Clin Exp Immunol 2004; **137**:8–11 doi:10.1111/j.1365-2249.2004.02526.x

EDITORIAL REVIEW

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

T. HUSSELL & A. WILLIAMS *Centre for Molecular Microbiology and Infection, Imperial College London, London, UK*

(Accepted for publication 4 May 2004)

Keywords coinfection respiratory tract influenza bacterial pneumonia disease susceptibility

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-haemoltic 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 heamagglutinin 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

Correspondence: Dr Tracy Hussell, Centre for Molecular Microbiology and Infection, Lord Flowers Building, Imperial College London, Exhibition Road, London, SW7 2AZ, UK.

E-mail: t.hussell@imperial.ac.uk.

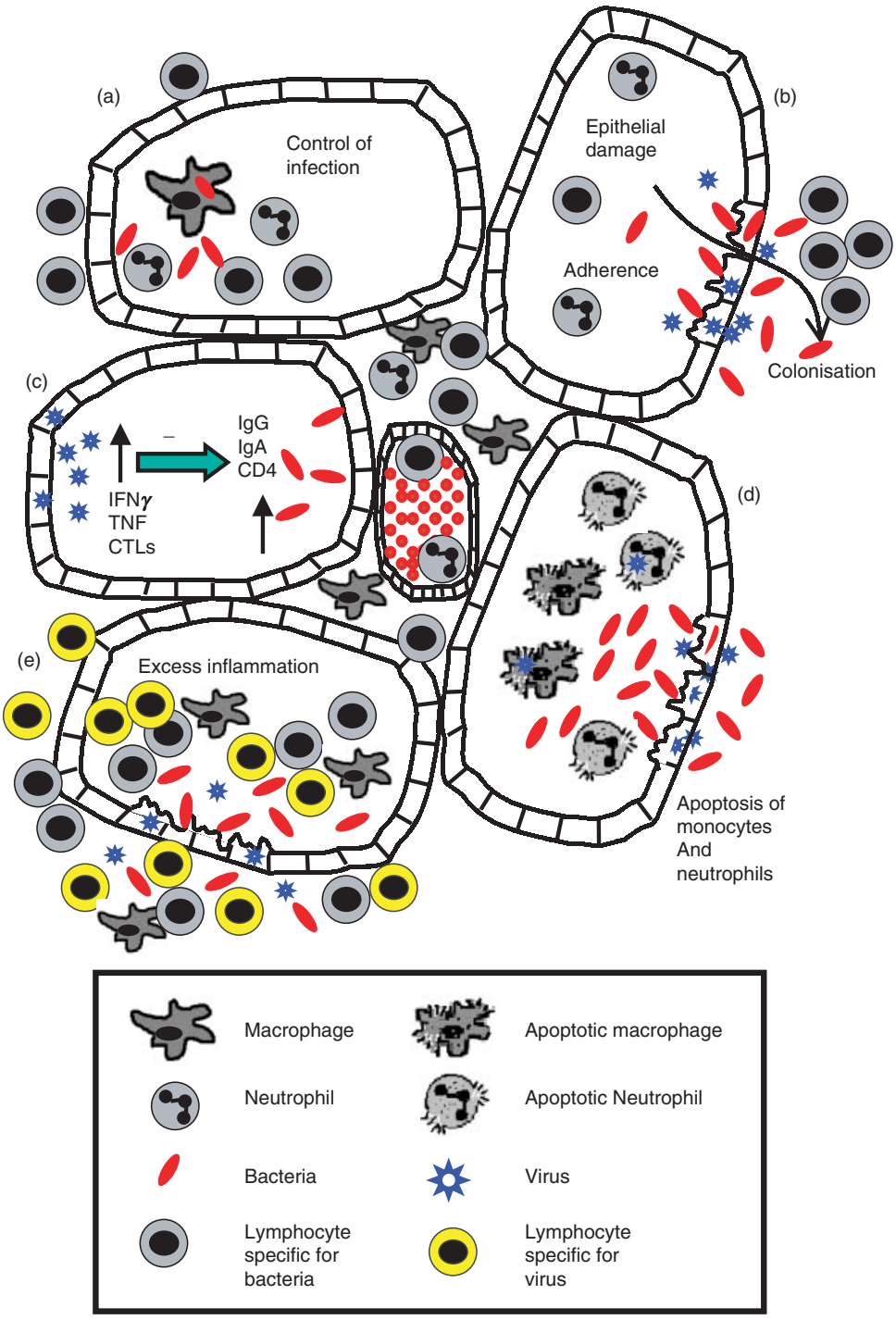

Fig. 1. Mechanisms responsible for exacerbation of bacterial infection by influenza virus. (a) Respiratory bacteria replicate in the extracellular spaces and/or intracellularly. These are usually prevented from reaching submucosal tissues by the impervious respiratory epithelium. Bacteria are cleared by antibody and/or by 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 up regulated 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-g 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 (PAFr) [28,29] to which certain strains of pneumococci expressing phosphorylcholine can bind [30–32]. Indeed inhibition of PAFrs 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 [33]. 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 nasopharygeal 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 1 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. 1e 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.

REFERENCES

1 Kremer L, Besra GS. Re-emergence of tuberculosis: strategies and treatment. Expert Opin Invest Drugs 2002; **11**:153–7.
2 Murray CJ, Lopez AD. Mortality by cause for eight regions of the world. Global Burden of Disease Study. L 1997; **349**:1269–76.
3 Lee N, Hui D, Wu A *et al.* A major outbreak of severe acute respiratory syndrome in Hong Kong. N Engl J Med 2003; **348**:1986–94.
