-induced endotoxemia is largely due to interferon-γ, and approximately 95% of this cytokine appears to be produced by virus-specific T cells [18].

**Therapeutic strategies**

Sepsis is a complex disease often driven by concomitant viral and bacterial infection, leading to severe immunopathology. Treatment strategies therefore need to be developed that encompass antiviral and antibacterial regimens, as well as a means to modulate a potentially excessive host immune response (Fig. 3). Several therapies have focused on the host response, in attempts to diminish immunopathology. However, these have not been successful, largely due to the difficulty in reversing the inflammatory process once it has reached such a late stage [31••,32•,33•].

Early therapeutic strategies designed to reduce inflammatory responses by antagonizing endotoxin or TNF-α generated rather disappointing results [31••]. Nine clinical trials that employed antibodies to neutralize endotoxin did not achieve statistically significant protection from septic shock [31••]. Compiled results of 10 trials that neutralized TNF-α showed a statistically significant, although only modest, 3.5% reduction in mortality [31••]. Endotoxin and TNF-α are two key contributors to inflammation, so the lack of a more pronounced effect of these therapies is somewhat surprising. One possible explanation may be that by the time sepsis is diagnosed, it is already too late for intervention to curtail the immunopathology and ensuing organ failure. In addition, inflammatory cytokines are required for clearance of microbial infections, so that too much suppression may preclude orchestration of a sufficient immune response [31••,32•,33•]. More recently, studies have investigated other means of modulating the host response in septic patients, including hemofiltration to remove inflammatory factors [34], administration of granulocyte-colony-stimulating factor to augment innate immunity [35], or corticosteroid therapy to curb excessive immune responses [36]. Unfortunately, none of these approaches has been very effective at reducing the mortality associated with sepsis.

The results of these studies highlight the challenges faced in treating infections once they have reached the level of sepsis and organ failure. One alternative may be to design therapeutics that can dampen, but not completely block, the host immune response. In a mouse model of influenza infection, blockade of the costimulatory molecule, OX40, with an OX40–immunoglobulin fusion protein reduces (but does not eliminate) host immunity [37*]. Thus, while viral clearance is normal after both primary and secondary influenza infection in OX40–immunoglobulin-treated animals, cachexia and weight loss are prevented, even when OX40–immunoglobulin administration is delayed until 3 days after infection. Therefore, administration of an agent that can modulate a potentially deleterious inflammatory response may provide a means to allow sufficient immune reactivity to occur while reducing immunopathology.

Aggressive treatment of the causative agents that initiate respiratory infections may also preclude escalation to sepsis. Clearly, any treatment that diminishes the magnitude of the initial viral infection will help to protect from subsequent bacterial infection. Amantadine and rimantadine are two antiviral agents that inhibit influenza replication [38] and treatment of influenza-infected mice 24 h before bacterial exposure completely blocked the mortality observed in untreated animals [20]. Other antiviral drugs, including oseltamivir, target viral neuraminidase and inhibit influenza replication [38], improving survival of mice co-infected with influenza and S. pneumoniae [26••]. Similar benefits were found in a clinical trial in which oseltamivir treatment within 36 h of onset of influenza symptoms reduced the median duration of illness by approximately 30%, and, importantly, reduced the emergence of secondary complications of pneumonia, bronchitis, sinusitis, and otitis media by 50% [39]. This indicates that antiviral therapy not only diminishes the symptoms of influenza, but also reduces secondary bacterial infections.
Prevention: the best therapy?
It is said that ‘an ounce of prevention is worth a pound of cure’, and this perspective may be far more important in preventing sepsis than previously realized or appreciated. Viral and bacterial vaccines are efficacious in limiting disease severity, especially among the elderly and those with underlying medical conditions [40–42]. In one study, influenza vaccination reduced pneumonia and influenza hospitalizations by 52% and overall deaths by 70% among 1898 individuals 65 years of age or older with chronic lung disease [40]. Another study found that influenza vaccination reduced pneumonia and influenza hospitalizations by 20–24% and overall deaths by 39–60% among more than 120 000 elderly individuals [41].

When stratified among healthy individuals versus those with high-risk conditions, including cardiopulmonary disease, diabetes, and immunosuppression, the benefits of vaccination were greatest in the latter group [42]. RSV is also a major contributor to morbidity and mortality among the elderly and new sub-unit RSV vaccines induce neutralizing antibodies in individuals over 60 years old [43,44], and may prove beneficial in preventing disease in the elderly.

