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Viral–bacterial co-infections in the respiratory tract

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Preceding or concurrent viral respiratory tract infection can predispose to secondary bacterial co-infection throughout the airway. The mechanisms by which viruses promote these superinfections are diverse and replete. Whereas we understand much as to how viruses damage the airway and dysregulate both innate and acquired immune responses which, in turn, supports bacterial growth, adherence and invasion into normally sterile sites within the respiratory tract, new information regarding these co-infections is being gained from recent advances in microbiome research and our enhanced appreciation of the contribution of bacterial biofilms, among others. The advanced understanding obtained by continued research efforts in all aspects of viral–bacterial co-infections of the respiratory tract will allow us to devise novel approaches for disease prevention as well as to develop more effective therapeutics.

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Viral–bacterial co-infections throughout the respiratory tract

In healthy persons, seasonal influenza usually resolves without consequence, however each year approximately 200 000 hospitalizations and 36 000 deaths occur in the U.S. alone [1]. At greatest risk for secondary bacterial pneumonia are children under the age of 1, adults over 65, pregnant women and individuals of any age with comorbid illnesses [2]. Longstanding evidence for the role of influenza virus in bacterial pneumonia derive from studies of the four well-documented pandemics of the last 100 years. Whereas *Streptococcus pneumoniae* (Spn) was the predominant bacterial pathogen associated with both the 1918 and 1968 pandemics, *Staphylococcus aureus* accounted for 44% of deaths in 1957. In the most recent pandemic (2009), interestingly the majority of deaths were now in persons under 65 [3•] with *Streptococcus pyogenes*, *Haemophilus influenzae* and other Gram negative rods identified as causative agents in addition to Spn and *S. aureus*. Despite the introduction of antibiotics and influenza vaccines in the period between the 1918 and 1957 pandemics, death from secondary bacterial pneumonia remains a significant problem and in fact, in part due to the rapid aging of the U.S. population, associated mortality has increased [2].

Multiple additional infections of the airway are predicated on a bacterial superinfection either subsequent to, or concurrent with, an ongoing upper respiratory tract (URT) viral infection due to influenza A, influenza B, respiratory syncytial virus (RSV), rhinovirus (RV), human coronavirus, parainfluenza virus and adenovirus (AV), among others [4]. Viral 'colds' predispose to bacterial rhinosinusitis in both adults and children [5,6] and are among the most common infections seen in primary care [7]. In one prospective longitudinal study in children, 8% of viral URTI were complicated by acute bacterial rhinosinusitis [6]. Spn, *H. influenzae*, *Moraxella catarrhalis*, *Staphylococci* and respiratory anaerobes predominate in both acute and chronic rhinosinusitis. In otitis media, or middle ear infection, virtually any URT virus, as well as some enteroviruses, can predispose the middle ear to invasion by bacteria that normally reside in the nasopharynx (NP) [8,9]. The predominant bacterial pathogens of OM are Spn, nontypeable *H. influenzae* and *M. catarrhalis*. Whereas the URT viruses are unique in terms of specific histopathology and nature of the evoked immune response, the net effect of all viral infections that lead to OM is compromise of the protective functions of the Eustachian tube and thus there is a bounty of evidence to support the parental lore that '*my kid gets a cold and a week later has an ear infection*' [10].

A positive association between viral RTI and bacterial superinfection has also been demonstrated in rhinitis [11,12], RSV-induced bronchiolitis [13], and acute expiratory wheezing [14]. Cystic fibrosis, an autosomal inherited disease that affects >60 000 persons worldwide is characterized by recurrent and chronic RTI [15] that are exacerbated by infection with common respiratory tract viruses [16]. *Pseudomonas aeruginosa* and *S. aureus* are the predominant causative agents of these exacerbations. Both viral and bacterial infections are also associated with exacerbations of chronic obstructive pulmonary disease (COPD) [17–19] with COPD expected to rank as the

third leading cause of death worldwide by 2030 (WHO, http://wwwwhoint/respiratory/copd/burden/en/.2015). A wide variety of URT viruses have been implicated as playing a role in bacterial exacerbations of COPD due to the 'usual suspects': *H. influenzae*, Spn, *M. catarrhalis*, *S. aureus*, *P. aeruginosa* and *Enterobacter* spp. In further support, a recent study [20] found that 15 days after experimental RV infection of subjects with COPD there was a sixfold increase in both the 16S copy number and a 16% rise in the number of proteobacterial sequences detected in sputum compared to baseline values obtained from sputum collected before RV inoculation, with *H. influenzae* predominating.

