Pathogenesis of influenza-induced acute respiratory distress syndrome

Kirsty R Short, Edwin J B Veldhuis Kroeze, Ron A M Fouchier, Thijs Kuiken

Acute respiratory distress syndrome (ARDS) is a fatal complication of influenza infection. In this Review we provide an integrated model for its pathogenesis. ARDS involves damage to the epithelial–endothelial barrier, fluid leakage into the alveolar lumen, and respiratory insufficiency. The most important part of the epithelial–endothelial barrier is the alveolar epithelium, strengthened by tight junctions. Influenza virus targets these epithelial cells, reducing sodium pump activity, damaging tight junctions, and killing infected cells. Infected epithelial cells produce cytokines that attract leucocytes—neutrophils and macrophages—and activate adjacent endothelial cells. Activated endothelial cells and infiltrated leucocytes stimulate further infiltration, and leucocytes induce production of reactive oxygen species and nitric oxide that damage the barrier. Activated macrophages also cause direct apoptosis of epithelial cells. This model for influenza-induced ARDS differs from the classic model, which is centred on endothelial damage, and provides a rationale for therapeutic intervention to moderate host response in influenza-induced ARDS.

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Department of Viroscience, Erasmus Medical Center, Rotterdam, Netherlands
(K R Short PhD, E J B Veldhuis Kroeze DVM, Prof R A M Fouchier PhD, Prof T Kuiken PhD)

Correspondence to:
Prof Thijs Kuiken, Department of Viroscience, Erasmus Medical Center, Dr Molewaterplein 50, 3015GE Rotterdam, Zuid Holland, Netherlands
t.kuiken@erasmusmc.nl

Introduction

Influenza A virus is a respiratory pathogen that substantially affects human health worldwide. Seasonal viruses circulating in the human population cause annual epidemics with about 500 000 deaths per year.[1] A novel strain of influenza A virus without pre-existing immunity in the population could cause a global pandemic with variable mortality; the 2009 H1N1 pandemic caused 151700–575 400 deaths in its first year of circulation,[2] whereas the 1918 H1N1 pandemic caused more than 40 million deaths.[3] There is concern that newly emerging strains of influenza A virus from animal reservoirs (eg, avian-origin H5N1 and H7N9 viruses) could gain efficient transmissibility in human beings and cause a severe pandemic.[4,5] To mitigate the severity of such a pandemic, the mechanisms by which influenza A causes respiratory disease need to be understood.

The main complication of influenza virus infection is viral pneumonia, which often occurs together with, or is followed by, bacterial pneumonia.[6–11] In this Review, we focus on primary viral pneumonia, which can then lead to acute respiratory distress syndrome (ARDS).[12–16] Clinically, the acute phase of ARDS is characterised by cyanosis, hypoxaemia, pulmonary oedema, and increasing respiratory failure over time, resulting in multiorgan failure and a high mortality rate (up to 60%).[17] In addition to influenza A virus, various other disorders—eg, sepsis, pneumonia, trauma, and aspiration of gastric contents—can cause ARDS.[18] A major cause of respiratory failure in the acute phase of ARDS is damage to the epithelial–endothelial barrier of the pulmonary alveolus, where gas exchange takes place (figure 1). Damage to this barrier results in flooding of the alveolar lumen with proteinaceous oedema fluid containing fibrin, erythrocytes, and inflammatory cells. This oedema fluid reduces alveolar gas exchange and can result in severe respiratory insufficiency, as noted in patients with ARDS.[18]

Much of the understanding of damage to the epithelial–endothelial barrier in the acute phase of ARDS comes from studies of bacterial sepsis, in which the first site of damage is the endothelium.[18–20] This model might not be appropriate for influenza virus, which first infects the epithelium. Additionally, although some reviews have focused on different factors of influenza-virus-induced lung damage (including viral virulence factors,[21] cytokine production,[22] and pathological changes[23]) no review has brought these features together. We review the available literature on the interaction between influenza A virus and key cell types present in the pulmonary alveolus in the acute phase of ARDS. Specifically, we focus on the roles of pulmonary epithelial and endothelial cells, neutrophils, and macrophages in damage to the epithelial–endothelial barrier to develop a new conceptual framework for influenza-induced ARDS.

Epithelial cells

Among the first cells that influenza virus encounters after entering the alveolus are epithelial cells, either type 1 or type 2 pneumocytes. Type 1 pneumocytes are flat cells that cover 95% of the alveolar surface and allow easy diffusion of gas between air in the alveolar lumen and blood in the capillaries. Type 2 pneumocytes are cuboidal cells that secrete lung surfactant, which is important to reduce alveolar surface tension. Tight junctions at the sites where adjoining epithelial cells meet reduce the permeability of the alveolar epithelial cell layer. Tight junctions are composed of different proteins, including claudins and zona occludens 1, 2, and 3.[24] More than 90% of the resistance to protein transport across the epithelial–endothelial barrier arises from the alveolar epithelium.[25] By limiting protein transport, the epithelium maintains osmotic pressure across the barrier and prevents pulmonary oedema.[17]

A second mechanism by which the alveolar epithelium keeps the alveolar lumen free of fluid is through the action of ion channels and membrane proteins, which include the amiloride-sensitive epithelial sodium channels (ENaCs), the cystic fibrosis transmembrane conductance regulator, and many different aquaporins. The best characterised ion