Infection with pneumococcus is common among the elderly and especially those with preexisting conditions, so pneumococcal vaccination is also warranted. A retrospective analysis of 47,365 individuals 65 years of age or older found that while a pneumococcal polysaccharide vaccine did not affect the incidence of pneumonia, it did reduce the incidence of bacteremia by 44%, and also significantly reduced all-cause mortality [45**]. Another study of aged individuals found that influenza and pneumococcal polysaccharide vaccines reduced hospital admissions for influenza, pneumonia, and invasive pneumococcal disease by 32, 22 and 54%, respectively. Overall mortality was also reduced by 27% [46*]. A protein–polysaccharide conjugate pneumococcal vaccine reduced the rate of invasive disease in children under the age of 2 years by 69%, and may prove equally beneficial in the elderly [47*]. It is clear that vaccination significantly reduces disease risk and efforts should be made to ensure extensive vaccination coverage, particularly among high-risk individuals.

The current recommendations against using antibiotics in patients experiencing cold or flu-like symptoms are based upon a desire to reduce antibiotic resistance and clearly antibiotics are unnecessary in healthy children and younger adults. However, in vulnerable populations such as the elderly, aggressive use of antivirals and initiation of antibiotic therapy at the first signs of bacterial respiratory infection could significantly prevent secondary bacterial progression. Although the possibility of overtreating some of these individuals and increasing the pressure for antibiotic resistance are valid concerns, this should be considered in the context of the thousands of patients who succumb to secondary bacterial infections associated with RSV and influenza infections each year – deaths that might otherwise be prevented if early antiviral and antibiotic therapy is instituted.

Conclusion
In vulnerable populations, the unfortunate combination of viral and bacterial infection often results in sepsis, a major cause of death worldwide. Approximately half of sepsis cases initially involve respiratory infection and these viral infections can predispose a patient to subsequent protracted bacterial infection. Therefore, a better understanding of the nature of viral/bacterial synergism will provide insight into improving therapeutic strategies. Animal models of dual infection have identified mechanisms whereby viral infection induces heightened bacterial infiltration/replication, and has increased our knowledge of how an otherwise protective antimicrobial immune response can be pushed to pathological levels via the overproduction of inflammatory cytokines. This suggests that an effective therapy for sepsis will likely need to be combinatorial, targeting both the initial causative microbes (by vaccination or antimicrobial drugs), as well as the consequent host inflammatory immune response.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
* of special interest
** of outstanding interest

This is an extensive compilation of epidemiological data from over 10 million cases of sepsis.


This analysis uses virus surveillance data to calculate the number of deaths due to influenza and RSV from 1976 to 1999, and brings to light the high mortality rates observed in the elderly.


This study demonstrated the high risk of influenza and RSV infection among the elderly, and emphasized the need for disease prevention among this group.


This study provided an excellent analysis of the lethal synergism between influenza and S. pyogenes infections. The paper contains extensive pathological and histological data detailing bacterial infiltration of the lungs and quantitates systemic spread of bacteria to other tissues during co-infection.


This study defined a powerful murine model system for the testing of therapeutics targeting viral/bacterial co-infections.


These authors describe a novel murine model system for the analysis of measles virus infection in vivo and demonstrate that direct measles virus infection of hSLAM+ T cells inhibits their ability to proliferate after mitogen-mediated activation.


The authors developed a new transgenic mouse model that is permissive to measles virus infection in vivo and demonstrates that direct measles virus infection of hSLAM+ T cells inhibits their ability to proliferate after mitogen-mediated activation.


This paper describes how administration of a neuraminidase inhibitor during influenza infection prevents subsequent bacterial superinfection. This is an important model for testing antiviral agents that may preclude secondary bacterial infections by lessening the severity of the initiating viral infection.


This article describes the difficulties associated with sepsis therapies that target mediators of the host immune response or bacterial components, and provides a comprehensive meta-analysis of many recent clinical trials. Moreover, it describes the potential pitfalls associated with poorly designed clinical trials and suggests a rational approach to future clinical trial development.


This paper illustrates the many inflammatory mediators and signal transduction pathways that comprise the host immune response during sepsis, and points out potential and novel targets for therapeutic intervention.


The authors provide a detailed description of alterations in the host immune response associated with sepsis, and use this as a basis to outline potential therapeutic strategies.


This study identified OX40-immunoglobulin as an effective immunosuppressive agent that targets the co-stimulatory molecule, OX40. Importantly, OX40-immunoglobulin treatment allows sufficient activation of the host immune response to clear an influenza infection, but suppresses the response enough to prevent severe immunopathology.


Jackson LA, Neuzil KM, Yu O, et al. Effectiveness of pneumococcal polysaccharide vaccine in older adults. N Engl J Med 2003; 348:1747–1755. This analysis shows that pneumococcal vaccination of elderly individuals significantly reduces the occurrence of bacteremia. This is an important demonstration of the value of preventive measures in protecting this high-risk group.