Mechanisms for viral predisposition to bacterial superinfection of the respiratory tract

Much of what we have learned to date has been gleaned from animal models which have shown that complex molecular mechanisms underlie the ability of viruses to predispose to bacterial superinfection (see Table 1). For detailed description, readers are referred to several reviews [21,22•]. Briefly however, as a general outcome, viral infection can induce destruction of the airway both histologically and functionally. Depending on the virus, the histopathology induced can be relatively mild or severe and include evidence of cell loss, goblet cell hyperplasia, altered mucus secretion and/or biochemistry, disruption of surfactant, reduced ciliary beat frequency, dis-coordinated mucociliary clearance function and reduced oxygen exchange [23]. Each of these effects has long been associated with potential mechanisms by which viruses predispose the respiratory tract to bacterial superinfection. Additional specific mechanisms associated with viral-bacterial co-infection are discussed in greater detail below.

Augmented bacterial adherence and colonization

Depending on the virus, bacterial species/strain and experimental system used, viral infection has been shown to promote bacterial adherence and airway colonization via a number of mechanisms. Using a ferret model, Peltola *et al.* [24] reported that influenza viruses of any subtype increased colonization of the NP by Spn, however only specific subtypes were associated with development of bacterial sinusitis or OM. These findings contribute to our understanding of why bacterial complication rates are greater during seasons when a particular influenza subtype predominates. As to mechanism, influenza viral neuraminidase has been shown to expose host cell receptors used for bacteria adherence due to its sialidase activity which alters epithelial cell surface carbohydrate moieties [1,23] and is also known to enhance bacterial adherence via activation of TGF-β which induces upregulation of expression of fibronectin and integrins to which bacteria bind [25••]. Moreover, stimulation of type I interferons (IFNs) by influenza virus leads to decreased production of the chemokine CCL2 resulting in impaired recruitment of macrophages (required for pneumococcal clearance) thereby promoting Spn colonization in mice [26]. Influenza virus also primes mice for pneumonia due to *S. aureus* [27] where both bacterial and viral titers are enhanced during co-infection. The investigators of this latter study hypothesized that viral titers increase after bacterial co-infection due to enhanced virus release from infected cells, but that bacterial titers increase due to alveolar macrophage (AM) impairment [28].

Other URT viruses also enhance bacterial adherence to both primary and immortalized epithelial cells with distinct differences noted amongst epithelial cell types in terms of response to infection with RSV, parainfluenza virus-3 or influenza virus [29]. Novotny and Bakaletz [30] recently showed that both RSV and AV induced upregulated expression of the cell surface glycoprotein intercellular adhesion molecule 1 (ICAM-1) by primary respiratory tract epithelial cells and that ICAM1 served as a cognate ligand for the Type IV pilus of nontypeable *H. influenzae*, thus promoting adherence of this Gram negative pathogen. RSV infection also enhances adherence of *P. aeruginosa* to both normal and CF epithelial cells [31] and this effect can be extended to Gram positive

Table 1

Mechanisms by which viruses predispose to secondary bacterial infection
Damage to airway epithelium/induction of hyperplasia/cell loss/exposure of basement membrane
Diminished ciliary beat frequency/disruption of mucociliary clearance/altered mucus rheology
Increased receptor availability on epithelial cells promotes augmented bacterial adherence
Dysregulated activation, migration and function of antigen presenting cells (alveolar macrophages, dendritic cells, tissue resident macrophages and T-cells)
Disruption of phagocyte function
Abnormal expression of antimicrobial/host defense peptides
Virus-induced type I interferons alter the phenotype of the immune response
Enhanced production of inflammatory mediators (cytokines, chemokines, acute phase reactants)
Generalized immunosuppression that leads to immune paralysis
Virus-mediated release of bacteria from biofilms
Viral dysregulation of nutritional immunity
Virus induced alteration of the microbiome with increase in pathogens associated with secondary infections

bacteria. After RSV infection, all Spn serotypes tested showed a significant 2-fold to 10-fold increase in adherence to two cell lines [32]. Similarly, in children, the load of Spn in the NP increased coincident with community acquired pneumonia or infection with either RSV or RV [33]. High NP colonization density with Spn is also observed in adults with viral URTI or HIV infection [34].