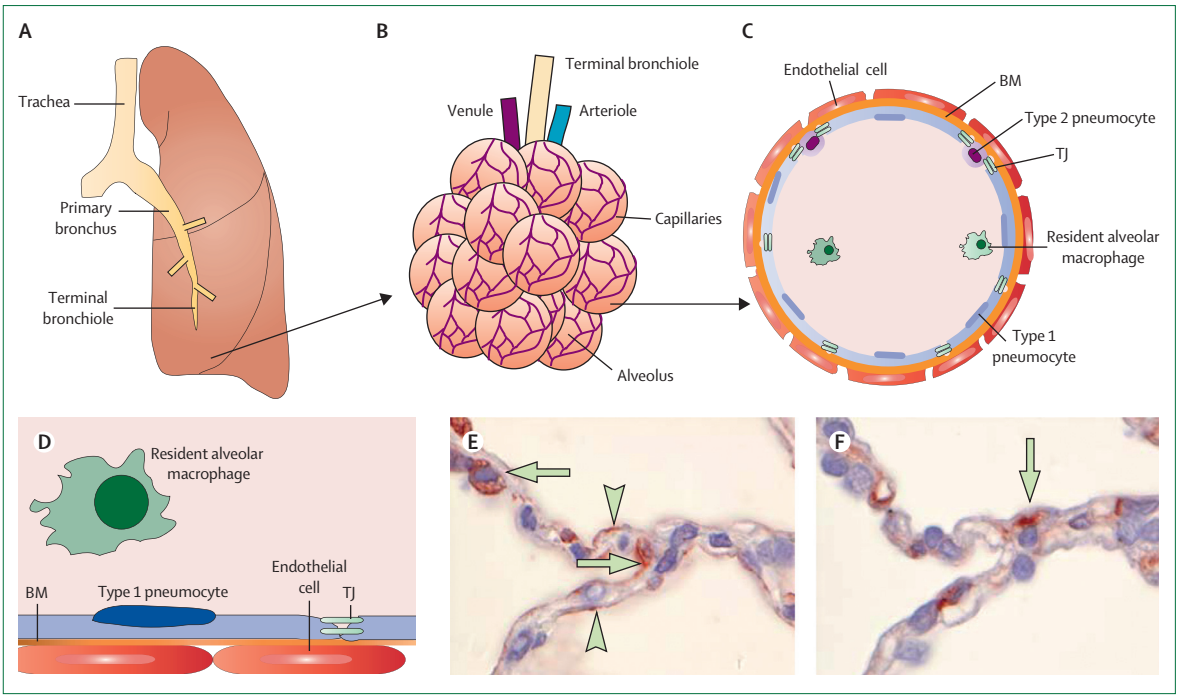

Figure 1: **Schematic representation of the epithelial–endothelial barrier in the human respiratory tract**
(A) In the lower respiratory tract, the trachea divides into primary bronchi and several levels of bronchi and bronchioles until the terminal bronchioles. (B) Each terminal bronchiole supplies an acinus of about 2000 alveoli. The alveolar walls contain a network of pulmonary capillaries, supplied with blood coming from pulmonary arterioles and draining into pulmonary venules. (C) Each alveolus contains several alveolar macrophages and is lined by flat type 1 pneumocytes and cuboidal type 2 pneumocytes. The pneumocytes lie on a basement membrane, which is directly juxtaposed to or fused with the basement membrane of the pulmonary capillaries, which are lined by endothelial cells. (D) Gas exchange between blood in pulmonary capillaries and air in alveolar lumina takes place across the epithelial–endothelial barrier, consisting of the alveolar epithelial layer, basement membrane or membranes, and pulmonary endothelial layer. (E) Specific staining for pankeratin stains type 1 pneumocytes (arrowheads) and type 2 pneumocytes (arrows) in a normal human alveolus (reduced by 13% from ×1000). (F) Specific staining for von Willebrand factor stains capillary endothelial cells (arrow) in a serial section of the same tissue shown in part E (reduced by 13% from ×1000). BM=basement membrane. TJ=tight junction.

channel in the lung is the ENaC, which is present on the apical surface of both type 1 and type 2 pneumocytes.[26,27] This channel is complemented by an Na^+/K^+ ATPase on the basolateral cell surface. Na^+ ions entering the channel at the apical surface are translocated to the basolateral cell surface, where Na^+/K^+ ATPase pumps them into the underlying interstitium (where present). The presence of Na^+ in the interstitium creates an osmotic gradient that removes water from the alveolar lumen through aquaporins and intracellular pathways in alveolar epithelial cells, thus preventing pulmonary oedema.[26,27]

Not only are pneumocytes a crucial component of the epithelial–endothelial barrier, but they are also target cells for infection by influenza A virus. Influenza A binds to its target cell by attachment of viral haemagglutinin to a sialosaccharide on the cell surface. The expression of sialosaccharides differs for different pneumocytes: type 1 pneumocytes express predominantly α-2,6-linked sialosaccharides, generally preferred by human influenza viruses, whereas type 2 pneumocytes express mainly α-2,3-linked sialosaccharides, generally preferred by avian influenza viruses.[28–30] Consistent with the tropism of avian influenza viruses for α-2,3-linked sialosaccharides, autopsy findings of patients who died from influenza H5N1 showed that H5N1 virus antigens are most prominent in type 2 pneumocytes.[31–33] By contrast, autopsy findings of fatal cases of infection with 2009 pandemic H1N1 virus (which has dual tropism for both α-2,3-linked and α-2,6-linked sialosaccharides[34]) showed antigen expression for pandemic H1N1 virus in both type 1 and type 2 pneumocytes.[35] These virus-dependent differences in attachment are dependent on specific aminoacid residues in the receptor-binding domain of the haemagglutinin globular head. Positions 190 and 225 (influenza A H1 virus) and positions 226 and 228 (influenza A H2 and H3 viruses) are the main determinants of receptor specificity for pandemic influenza viruses.[36] Mutations at these positions also define the receptor-binding preference of the influenza A H5N1 virus.[37] Thus, the type of alveolar epithelial cell targeted might differ between influenza A strains.

Very early after infection, influenza A virus causes fluid accumulation in the alveolar lumen by direct inhibition of ENaCs (figure 2).[38] In-vitro exposure of type 2 pneumocytes to influenza A/Puerto Rico/8/1934 (H1N1) for 1 h decreased ENaC activity while maintaining the integrity of the epithelium.[38] This decrease in activity was

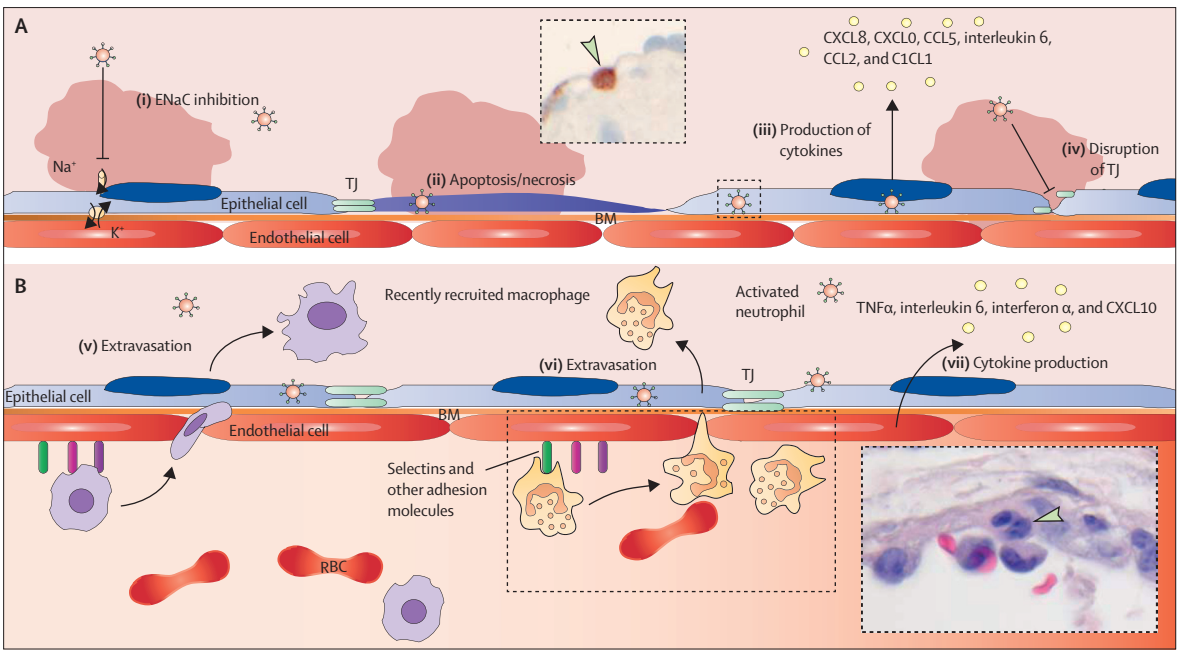

Figure 2: **The role of alveolar epithelial and endothelial cells in influenza-induced ARDS**
(A) In the early stages of ARDS, influenza A virus can inhibit the action of ENaCs (i) on alveolar epithelial cells. Later in infection, influenza virus can trigger the apoptosis or necrosis (ii) of alveolar epithelial cells, cytokine production (iii), and disruption of tight junctions (iv). Ultimately, these changes lead to the accumulation of proteinaceous fluid in the alveolus (pink). Specific staining for influenza A nucleoprotein shows influenza A H5N1 infection (inset) of an alveolar epithelial cell (arrowhead) in human lung tissue after 24 h (reduced by 22% from ×400). (B) After influenza A virus infection, the capillary endothelial cells enable the extravasation of monocytes (v) and neutrophils (vi) into the alveolus via the upregulation of adhesion molecules such as E-selectin and P-selectin. The inset shows adhesion of neutrophils (arrowhead) and other leucocytes to the endothelium of a pulmonary blood vessel from a ferret, 4 days after in-vivo infection with influenza A H5N1 (haematoxylin and eosin stain, reduced by 22% from ×800). The endothelium can produce various proinflammatory cytokines (vii) in response to influenza A. In both (A) and (B), epithelial cells represent both type 1 and type 2 pneumocytes. ARDS=acute respiratory distress syndrome. ENaC=amiloride-sensitive epithelial sodium channel. BM=basement membrane. RBC=red blood cell. TJ=tight junction. TNF=tumour necrosis factor.

