Ménage à trois of bacterial and viral pulmonary pathogens delivers coup de grace to the lung

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Respiratory infections account for more than 4 million deaths worldwide and the inclusion of tuberculosis related deaths pushes this figure close to 8 million [1]. Lower respiratory tract infections are the third leading cause of death worldwide [2] and are a WHO priority for vaccine development. Research into respiratory immune responses is therefore central to the campaign to decrease the global burden of disease. Public recognition of the threat of respiratory infection has recently increased due to publicity surrounding the emergence of the severe acute respiratory syndrome-associated coronavirus (SARS-CoV), which causes atypical pneumonia with high mortality [3–6].

Another significant respiratory pathogen that has also captured media interest is the influenza virus, which uses the surface expressed proteins haemagglutinin and neuraminidase to penetrate host cells [7]. Since these proteins are expressed on the surface of the virus they are exposed to the immune system. Mutations introduced into the viral genome during replication by the RNA polymerase results in antigenically distinct influenza strains (termed antigenic drift), thereby avoiding immune recognition. More alarmingly however, is the swapping of whole gene segments between two different influenza strains infecting the same cell (termed antigenic shift) [8]. Antigenic shift is thought to be responsible for influenza pandemics, including the 1918 ‘Spanish flu’ that killed at least 20 million people (more than that of the first world war) and infected approximately half of the world’s population. Unusually, this pandemic was particularly fatal in young adults. Although the sequence of the haemagglutinin gene from the virus is known [9], live virus is not available for study, making the basis for its high pathogenicity so far elusive.

The source of Spanish flu is thought to be an avian reservoir and a similar clinical outcome was observed during the ‘chicken flu’ outbreak in Hong Kong in 1997, caused by an avian influenza A (H5N1) strain. Six of 18 infected people died and symptoms associated with this infection were febrile influenza-like illness, upper and lower respiratory tract illness and multiorgan failure [10,11].

Unlike most animal models used to examine infectious disease, humans are never free of previous or concurrent infections. Additional complicating cofactors include malignancy (cancer or autoimmune conditions), toxin ingestion or inhalation (smoking, alcohol or drug abuse), trauma (for example surgery), and congenital defects. The precise combination of events required to cause mortality is often overlooked by focusing only on one pathogen. No one beyond infancy is naïve with respect to infection [12]. Indeed the sequence of infection history alters the response to subsequent unrelated organisms [13,14]. Simultaneous infections are the norm rather than the exception. Immune mediated elimination of one pathogen may allow another to escape unchecked leading to localized or systemic disease [15].

Studies performed during influenza pandemics showed that the incidence of associated bacterial pneumonia ranged from 2 to 18% depending on the population studied [16–18], Streptococcus pneumoniae, Haemophilus influenzae, Staphylococcus aureus, α-haemolitic streptococci, Escherichia coli, Neisseria meningitidis, Aspergillus fumigatus and Branhamella catarrhalis are the most prominent described to date [19,20]. This raises a ‘chicken and egg’ question. Does the viral infection predispose to bacterial super infection or vice versa? Also, is it the combination of infections that results in death and disease or an altered reaction to one pathogen? Though bacterial proteases may assist influenza infection by cleaving the haemagglutinin precursor into a form that allows viral attachment [21], more evidence suggests that influenza virus inadvertently assists bacterial replication in the respiratory tract. A paper in this issue of Clinical and Experimental Immunology by Seki et al. [22] investigates the interaction between 3 significant respiratory pathogens: S. pneumoniae, P. aeruginosa and influenza virus. Fatality only occurred in mice infected with all three pathogens and not in those with any two in combination. This is unlike the scenario reported previously in mice coinfected with S. pneumoniae and influenza virus [23,24]; a difference likely attributable to the dose of each pathogen used.