Dysregulation of the innate and adaptive immune response

Viral–bacterial co-infection subverts many aspects of mucosal immunity, of which the net result is a failure to control bacterial replication [35,36]. The complexity of this field is far beyond the scope of this brief review and as such, the reader is directed to several excellent comprehensive reviews [1,3•,10,23,36,37••,38•], however several of the main conclusions in the field are discussed here including the effect of viral infection on phagocyte function. Influenza virus induced depletion of AMs via promotion of apoptosis is well-known to facilitate bacterial superinfection [36,39]. Via tracking of dye-labeled AMs in a murine model, investigators showed that >90% of resident AMs were lost in the first week after influenza, with the remaining cells demonstrating a necrotic phenotype. Whereas >95% of the initial bacterial challenge dose was cleared within three hours by AM in non-influenza infected mice, in those with influenza co-infection ~50% of the bacterial inoculum remained recoverable at the same time point. In addition to reduction in cell numbers via depletion, phagocyte function can also be affected by viral infection. Influenza virus infection of AMs results in low level production of cytokines and chemokines necessary for recruitment and activation of neutrophils [36] and can suppress NADPH oxidase-dependent phagocytic bacterial clearance, thereby enhancing susceptibility to secondary bacterial infection [40].

As mentioned above, virus infection induced dysregulation of the proinflammatory cytokine response, including but not limited to that induced by influenza virus, is generally believed to play a major role in predisposing to secondary bacterial infection [3•,37••,41,42]. Whereas type I IFNs have well-characterized antiviral and immunostimulatory properties, when IFN production is mistimed, inappropriate and/or excessive, there can be detrimental effects. Key observations include the role of IFNs in promotion of production of specific cytokines such as immunosuppressive IL-10 and pro-inflammatory IL-6, suppression of cytokines key to the linkage between the innate and adaptive immune response including IL-17 and IL-23; reduced function of dendritic cells, macrophages, natural killer cells, CD4+ and CD8+ T-cells, all of which leads to impaired ability to effectively eradicate bacterial co-pathogens [1,37••,41,42].

There are also phagocyte independent mechanisms by which virus infection can predispose to secondary bacterial infection. Expression of antimicrobial (or host defense) peptides such as lipocalin2, CAMP, REG3B, S100A8 and S100A9, among others, can be dysregulated by URT viruses [37••]. In a chinchilla model [43], RSV infection reduced the transcript abundance of chinchilla beta-defensin 1 (cBD-1, an ortholog of human β-defensin 3, hBD-3) and further, concurrent RSV infection induced a 10–100-fold increase in the NP load of nontypeable *H. influenzae*. Delivery of anti-cBD1 intranasally (to inactivate available cBD-1 locally) or of either hBD-3 or recombinant cBD-1 (to augment available host defense peptides) demonstrated that disruption in availability of even a single innate immune effector due to a viral infection had a significant impact on the relative load of *H. influenzae* within the airway.

Immunosuppressive viruses

Whereas bacterial superinfections associated with generally immunosuppressive viruses (e.g. HIV, CMV, measles virus (MeV), among others) are not restricted to the respiratory tract, the role of these viruses need to be considered. Prior to the use of combination anti-retroviral therapy, pneumocystis pneumonia was the most prevalent HIV-associated pulmonary disease, however since broad use of these potent drug combinations, in high income countries this has changed to community acquired bacterial pneumonia and in low income countries, tuberculosis (TB) prevails [44]. The hallmark of an HIV infection is severe depletion of CD4+ T cells which contributes to the fact that persons living with HIV are 20–37 times more likely to develop TB than those without HIV [45]. HIV virus has also been reported to facilitate infection by *Mycobacterium tuberculosis* by a variety of effects on macrophage function including upregulated expression of *Mycobacterium* entry receptors, manipulation of macrophage bactericidal pathways, altered chemotaxis, induction of an immune response that results in a Th1/Th2 imbalance and an impaired tumor necrosis factor-mediated apoptotic response, each of which inhibits bacterial clearance [46]. Similarly, cytomegalovirus (CMV) infection is associated with immune system paralysis, characterized by increased IL-10 and NFκβ production, lymphopenia affecting natural killer cells specifically, depletion of IFN-γ producing T-cells early in infection and dysregulated cytokine production that results in increased tissue damage; each of which may enhance susceptibility to secondary bacterial infection [47]. MeV is another that results in generalized immunosuppression and, in severe cases, can predispose to bacterial pneumonia due to its ability to infect SLAM-positive lymphocytes and dendritic cells, induce T-cell apoptosis, alter lymphocyte trafficking, suppress proliferation of lymphocytes and result in a cytokine imbalance with increased levels of IL-4, IL-10 and IL-13, but suppressed levels of IL-12, the latter of which is associated with a prolonged Th2-biased immune response that leads to suppression of cellular immunity [48].