due to activation of protein kinase C and phospholipase by the viral haemagglutinin.[38,39] The M2 ion channel of influenza virus might also inhibit the activity of ENaCs on epithelial cells by triggering the production of reactive oxygen species and subsequently activating protein kinase C.[40] Consistent with these findings, rats infected with influenza virus and then intratracheally given saline solution had significantly less fluid clearance from the lungs at 2 h after infection than did uninfected rats.[38]

At a later stage of infection, death of epithelial cells induced by influenza A virus plays a major part in damage to the epithelial–endothelial barrier by destruction of the physical epithelial layer.[41] Both types of cell death—apoptosis and necrosis—have been reported in post-mortem tissues of patients with ARDS induced by influenza virus.[42,43] The mechanisms by which influenza virus induces cell death have been shown in vitro,[44–46] although typically for bronchial or bronchiolar epithelial cells rather than alveolar epithelial cells. Infection of a human bronchiolar epithelial cell line (NCL-H292) with reassortant influenza A/England/939/69 (H3N2) resulted in caspase-8-dependent apoptosis.[44] Influenza virus can induce epithelial cell apoptosis through various different pathways, including the activation of protein kinase R, which subsequently upregulates proapoptotic genes (eg, *FAS*).[47] Alternatively, viral neuraminidase can activate transforming growth factor β to its active form, which then induces apoptosis by interacting with its cognate receptors.[48] The expression of non-structural protein 1 (NS-1), derived from influenza A/Hong Kong/483/97 (H5N1), is also sufficient to induce epithelial cell apoptosis in a caspase-dependent manner.[46] By contrast with influenza-virus-induced apoptosis of NCL-H292 cells, infection of primary human bronchial epithelial cells with a mouse-adapted strain of influenza A/Puerto Rico/8/1934 (H1N1) resulted in necrosis.[45] The viral polymerase complex (particularly the nucleoprotein) could contribute to influenza-virus-induced cell death by removal of the cap structures of host-cell mRNA (so-called cap snatching), which reduces the amounts of capped host mRNAs that are translated into functional proteins.[49] Because of the important role of the epithelium in minimisation of pulmonary oedema, the loss of epithelial cells as a result of influenza-virus-induced cell death has a major role in damaging the architecture of the lung.

The effects of epithelial cell death on damage to the epithelial–endothelial barrier could be compounded by the ability of influenza virus to damage tight junctions of epithelial cells. Specifically, some H5N1 strains might

	Cell description or source	Influenza A strain	Cytokines produced upon influenza virus infection
Human primary airway epithelial cells[52]	Derived from main bronchi and lobar or segmental bronchi of patients after surgical lung resection	A/Port Chalmers/72 (H3N2)	Interleukin 8
Human primary type 2 pneumocytes[53]	Isolated from human non-tumour lung tissue	A/Hong Kong/483/97 (H5N1), A/Vietnam/1194/04 (H5N1), A/Vietnam/3046/04 (H5N1), A/Hong Kong/54/98 (H1N1)	Interleukin 6, interferon β,* CXCL10, and CCL5 (Hong Kong/483/97 strain, Vietnam/1194/04 strain); interleukin 6, interferon β,* CXCL10, and CCL5* (Vietnam/3046/04 strain, Hong Kong/54/98 strain)
Primary mouse airway epithelial cells[54]	Derived from the lungs of uninfected BALB/c mice	A/PR/8/34 (H1N1)	Ccl2 and Ccl5
Normal human bronchial or tracheal epithelial cells[45]	Commercially available primary cells	A/PR/8/34 (H1N1)	Interleukin 8
U1752[45]	Cell line derived from a lung tumour originally diagnosed as a small-cell carcinoma	A/PR/8/34 (H1N1)	Interleukin 8, CXCL1, and CCL5
BEAS-2B[55]	Cell line derived from from normal human bronchial epithelium obtained from autopsy of non-cancerous individuals	A/Scotland/20/74 (H3N2)	Interleukin 8, CCL5, and interleukin 6
Primary ferret pulmonary epithelial cells[56]	Derived from the trachea of uninfected ferrets	A/Brisbane/59/07 (H1N1), A/Mexico/4482/09 (H1N1), A/Wisconsin/67/05 (H3N2), A/Vietnam/1204/03 (H5N1)	Interferon β,* Cxcl9,* Cxcl11,* Cxcl10,* TNFα,* interleukin 6,* interleukin 8,* and Cxcr3* (Brisbane strain); interferon β,*Cxcl9,* Cxcl10,* TNFα,* interleukin 6,* interferon α, and Cxcr3* (Mexico strain); interferon β,* Cxcl9,* Cxcl10,* TNFα,* interleukin 6,* interferon α* Cxcl11,* interleukin 8*and Cxcr3* (Wisconsin strain); interferon β,*Cxcl9,* Cxcl10,* TNFα,* interleukin 6,* interferon α,* Cxcl11,* interleukin 8,* and Cxcr3* (Vietnam strain)
A549[57]	Cell line derived from lung carcinomatous tissue	A/WSN/33 (H1N1)	TNFα,* interleukin 1β,* interleukin 6,* interleukin 8, and CCL5*
A549[58]	Cell line derived from lung carcinomatous tissue	A/New Caledonia/20/99 (H1N1), A/Beijing/353/89 (H3N2)	CCL2, CCL5, CXCL10, and interleukin 8
A549[45]	Cell line derived from lung carcinomatous tissue	A/PR/8/34 (H1N1)	Interleukin 8, CXCL1, CCL5, and CCL2
NCI-H292[59]	Cell line derived from a lymph node metastasis of a pulmonary mucoepidermoid carcinoma	H3N2	Interleukin 6, interleukin 8, and CCL5
NCI-H292[60]	Cell line derived from a lymph node metastasis of a pulmonary mucoepidermoid carcinoma	A/Sisen/2/92 (H3N2)	Interleukin 6, interleukin 8, and CCL5
NCI-H292[44]	Cell line derived from a lymph node metastasis of a pulmonary mucoepidermoid carcinoma	Reassortant virus: A/Puerto Rico/8/34 (H1N1) x A/England/939/69 (H3N2)	Interleukin 6, interleukin 8, and CCL5

*Determined by mRNA expression. TNF=tumour necrosis factor.

Table 1: **Cytokine production by cell type after influenza A virus infection of epithelial cells**

directly disrupt tight junctions through a PDZ-ligand-binding motif present in the ESEV consensus sequence in the carboxyl terminus of viral NS-1.[50] This sequence mediates binding of viral NS-1 to host proteins such as scribble and DLG1, which are important to the formation of tight junctions.[50] Accordingly, infection of Madin-Darby canine kidney cells with viruses possessing the ESEV motif disrupts the formation of tight junctions.[50] However, the expression of the ESEV motif is limited to a subset of influenza A strains.[50] Thus, direct targeting of tight junctions by viral NS-1 might not occur in all influenza virus infections.