4 Poutanen SM, Low DE, Henry B *et al.* Identification of severe acute respiratory syndrome in Canada. N Engl J Med 2003; **348**:1995–2005.
5 Ksiazek TG, Erdman D, Goldsmith CS *et al.* A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med 2003; **348**:1953–66.
6 Peiris JS, Lai ST, Poon LL *et al.* Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet 2003; **361**:1319–25.
7 Gimsa U, Grotzinger I, Gimsa J. Two evolutionary strategies of influenza viruses to escape host non-specific inhibitors: alteration of hemagglutinin or neuraminidase specificity. Virus Res 1996; **42**:127–35.
8 Ferguson NM, Galvani AP, Bush RM. Ecological and immunological determinants of influenza evolution. Nature (London) 2003; **422**:428–33.
9 Reid AH, Fanning TG, Janczewski TA, Taubenberger JK. Characterization of the 1918 'Spanish' influenza virus neuraminidase gene. Proc Natl Acad Sci USA 2000; **97**:6785–90.
10 Yuen KY, Chan PK, Peiris M *et al.* Clinical features and rapid viral diagnosis of human disease associated with avian influenza A H5N1 virus. Lancet 1998; **351**:467–71.
11 Webby RJ, Webster RG. Are we ready for pandemic influenza? Science 2003; **302**:1519–22.
12 Welsh RM, Selin LK. No one is naive. the significance of heterologous T-cell immunity. Nat Rev Immunol 2002; **2**:417–26.
13 Walzl G, Tafuro S, Moss P, Openshaw PJ, Hussell T. Influenza virus lung infection protects from respiratory syncytial virus-induced immunopathology. J Exp Med 2000; **192**:1317–26.
14 Selin LK, Varga SM, Wong IC, Welsh RM. Protective heterologous antiviral immunity and enhanced immunopathogenesis mediated by memory T cell populations. J Exp Med 1998; **188**:1705–15.
15 Openshaw PJ. When we sneeze, does the immune system catch a cold? Br Med J 1991; **303**:935–6.
16 Abrahams A, Hallows N, French H. A further investigation into influenzo-pneumococcal and influenza-streptococcal septicaemia. Lancet 1919; **1**:1–11.
17 Guthire J, Forsyth DM, Montgomery H. Asiatic influenza in the Middle East: an outbreak in a small community. Lancet 1957; **2**:590–2.
18 Gilroy J. Asian influenza. Br Med J 1957; **2**:997–8.