The mechanisms by which influenza virus increases the occurrence of secondary bacterial infections are numerous (Fig. 1). The epithelial layer covering the mucosa provides an effective first line barrier preventing pathogen (or commensal) entry (Fig. 1a). Respiratory viruses commonly replicate within the epithelium and may cause cell death by exhaustion of host protein synthesis during replication of the viral genome or direct lysis of the infected cell. Adherent staphylococci are visible in lung autopsy specimens from the 1957–58 pandemic in areas where virus replication had caused death of the respiratory epithelium [25]. Pneumococci also adhere in vitro to cultured tracheal biopsies...
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Fig. 1. Mechanisms responsible for exacerbation of bacterial infection by influenza virus. (a) Respiratory bacteria replicate in the extracellular spaces and/or intracellularly. These are usually prevented from reaching submucosal tissues by the impervious respiratory epithelium. Bacteria are cleared by antibody and/or intracellular killing. (b) Viral replication often results in damage or death of the infected cell. In the case of the respiratory epithelium this exposes areas to which bacteria can bind that would otherwise be masked. Bacterial adhesion molecules may also be upregulated by viral infection. Increased adherence and destruction of the epithelial barrier results in bacterial colonization of submucosal tissues. (c) The immune response to most viral infections is dominated by cytotoxic T cells (CTLs) and CD4 T cells secreting IFN-γ and TNF. These may not be appropriate for bacterial clearance. The immune response induced by the virus may even inhibit that to the bacteria. (d) Some viral infections cause apoptosis of macrophages and neutrophils both of which are vital for bacterial clearance. (e) Regardless of the mechanism of uncontrolled bacterial growth the outcome of two or more infections is excessive inflammation. This occludes the lung leading to disability and in extreme circumstances, death.
devoid of epithelium due to previous influenza infection in vivo [26] and to A459 cells preincubated with influenza [27]. A cytopathic virus can therefore ‘open the flood gates’ allowing access to submucosal layers or receptors to which bacteria may bind (Fig 1b).

Infected respiratory epithelial cells alert the immune system to the presence of an invader by release of inflammatory cytokines/chemokines. To engage recruited cells, the infected cell must also up-regulate a battery of adhesion, MHC and complement binding molecules. This is an evolutionary relationship between host and pathogen that allow certain bacterial strains to use these up-regulated molecules as a binding receptor. For example, influenza virus up-regulates platelet activating factor receptor (PAF-R) [28,29] to which certain strains of pneumococci expressing phosphocholine can bind [30–32]. Indeed inhibition of PAF-R delays mortality in influenza and pneumococcus dual infected mice [33]. This ability of viruses to enhance bacterial adhesion is not restricted to influenza, since adenovirus infection of a lung epithelial cell line also increases pneumococcus adherence [35]. The complement protein C3 acts as a substrate for adhesion of S. pneumoniae [34] and the polymeric receptor responsible for translocating dimeric IgA or pentameric IgM across mucosal epithelium inadvertently assists the movement of pneumococci across nasopharyngeal epithelial cells [35]. The action of influenza neuraminidase may directly expose critical structures required for secondary bacterial adherence [33]. Neuraminidase releases newly synthesized virus by cleaving sialic acid from host glycoproteins during budding, to prevent viral aggregation and inhibit virus binding to respiratory tract mucins. Consequently the pathogenic and commensal bacterial vista suddenly changes. Information regarding the ability of viruses to modulate bacterial attachment or adhesion molecules however, is limited by the incomplete knowledge of the precise receptors involved.

In addition to exposure of potential receptors, secondary pathogens may escape elimination because the immune system is otherwise occupied or of inappropriate make up (Fig 1c). Many interconnecting pathways exist to either prevent exaggerated immune responses or elicit the correct immunological environment. Type I cytokines produced by Th1 cells inhibit the development of Th2 cells [36] ensuring that the dominant response to infection is appropriate. Regulatory T cells produce immune suppressive cytokines that limit the extent of immunity and prevent bystander tissue damage and autoimmunity [37]. An imbalance of signals required to prevent bacterial infection may occur indirectly during influenza virus infection. In addition to inducing environmental alterations viruses have evolved numerous strategies to inhibit specific immune compartments. Macrophages are a prominent antigen presenting cell in the airways but their recruitment and activation are inhibited during influenza infection [38,39]. Neutrophil apoptosis [40,41] is also a feature of influenza virus infection. Since both of these immune compartments are obviously critical for clearance of bacteria any dysfunction will favour bacterial proliferation (Fig 1d).

The outcome of multiple infections may depend on timing. An initial infection with pneumococcus dampens mortality to subsequent influenza virus challenge [32]. Though the mechanism(s) responsible are not known the results generated are reminiscent of studies showing that previous influenza virus infection improves immunopathology to subsequent respiratory syncytial virus infection [13] in the lung. In the latter study TNF production during the second infection was reduced and may therefore explain the reduced cachexia and weight loss. However, pneumococcal infection 7 days after influenza virus infection severely exacerbates the disease otherwise observed with either infection alone, and leads to extensive pulmonary consolidation, enhanced bacteraemia and death [32]. Whatever the mechanism or pathogens involved, the outcome is usually occlusion of respiratory airways by excessive inflammatory cells (Fig 1c and Seki et al. [22]). A reduction of inflammation by depletion of cell subsets, inhibition of inflammatory cytokines or blockade of signals required for T cell activation [42], though beneficial, have yet to be tested in experimental systems containing multiple pathogens. We are rarely infected with a pathogen in the absence of other underlying infections or complications. The challenge in the future therefore will be to take these into consideration when designing and testing future immune therapeutics.

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