Finally, in influenza virus infection, epithelial cells produce cytokines that can subsequently damage the epithelial–endothelial barrier. Post-mortem analysis of a fatal case of influenza virus H5N1 pneumonia showed the production of tumour necrosis factor α (TNFα) by alveolar epithelial cells.[51] Findings from in-vitro studies suggest that influenza-virus-infected alveolar epithelial cells produce other cytokines in addition to TNFα (table 1).[44,45,52–60] Generally, data from in-vitro studies must be interpreted with caution because results can differ dependent on the influenza virus strain and cell type used, and because many studies use primary cells or cell lines derived from the trachea, bronchus, or bronchiole (rather than from the alveolus). Findings of studies with primary human type 2 pneumocytes suggest that the increased production of CXCL10, interleukin 6, and CCL5 after infection with H5N1 (compared with infection with a seasonal H1N1 strain) could contribute to increased disease severity.[53] However, the mechanism by which these or other cytokines damage the epithelial–endothelial barrier is unclear. ENaC activity can be decreased by cytokines such as interleukin 1β.[61] Tight junctions can also be disrupted by cytokines such as TNFα.[62] Several cytokines produced by alveolar epithelial cells in response to influenza virus infection are also chemotactic, or can upregulate the expression of cell adhesion molecules on pulmonary endothelial cells, and thus enable the extravasation of leucocytes.[63] These cells can then damage the epithelial–endothelial barrier.

Endothelial cells

Endothelial cells are the most abundant cell type in the lung, constituting 30% of the total cell population.[64] In the alveolus, endothelial cells line the capillaries that form a

network in the alveolar walls. On their apical side, endothelial cells are in direct contact with circulating blood and form the site of attachment for recruited inflammatory cells. After appropriate activation, endothelial cells express cell adhesion molecules, which bind to their cognate ligands on leucocytes and mediate leucocyte extravasation.[17] On their basolateral side, endothelial cells lie on a basement membrane, which is closely apposed to (or even fused with) the epithelial basement membrane.[64] This intimate contact suggests that endothelial cells are strongly affected by signals and virus particles released from epithelial cells and inflammatory cells in the alveolar lumen. Activation of the endothelium by these signals and virus particles is essential to mediate an effective immune response against influenza A. However, activation of the endothelium might play a part in damage to the epithelial–endothelial barrier and contribute to pulmonary oedema.

Influenza virus infection can upregulate the expression of endothelial adhesion molecules (eg, E-selectin, P-selectin, ICAM1, and VCAM1), thereby enabling the recruitment of leucocytes to the alveolus (figure 2).[65,66] The presence of a large number of neutrophils and macrophages could damage the epithelial–endothelial barrier through various mechanisms. Infection with influenza A H5N1 virus results in increased expression of E-selectin and P-selectin on human endothelial cells compared with infection with 1918 pandemic H1N1 or a seasonal H1N1 strain.[66] This differential activation of the endothelium might help to account for the increased leucocyte recruitment that occurs in H5N1 influenza virus infection[67] and the consequent increase in alveolar damage. Influenza virus infection can also trigger cytokine production by pulmonary endothelial cells. Seasonal H3N2 and H1N1 viruses trigger the production of interleukin 6, CXCL9, and CXCL10 by human umbilical-vein endothelial cells,[68,69] whereas infection with H5N1 virus can trigger the production of CXCL10, TNFα, and interleukin 6.[66] Findings of murine studies have shown that influenza-virus-induced activation of the pulmonary endothelium contributes to disease severity in mice infected with 2009 pandemic H1N1 or seasonal H1N1 viruses.[70] Specifically, the pulmonary endothelium produced cytokines such as CCL2, CXCL10, interferon α, interleukin 6, TNFα, and interferon γ in response to influenza A virus and recruited leucocytes to the lung during infection.[70] Accordingly, inhibition of endothelial-induced cytokine production and leucocyte recruitment by treatment with a sphingosine-1-phosphate agonist significantly improved survival rates after infection with influenza virus.[70] Unfortunately, the mechanism by which endothelial-derived cytokines could have damaged the epithelial–endothelial barrier in influenza virus infection is unknown.[70] Nevertheless, these findings emphasise the important role that endothelial activation has in influenza-virus-induced damage of the alveolus.

Influenza A virus might also damage the epithelial–endothelial barrier by directly infecting endothelial cells, triggering cell death, and thereby creating sufficient endothelial damage to mediate pulmonary oedema.[65,71,72] In vitro, the presence of a multibasic cleavage site in haemagglutinin enables the productive replication of H5N1 viruses in endothelial cells.[66] In poultry, haemagglutinins with a multibasic cleavage site enable systemic virus replication because these haemagglutinins can be cleaved by ubiquitously expressed proteases, by contrast with haemagglutinins with monobasic cleavage sites that depend on trypsin-like proteases expressed only in the airways and gastrointestinal tract.[73] However, unlike the epithelial layer, the endothelial layer is not as important a barrier to fluid leakage from the capillary lumen to the alveolar lumen. Together with the basement membrane, the endothelial layer constitutes only 10% of the resistance to protein transport (which maintains osmotic pressure) across the epithelial–endothelial barrier.[25] Therefore, damage to the endothelial layer alone is not a key cause of pulmonary oedema. Exposure of sheep to endotoxin caused moderately severe injury to the lung endothelium but no pulmonary oedema, probably because the alveolar epithelium remained morphologically and functionally intact.[74] Furthermore, although H5N1 influenza virus and other influenza virus strains infect endothelial cells of human beings and laboratory mammals in vitro,[65,66,68,71,75,76] little evidence suggests that influenza virus infection of the endothelium occurs in vivo. Findings of post-mortem studies of fatal human cases show no infection or rare infection of endothelial cells by H5N1 and other influenza virus strains.[11,35,77] Similarly, findings of many detailed pathological studies of H5N1 and other influenza virus strains in laboratory mammals also show no evidence for endotheliotropism,[78] and extensive infection of the endothelium is seen only when H5N1 virus is given to cats through the intestine.[79] Together, these results suggest that influenza virus infection of endothelial cells is absent or rare in human beings and other mammals. Thus, endothelial activation plays a more important part than does direct infection of endothelial cells with influenza virus in damage to the alveolar epithelial–endothelial barrier.

Neutrophils

Neutrophils arrive in the pulmonary alveolus within 1 day of influenza virus infection,[67] where they join residential leucocytes such as alveolar macrophages. Neutrophils are short-lived, phagocytic granulocytes that, in response to proinflammatory stimuli, extravasate from the blood into the alveolar lumen. Extravasation of neutrophils has several steps: rolling along the endothelium, adherence to selectins and adherins on endothelial cells, and migration through both the endothelial and the epithelial layer into the alveolar lumen.[80] This migration can cause temporary damage to the epithelial–endothelial barrier, but is not sufficient to cause pulmonary oedema.[80–82]

After entering the alveolar lumen, neutrophils become activated in response to locally present cytokines and pathogens. Activated neutrophils can phagocytose pathogens. The resulting phagosome can fuse with intracytoplasmic primary, secondary, and tertiary granules, which contain various toxic compounds to kill phagocytosed pathogens. These toxic compounds can be released extracellularly after contact with indigestible material (eg, immune complexes deposited on a basement membrane), phagocytosis of membranolytic substances (eg, urate crystals), or fusion of the granules with the phagosome before it is completely closed.[17,83–85] In addition to phagocytosis, neutrophils can trap and kill pathogens by forming neutrophil extracellular traps, which consist of extracellular DNA studded with histones, chromatin, and antimicrobial compounds.[86] The importance of neutrophils in limiting the replication and spread of influenza virus is shown by the fact that mice depleted of neutrophils have increased virus titres in the lungs and extrapulmonary sites compared with control mice.[87]