19 Louria D, Blumenfeld H, Ellis J, Kilborne ED, Rogers D. Studies on influenza in the pandemic of 1957-58. II. Pulmonary complications of influenza. J Clin Invest 1959; **38**:213–65.
20 Mills EL. Viral infections predisposing to bacterial infections. Annu Rev Med 1984; **35**:469–79.
21 Tashiro M, Ciborowski P, Klenk HD, Pulverer G, Rott R. Role of Staphylococcus protease in the development of influenza pneumonia. Nature (London) 1987; **325**:536–7.
22 Seki M, Higashiyama Y, Tomono K *et al.* Acute infection with influenza virus enhances susceptibility to fatal pneumonia following *Streptococcus pneumoniae* infection in mice with chronic pulmonary colonization with *Pseudomonas aeruginosa*. Clin Exp Immunol 2004; **137**:35–40.
23 McCullers JA, Bartmess KC. Role of neuraminidase in lethal synergism between influenza virus and Streptococcus pneumoniae. J Infect Dis 2003; **187**:1000–9.
24 LeVine AM, Koeningsknecht V, Stark JM. Decreased pulmonary clearance of S. pneumoniae following influenza A infection in mice. J Virol Meth 2001; **94**:173–86.
25 Hers JF, Masurel N, Mulder J. Bacteriology and histopathology of the respiratory tract and lungs in fatal Asian influenza. Lancet 1958; **2**:1141–3.
26 Plotkowski MC, Puchelle E, Beck G, Jacquot J, Hannoun C. Adherence of type I Streptococcus pneumoniae to tracheal epithelium of mice infected with influenza A/PR8 virus. Am Rev Respir Dis 1986; **134**:1040–4.
27 Peltola VT, McCullers JA. Respiratory viruses predisposing to bacterial infections: role of neuraminidase. Pediatr Infect Dis J 2004; **23**:S87–S97.
28 Hament JM, Kimpen JL, Fleer A, Wolfs TF. Respiratory viral infection predisposing for bacterial disease: a concise review. FEMS Immunol Med Microbiol 1999; **26**:189–95.
29 Tong HH, McIver MA, Fisher LM, DeMaria TF. Effect of lacto-N-neotetraose, asialoganglioside-GM1 and neuraminidase on adherence of otitis media-associated serotypes of Streptococcus pneumoniae to chinchilla tracheal epithelium. Microb Pathog 1999; **26**:111–9.
30 Cundell DR, Gerard C, Idanpaan-Heikkila I, Tuomanen EI, Gerard NP. PAf receptor anchors Streptococcus pneumoniae to activated human endothelial cells. Adv Exp Med Biol 1996; **416**:89–94.
31 Ishizuka S, Yamaya M, Suzuki T *et al.* Acid exposure stimulates the adherence of Streptococcus pneumoniae to cultured human airway epithelial cells: effects on platelet-activating factor receptor expression. Am J Respir Cell Mol Biol 2001; **24**:459–68.
32 McCullers JA, Rehg JE. Lethal synergism between influenza virus and Streptococcus pneumoniae: characterization of a mouse model and the role of platelet-activating factor receptor. J Infect Dis 2002; **186**:341–50.
33 Hakansson A, Kidd A, Wadell G, Sabharwal H, Svanborg C. Adenovirus infection enhances in vitro adherence of Streptococcus pneumoniae. Infect Immun 1994; **62**:2707–14.
34 Smith BL, Hostetter MK. C3 as substrate for adhesion of Streptococcus pneumoniae. J Infect Dis 2000; **182**:497–508.
35 Zhang JR, Mostov KE, Lamm ME *et al.* The polymeric immunoglobulin receptor translocates pneumococci across human nasopharyngeal epithelial cells. Cell 2000; **102**:827–37.
36 Mosmann TR, Sad S. The expanding universe of T-cell subsets. Th1, Th2 More Immunol Today 1996; **17**:138–46.
37 von Herrath MG, Harrison LC. Antigen-induced regulatory T cells in autoimmunity. Nat Rev Immunol 2003; **3**:223–32.
38 Kleinerman ES, Daniels CA, Polisson RP, Snyderman R. Effect of virus infection on the inflammatory response. Depression of macrophage accumulation in influenza-infected mice. Am J Pathol 1976; **85**:373–82.
39 Nickerson CL, Jakab GJ. Pulmonary antibacterial defenses during mild and severe influenza virus infection. Infect Immun 1990; **58**:2809–14.
40 Hinshaw VS, Olsen CW, Dybdahl-Sissoko N, Evans D. Apoptosis. a mechanism of cell killing by influenza A and B viruses. J Virol 1994; **68**:3667–73.
41 Colamussi ML, White MR, Crouch E, Hartshorn KL. Influenza A virus accelerates neutrophil apoptosis and markedly potentiates apoptotic effects of bacteria. Blood 1999; **93**:2395–403.
42 Humphreys IR, Walzl G, Edwards L, Rae A, Hill S, Hussell T. A critical role for OX40 in T cell-mediated immunopathology during lung viral infection. J Exp Med 2003; **198**:1237–42.