Virus-induced release of planktonic bacteria from a biofilm

To better understand the mechanisms responsible for the intermittent exacerbations of disease observed in patients with CF, RV was found to induce H_2O_2 via the action of dual oxidase 2 in primary CF airway cells. This release of H_2O_2 was sufficient to disperse planktonic bacteria from a biofilm formed on those cells by mucoid *P. aeruginosa* [49]. Similarly, influenza A virus mediates release of pneumococci from a biofilm [50••]. Intriguingly, when dispersed in this manner, the pneumococcal transcriptome undergoes tremendous and complex changes [51] with newly released bacteria demonstrating a more virulent phenotype. These released bacteria are then capable of re-initiating infection within the respiratory tract.

Viral dysregulation of nutritional immunity

Nutritional immunity is defined as host sequestration of trace minerals and other growth factors needed by bacteria in an effort to limit pathogenicity [52]. However, influenza virus can subvert this defense to promote pneumococcal growth during co-infection by providing host sialylated substrates as a bacterial nutrient source via desialylation of host glycoconjugates [53]. Similarly, Hendricks *et al.* [54••] showed that viral RTI, with induction of anti-viral interferons, promoted robust biofilm formation by *P. aeruginosa* via a mechanism of dysregulated iron homeostasis. RSV-infected cells increased apical release of the host iron-binding protein transferrin which was utilized by *P. aeruginosa* and induced biofilm formation by this microbe. These latter observations suggest a likely mechanism for the clinical observation that links URT virus infection with enhanced *P. aeruginosa* colonization in chronic diseases such as CF and COPD.

Virus induced alteration of the microbiome

The composition of the microbiome of the respiratory tract can be enriched for pathogens associated with secondary bacterial infection by a viral RTI [55]. Evaluating a cohort of children ages 6–36 months prospectively to determine changes in their NP microbiome co-incident with viral URI, Pettigrew *et al.* [56] reported shifting complex competitive relationships (both positive and negative) amongst resident bacteria. In a study of NP bacteria and respiratory viruses in symptomatic children with and without acute OM, Ruohola *et al.* [57] found that colonization of the NP with both Spn and *H. influenzae* increased the risk of acute OM and that either the presence of *M. catarrhalis* in the NP or RSV infection further increased this risk.

Evidence for superinfection wherein bacteria augment viral infection of the airway

Whereas more typically we think of viral URT infection predisposing to bacterial superinfection, recently we've learned that pre-existing bacterial infection can promote viral shedding [58••,59]. As an example, Spn was shown to augment human RSV infection *in vitro* and *in vivo* [60]. In this study, three pneumococcal strains enhanced RSV infection of primary normal human bronchial epithelial cells. Further, nasal colonization of cotton rats, followed three days later by intranasal challenge with RSV, resulted in strain-specific enhancement of RSV replication *in vivo*. Additionally, *H influenzae* increased the susceptibility and inflammatory response of airway epithelial cells to viral infections [61] via its ability to enhance expression of ICAM-1, which can in turn be used by RV for attachment.

Opportunities for development of novel treatment and/or prevention strategies

As we gain an increased understanding of the molecular mechanisms that underlie viral–bacterial superinfection, this improved knowledge provides opportunities to develop novel treatment modalities and prevention strategies. Whereas viral vaccine development can present some formidable obstacles, vaccination against those viruses associated with increased risk for secondary bacterial infections is believed to be an ideal strategy to mitigate this risk [62]. In addition to prevention, early antiviral therapy (with agents such as rimantadine, zanamivir, and oseltamivir) has been proposed for several viral–bacterial co-infections [2,63]. There is also interest in attempting to abrogate the overly robust host inflammatory response commonly elicited during viral–bacterial co-infection, wherein both controlling bacterial replication and quieting the host immune response has been suggested as an improved treatment strategy. Using a co-infection mouse model of influenza virus and *Legionella pneumophila* to separate 'resistance' (pathogen detection and elimination) from 'tolerance' (host adaptation to a given level of pathogen burden), Jamieson *et al.* [64] demonstrated that influenza can promote susceptibility to lethal bacterial co-infection even if that bacterial species is controlled by the host's immune system and suggest that this is due to the host's inability to tolerate tissue damage to the lung. Damjanovic *et al.* [65] showed that combined treatment with both azithromycin and dexamethasome best improved clinical outcome, bacterial clearance, cytokine reponses and immunopathology in a murine model of dual infection.

Conclusions

The ultimate goal of research in the field of viral–bacterial co-infections of both the upper and lower respiratory tract is to translate our improved understanding of the molecular mechanisms that underlie these superinfections into development of better diagnostics, treatment modalities and prevention strategies. This is particularly important as we anticipate the potential for future pandemics and emergence of novel viruses, the expansion of antibiotic resistance by bacterial pathogens and consider the impact of global travel on relative ease of transmission.

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