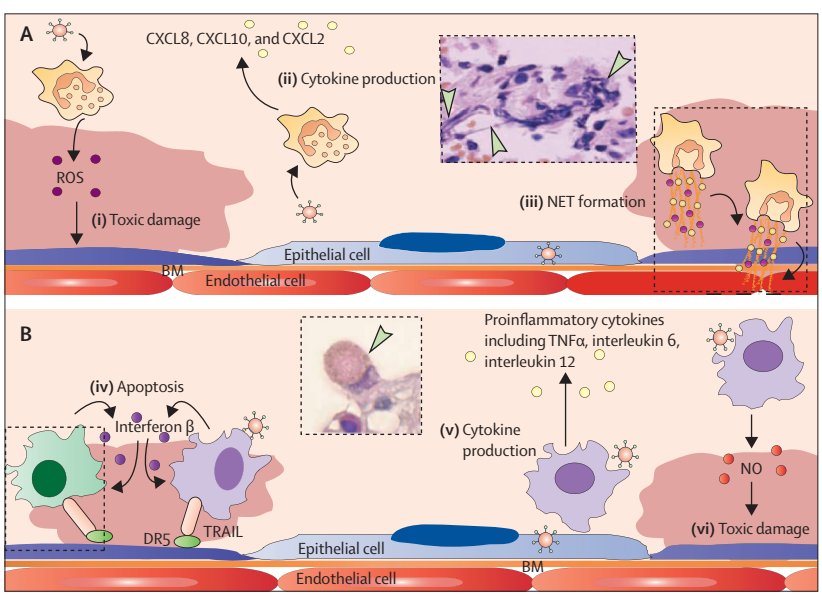

Figure 3: **The role of neutrophils and macrophages in influenza-induced ARDS**
(A) Neutrophils can damage the epithelial–endothelial barrier in influenza virus infection via the production of reactive oxygen species (i). In response to influenza virus infection, neutrophils also produce cytokines (ii), which can then indirectly damage the epithelial–endothelial barrier. The production of NETs (iii) can also damage epithelial and endothelial cells. The inset shows NETs (arrowheads) in large blood vessels of a mouse after in-vivo infection with influenza virus (haematoxylin and eosin stain). (B) Macrophages can damage the epithelial–endothelial barrier directly by influenza-induced expression of TRAIL, which interacts with DR5 (iv) to cause epithelial cell apoptosis. TRAIL expression is triggered by interferon β. In response to influenza virus, macrophages also produce various cytokines (v) that can indirectly damage the epithelial–endothelial barrier. The inset shows a macrophage (arrowhead) interacting with an alveolar epithelial cell in human lung tissue, 24 h after ex-vivo infection with influenza A H5N1 virus (haematoxylin and eosin stain, reduced by 33% from ×500). Finally, influenza A induces macrophages to mediate the production of NO (vi) and the subsequent formation of peroxynitrite, which can damage the epithelial–endothelial barrier. In both (A) and (B) epithelial cells represent both type 1 and type 2 pneumocytes. Newly recruited macrophages are depicted in dark pink, and resident alveolar macrophages are depicted in green. Accumulation of proteinaceous fluid in the alveolus is shown in pink. ARDS=acute respiratory distress syndrome. BM=basement membrane. ROS=reactive oxygen species. ROS=reactive oxygen species. NET=neutrophil extracellular trap. TNF=tumour necrosis factor. NO=nitric oxide. DR5=death receptor 5. TRAIL=tumour-necrosis-factor-related apoptosis-inducing ligand. Inset of part iii reproduced from Narasaraju and colleagues[90] by permission of Elsevier.

However, neutrophils might also have an important role in damage to the epithelial–endothelial barrier. In human cases of ARDS, neutrophil concentrations in bronchoalveolar lavage fluid are positively correlated with disease severity.[88] Similarly, pulmonary lesions in influenza-virus-infected mice are reduced by blocking of neutrophil recruitment to the lungs.[89] Although several mechanisms have been proposed as to how neutrophils might damage the epithelial–endothelial barrier in ARDS, those best characterised for influenza virus infection are the ability of neutrophils to produce reactive oxygen species, cytokines, and neutrophil extracellular traps (figure 3).

Neutrophils can produce reactive oxygen species (eg, superoxide) through NADPH oxidase, a multicomponent enzyme complex consisting of proteins in the cytosol and cytochrome b558 on the membrane of secondary granules. Fusion of secondary granules with phagosomes or the plasma membrane results in production of reactive oxygen species in the phagosome or extracellular environment.[91] Accordingly, reactive oxygen species can not only kill pathogens such as influenza virus, but also damage the alveolus.[92,93] Consistent with this notion, removal of superoxide (by injection of superoxide dismutase) protected mice from lethal influenza virus infection.[94] Similarly, influenza virus infection caused less tissue damage in NADPH-oxidase-deficient mice than in wild-type mice.[95]

Myeloperoxidase is present in primary granules and enables the production of hypochlorous acid from hydrogen peroxide and free chloride ions. Myeloperoxidase, interacting with the macrophage mannose receptor, can trigger macrophages to release reactive oxygen species and cytokines.[96] These effects can not only inactivate influenza virus,[97] but also damage the epithelial–endothelial barrier.[98] Accordingly, influenza virus infection causes less disruption of the pulmonary architecture and less pulmonary oedema in myeloperoxidase-deficient mice than in wild-type mice.[99] Additionally, myeloperoxidase-deficient mice also showed increased expression of claudin 9 and claudin 18-1,[99] suggesting that myeloperoxidase could damage the epithelial–endothelial barrier by disrupting tight junctions. How important myeloperoxidase-induced damage is to influenza-virus-induced mortality is not clear; although survival did not differ between wild-type and *Mpo*$^{-/-}$ mice inoculated with a high viral dose, the knockout mice had a trend for prolonged survival at a lower dose.[99]

Neutrophils might indirectly damage the epithelial–endothelial barrier by producing cytokines. Influenza virus infection triggers neutrophils to produce CXCL2 and interleukin 8,[100] both of which recruit additional neutrophils to the site of infection. Neutrophils are also the key producers of CXCL10 after influenza virus infection.[101] Influenza virus infection caused less pulmonary injury and mortality in Cxcl10-deficient mice

than in wild-type mice. Neutrophil-derived CXCL10 might act in an autocrine fashion and bind to its receptor CXCR3, which is expressed on neutrophils. In doing so, CXCL10 triggers the generation of superoxide and enhances chemotaxis towards CXCL2.[101] Accordingly, treatment of ferrets with a Cxcr3 antagonist reduced the severity of clinical signs after H5N1 virus infection.[102]

Neutrophils might also damage the epithelial–endothelial barrier through the production of neutrophil extracellular traps.[90] Mice infected with influenza A/Puerto Rico/8/1934 developed neutrophil extracellular traps both in the lumina of alveoli and in terminal bronchioles in areas of tissue damage, and attached to the endothelium in areas of haemorrhage.[90] These results suggest that neutrophil extracellular traps could damage both alveolar epithelial and endothelial cells. The role of influenza virus in the production of neutrophil extracellular traps in vivo was supported in vitro by co-incubation of neutrophils and influenza-virus-infected epithelial cells.[90] However, the survival rate of mice deficient in peptidyl arginine deiminase 4 (an enzyme that is essential for formation of neutrophil extracellular traps) did not differ substantially from that of wild-type mice after influenza virus infection,[103] suggesting that formation of neutrophil extracellular traps might not be crucial for damage to the epithelial–endothelial barrier.

Macrophages

Like neutrophils, macrophages are an important component of the innate immune response against influenza virus infection. Macrophages ingest pathogens and infected or apoptotic cells. Phagocytosed pathogens are then killed through a so-called respiratory burst and the production of reactive oxygen species and nitric oxide.[93] Macrophages can also produce a broad range of both proinflammatory and anti-inflammatory cytokines. Within the alveolus, two types of macrophages need to be distinguished. The first type are the alveolar macrophages, which are long-lived resident cells that occur at a density of about seven per alveolus.[104] They typically express an alternatively activated phenotype to minimise pulmonary inflammation in response to innocuous pathogens, while still protecting the lung from more virulent pathogens.[105] Their importance in control of influenza virus infection is shown by the fact that depletion of alveolar macrophages before influenza virus infection leads to increased viral replication and disease severity.[90] In influenza virus infection, alveolar macrophages are soon outnumbered by monocytes that are attracted to the alveolus by chemokines (eg, CCL2, CCL5, and CXCL10). These monocytes migrate from the blood to the alveolar lumen, where they differentiate into macrophages. This second type of macrophages—recently recruited macrophages—typically have a classically activated phenotype. Although blocking of monocyte recruitment to the lung leads to increased virus replication,[106] recently recruited macrophages could also have an important role in damage to the epithelial–endothelial barrier. After influenza virus infection, mice deficient in CCR2 (the receptor for CCL2, which mediates monocyte chemotaxis) had reductions in both monocyte recruitment to the lungs and severity of pulmonary lesions.[107] The main mechanisms for damage by recently recruited macrophages are related to release of tumour-necrosis-factor-related apoptosis-inducing ligand (TRAIL, also known as TNFSF10), production of nitric oxide, and production of proinflammatory cytokines (figure 3).

After infection with influenza A/Puerto Rico/8/1934 recently recruited macrophages express TRAIL, which interacts with death receptor 5, a protein that is upregulated on alveolar epithelial cells in influenza virus infection; this interaction induces apoptosis of these cells. Accordingly, inhibition of TRAIL reduced the rate of epithelial cell apoptosis, the amount of pulmonary oedema, and the mortality rate of mice infected with influenza A virus.[108] TRAIL production is dependent on activation of protein kinase R by influenza A virus, and subsequent production of interferon β. Autocrine interaction of interferon β with its receptor (interferon α, β, and ω receptor) triggers the production of TRAIL in a JAK–STAT-dependent manner.[109] In accordance with this pathway, blocking of interferon β signalling in mice impaired TRAIL production and reduced alveolar epithelial damage after influenza virus infection.[109]

Macrophages—and, to a lesser degree, neutrophils—that are stimulated by proinflammatory cytokines express inducible nitric oxide synthase (NOS2), which mediates the production of nitric oxide.[93] Nitric oxide can combine with superoxide to form peroxynitrite, which can both kill pathogens[110] and damage cells.[111] Inhibition of NOS2 activity reduced the extent of pneumonia and mortality rate after lethal influenza virus infection.[112] These findings complement the finding that, unlike wild-type mice, NOS2-deficient mice do not develop pneumonia after influenza virus infection.[113] Karupiah and colleagues[113] suggested that NOS2 expression contributes more to severity of pneumonia than to cytopathic effects of influenza virus infection, because increasing viral titres in NOS2-deficient mice by blocking of interferon γ still did not induce mortality. The findings of this study emphasise the importance of pulmonary damage induced by nitric oxide in influenza virus infection.

The production of cytokines by macrophages is broad (table 2),[67,114–126] and depends on both strain of influenza virus and type of macrophage. The importance of these cytokines to protect against influenza virus is shown by the fact that viral NS-1 acts at several levels to interfere with interferon production and interferon-mediated induction of antiviral proteins.[127] For example, the Asp92Glu mutation in NS-1 renders influenza A H5N1 virus relatively insensitive to interferon α, interferon γ, and TNFα.[128] Typically, however, H5N1 strains induce higher concentrations and a more diverse repertoire of cytokines in macrophages than do other influenza A strains,[67,115,120,121] and whereas this

	Influenza A strain
Infection of monocyte-derived macrophages	
Interleukin 1β[67,114–116]	A/HK/486/97(H5N1),* A/Beijing/353/89(H3N2), A/PR/8/34(H1N1), A/Vietnam/1203/04(H5N1),* A/HK/54/98(H1N1),* A/Vietnam/3212/04(H5N1),* A/Thailand/16/04(H5N1), A/Thailand/SP/83/04(H5N1)
Interleukin 10[115]	A/HK/486/97(H5N1)*
Interleukin 12[67,115]	A/HK/486/97(H5N1),* A/HK/483/97(H5N1),* A/HK/1174/98(H3N2),* A/Thailand/16/04(H5N1), A/Thailand/SP/83/04(H5N1), A/South Carolina/1/18(H1N1), A/Texas/36/91(H1N1)
CCL3[115,117–119]	A/HK/486/97(H5N1),* A/HK/483/97(H5N1),* A/HK/1174/98(H3N2),* A/Beijing/353/89(H3N2), A/Vietnam/1203/04(H5N1),* A/Vietnam/3212/04(H5N1),* A/HH/01/09(H1N1), A/California/07/04(H3N2), A/Thailand/1(Kan-1)/04(H5N1)
CCL4[67,115]	A/HK/486/97(H5N1),* A/HK/483/97(H5N1),* A/Thailand/16/04(H5N1), A/Thailand/SP/83/04(H5N1)
CCL2[115,117,118,120]	A/HK/486/97(H5N1),* A/HK/483/97(H5N1),* A/Vietnam/HN3028(H5N1),* A/California/04/09(H1N1),* A/HK/403946(H1N1),* A/Beijing/353/89(H3N2), A/Vietnam/1203/04(H5N1),* A/Vietnam/3212/04(H5N1),* A/Thailand/16/04(H5N1), A/Thailand/SP/83/04(H5N1)
CCL5[115,117,118,121,122]	A/HK/486/97(H5N1),* A/HK/483/97(H5N1),* A/Beijing/353/89(H3N2), A/Vietnam/1203/04(H5N1), A/Thailand/1(Kan-1)/04(H5N1),* A/PR/8/34(H1N1)*
Interferon α[114–116,123,124]	A/HK/486/97(H5N1),* A/Beijing/353/89(H3N2), A/Finland/553/09(H1N1),* A/PR/8/34(H1N1), A/Thailand/1(Kan-1)/04(H5N1)*
Interleukin 6[67,116,119,120,122]	A/Vietnam/HN3028(H5N1),* A/California/04/09(H1N1),* A/HK/415742(H1N1),* A/HK/403946(H1N1),* A/PR/8/34(H1N1), A/Vietnam/1203/04(H5N1), A/HH/01/09(H1N1), A/California/07/04(H3N2), A/Thailand/1(Kan-1)/04(H5N1), A/Thailand/16/04(H5N1), A/Thailand/SP/83/04(H5N1)
Interferon β[114–117,123,124]	A/HK/486/97(H5N1),* A/HK/483/97(H5N1),* A/HK/54/98(H1N1),* A/HK/1174/98(H3N2),* A/Beijing/353/89(H3N2), A/Finland/553/09(H1N1),* A/PR/8/34(H1N1), A/Vietnam/1203/04(H5N1),* A/Vietnam/3212/04(H5N1),* A/Thailand/1(Kan-1)/04(H5N1)*
TNFα[114–117,120,121,123–126]	A/HK/486/97(H5N1), A/HK/483/97(H5N1), A/HK/54/98(H1N1), A/HK/1174/98(H3N2), A/Beijing/353/89(H3N2), A/Vietnam/1203/04(H5N1), A/Vietnam/1194/04(H5N1), A/Thailand/MK2/04(H5N1), A/Vietnam/3046/04(H5N1), A/Vietnam/HN3028(H5N1),* A/California/04/09(H1N1),* A/HK/415742(H1N1),* A/PR/8/34(H1N1), A/HK/403946(H1N1),* A/Vietnam/3212/04(H5N1),* A/HH/01/09(H1N1), A/California/07/04(H3N2), A/Thailand/1(Kan-1)/04(H5N1), A/Thailand/16/04(H5N1), A/Thailand/SP/83/04(H5N1), A/Finland/553/09(H1N1),* A/PR/8/34(H1N1)*
CFS2[67]	A/Thailand/16/04(H5N1), A/Thailand/SP/83/04(H5N1)
CFS3[67]	A/Thailand/16/04(H5N1), A/Thailand/SP/83/04(H5N1)
Interleukin 4[115]	A/HK/483/97(H5N1)*
Interferon γ[120]	A/Vietnam/HN3028(H5N1),* A/California/04/09(H1N1),* A/HK/403946(H1N1)*
TGFβ[126]	A/Netherlands/602/09(H1N1),* A/Vietnam/1194/04(H5N1)*
Interleukin 7[115]	A/HK/483/97(H5N1)*
Interleukin 18[114]	A/Beijing/353/89(H3N2)
Interferon λ[117,123]	A/Finland/553/09(H1N1),* A/Vietnam/3212/04(H5N1),* A/HK/483/97(H5N1)*
CXCL10[117–119,121–123]	A/Finland/553/09(H1N1),* A/Vietnam/1203/04(H5N1), A/New Caledonia/20/99(H1N1), A/HH/01/09(H1N1), A/California/07/04(H3N2), A/Thailand/1(Kan-1)/04(H5N1), A/HK/483/97(H5N1), A/Beijing/353/89(H3N2), A/Vietnam/3212/04(H5N1),* A/HK/54/98(H1N1)*
CCL8[124]	A/Thailand/1(Kan-1)/04(H5N1),* A/PR/8/34(H1N1)*
CXCL11[124]	A/Thailand/1(Kan-1)/04(H5N1),* A/PR/8/34(H1N1)*
Infection of alveolar macrophages	
CXCL10[121]	A/HK/483/97(H5N1)
TNFα[121]	A/HK/483/97(H5N1)
CCL5[121]	A/HK/483/97(H5N1)*
Interferon β[121]	A/HK/483/97(H5N1)*
Interleukin 6[121]	A/HK/483/97(H5N1)*
CCL2[121]	A/HK/483/97(H5N1)*

*Determined by mRNA expression. TNF=tumour necrosis factor. TGF=transforming growth factor.

Table 2: **Cytokine production after influenza A virus infection of human monocyte-derived and alveolar macrophages**

induction represents an important component of the antiviral response, it could also contribute to the hypercytokinaemia and subsequent pulmonary dysfunction reported in some human cases of influenza H5N1 infection.[115] Human monocyte-derived macrophages—perhaps representative of recently recruited macrophages—produce significantly higher concentrations of proinflammatory cytokines than do human alveolar macrophages upon ex-vivo influenza virus infection.[121,126] Any macrophage-mediated, cytokine-induced pulmonary damage could therefore come mainly from recently recruited macrophages. However, the many different roles of cytokines in influenza virus infection, and the redundancy in many cytokine-signalling pathways, results in contradictory findings from experimental inoculations of influenza virus in mice deficient in specific cytokines.[129,130] Which macrophage-induced cytokines are most important in damage to the epithelial–endothelial barrier, whether this damage is direct or indirect and the mechanism of this damage are unclear.

Conclusion and perspectives

In this Review, we set out the main cell types and mechanisms involved in influenza virus infection of the

lung that lead to the following model for influenza-virus-induced damage to the epithelial–endothelial barrier (figure 4). Upon entering the alveolus, influenza virus infects its main target, epithelial cells, which undergo apoptosis or necrosis, opening the epithelial layer. Influenza-virus-infected epithelial cells also produce cytokines (eg, CCL2, CCL5, and CXCL10) that recruit neutrophils and monocytes to the alveolus by direct chemotaxis. Activation of endothelial cells in influenza virus infection results in the upregulation of adhesion molecules for leucocyte extravasation and the production of cytokines such as CCL2, CXCL10, interferon α, interleukin 6, TNFα, and interferon γ. Both processes further attract neutrophils and macrophages to the alveolus. Recruited neutrophils produce reactive oxygen species, which can cause tissue damage. Neutrophils also produce cytokines in response to influenza virus infection. In particular, the production of CXCL10 could damage the epithelial–endothelial barrier by triggering the production of superoxide or enhancing neutrophil chemotaxis. Recruited macrophages can damage the epithelial–endothelial barrier in three ways. First, they can express TRAIL, which interacts with death receptor 5 on the epithelial cell and induces epithelial cell apoptosis. Second, by the activation of NOS2, recruited macrophages can increase concentrations of nitric oxide and peroxynitrite, which cause tissue damage. Third, they are important producers of proinflammatory cytokines, which further exacerbate the inflammatory response and could damage the epithelial–endothelial barrier.

The pathogenesis of influenza-virus-induced ARDS is centred on the alveolar epithelium. This framework contrasts with the classic model of ARDS pathogenesis, in which lung vascular endothelium is thought to be the main target. An important cause of ARDS in the classic model is bacterial sepsis, which differs in two important ways from influenza virus infection. First, the typical pathological agent in sepsis, lipopolysaccharide, enters the alveolus through the blood circulation,[19] whereas influenza virus enters through the airways. Second, the main target of lipopolysaccaride is the endothelial cell,[19] whereas that of influenza virus is the epithelial cell.

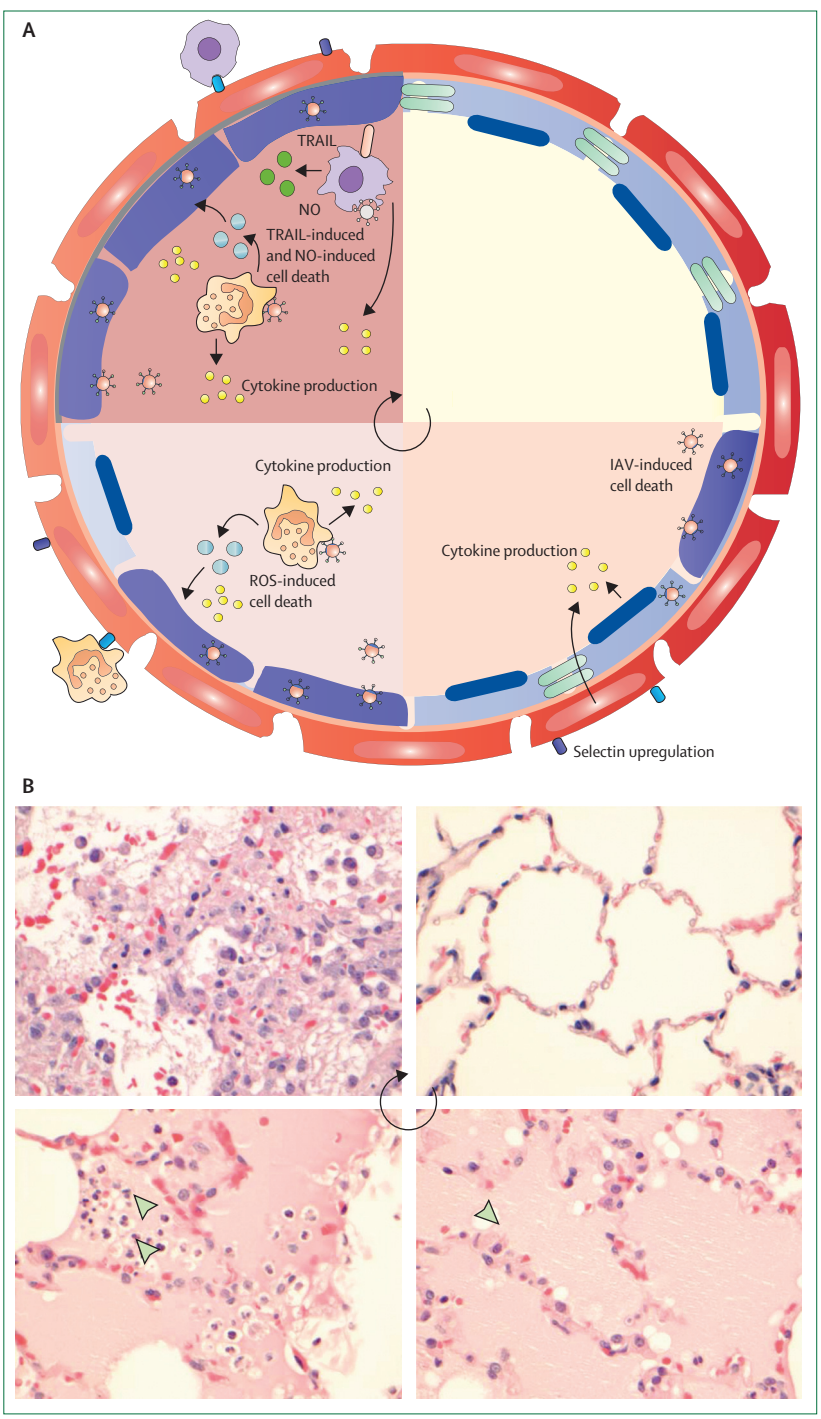

Figure 4: **Pathogenesis model and histopathology of influenza-induced ARDS**
(A) A schematic model of the pathogenesis of influenza-induced ARDS. In a healthy lung, the alveolar lumen is free of fluid to ensure optimum gas exchange (upper right quadrant). After infection, influenza A virus induces epithelial cell death, resulting in leakage of proteinaceous fluid into the alveolar lumen (lower right quadrant). Influenza virus also induces cytokine production by epithelial and endothelial cells, and selectin upregulation on endothelial cells. Selectin upregulation on endothelial cells enables extravasation of neutrophils, which are among the first cells recruited to the lung in influenza virus infection (lower left quadrant). Neutrophils damage the epithelial–endothelial barrier via the release of toxic granules and cytokine production, resulting in increased leakage of proteinaceous fluid into the alveolar lumen. Selectin upregulation on endothelial cells enables extravasation of macrophages, which damage the barrier via the production of NO, TRAIL, and cytokines (upper left quadrant). In addition to proteinaceous fluid and infiltrating leucocytes, the progressive damage to the epithelial–endothelial barrier results in fibrin deposition and haemorrhage into the alveolar lumen. (B) Histopathology panel shows representative stages of ARDS in a ferret lung 4 days after infection with influenza A H5N1. The upper right quadrant shows normal air-filled alveoli from an uninfected ferret. The lower right quadrant shows alveoli abundantly filled with proteinaceous fluid, visible as homogeneous pink material (arrowhead). The lower left quadrant shows alveoli filled with proteinaceous fluid mixed with infiltrating leucocytes, mainly neutrophils (arrowheads). The upper left quadrant shows alveoli filled with proteinaceous fluid mixed with macrophages, erythrocytes, and fibrin strands. Additionally, the alveolar walls are disrupted (haematoxylin and eosin stain, reduced by 24% from x400). ARDS=acute respiratory distress syndrome. IAV=influenza A virus. NO=nitric oxide. TRAIL= tumour-related apoptosis-inducing ligand.

Because many in-vitro and in-vivo models used to study the pathogenesis of ARDS make use of lipopolysaccharide, that the endothelial cell has received such attention is not surprising. However, irrespective of whether the cause of ARDS is sepsis, a respiratory virus such as influenza virus, or another pathological agent, its pathogenesis is highly dependent on damage to the alveolar epithelium, which is mainly responsible for maintaining the osmotic pressure across the epithelial–endothelial barrier.[25]

This new model for influenza-virus-induced ARDS has implications for pathogenesis research, both by identification of topics of particular interest and by exposure of gaps in our knowledge. Can this framework, which is based on information from various sources, be validated in one experimental system? By which mechanisms does influenza virus induce cell death in alveolar epithelial cells? Which factors establish whether apoptosis or necrosis occurs? How is this mechanism affected by cell type (ie, type 1 or type 2 pneumocytes)? By which mechanisms do cytokines produced by epithelial cells, endothelial cells, and recently recruited macrophages damage the epithelial–endothelial barrier? Which are the most important mechanisms by which endothelial cells are activated; direct contact with influenza virus, cytokines produced by influenza-virus-infected epithelial cells, or cytokines produced by other cell types? At which stage of damage to the epithelial–endothelial barrier are influenza virus particles released into the systemic circulation, and on which factors is this release dependent? Does influenza virus infection damage the basement membrane? If so, how? What effect does this damage have on re-epithelialisation? Which characteristics in influenza-virus-induced damage to the alveolar epithelium are crucial to establish whether re-epithelialisation or fibrosis occurs? How do the above mechanisms vary between different influenza virus strains? Which host factors define whether a patient with influenza goes on to develop ARDS? How is the difference in main target (epithelium *vs* endothelium) between influenza-virus-induced ARDS and sepsis-induced ARDS shown in disease progression, response to intervention, or clinical outcome? Findings that patients with sepsis-induced ARDS have higher concentrations of procalcitonin and interleukin 6 on days 1 and 2 after diagnosis of ARDS, more severe disease, and higher mortality than do patients with non-septic ARDS lend support to the notion that different causes of ARDS can result in different clinical outcomes.[131–133]

The model described in this Review also provides a rationale to choose therapeutic targets to minimise the tissue-damaging response against influenza virus infection of pulmonary alveoli.[134,135] Potential therapeutic targets for influenza-virus-induced ARDS are the alveolar epithelium, infiltrating leucocytes, and the overall proinflammatory response. Growth factors such as keratinocyte growth factor[134] and hepatocyte growth factor[136] help to protect and restore alveolar epithelium. Inhibitors of the CXCL10–CXCR3 axis reduce infiltration of neutrophils and induction of reactive-oxygen-species production.[101,102] Apocynin, an inhibitor of NADPH oxidase 2, also reduces production of reactive oxygen species.[137,138] Celecoxib and mesalazine, cyclo-oxygenase-2 inhibitors, reduce cytokine dysfunction and prevent apoptosis.[139] Gemfibrozil, an agonist of peroxisome proliferator-activated receptor α, reduces the release of proinflammatory cytokines.[139] Mesenchymal stem cells moderate inflammatory response, improve alveolar fluid clearance, and maintain integrity of lung epithelium and endothelium in pneumonia.[135] Lung stem cells have been identified that undergo rapid proliferation and radiate to areas of alveolar damage and express markers typical of alveoli after sublethal influenza virus infection in mice. Understanding of the signals that trigger this cell proliferation and radiation, and apparent regeneration of alveolar structures, could be useful to develop new therapies for treatment of influenza-virus-induced ARDS. Furthermore, therapies should promote re-epithelialisation in the absence of widespread fibrosis (which can result in decreased lung function and increased ventilator dependence).[140] However, treatments that limit influenza-virus-induced damage in mice might not necessarily produce equivalent effects in human beings. Therefore, prevention of damage to the alveolus in influenza virus infection needs an approach that combines knowledge obtained with use of experimental infection in laboratory animals together with clinical data.

Influenza-virus-induced ARDS has a high case fatality rate, despite intensive hospital care, development of lung-protective ventilation strategies, and the use of extracorporeal membrane oxygenation. Therapies directed against the virus are often not adequate to cure the disease because the lung damage remaining after the virus infection has been abrogated is too severe to resolve by itself. Only through better understanding of the mechanism by which this damage develops and is repaired can therapeutic strategies be developed to improve the outcome of this intractable complication of influenza.

Search strategy and selection criteria

We identified relevant articles by searching PubMed and Google Scholar with the search terms "epithelial cell", "influenza", "macrophages", "neutrophils", "endothelial cells", "H5N1", "tight junctions", "interferon", "alveolar macrophage", "sodium channel", "lung damage", "ENaC", "cytokines", "necrosis", "post-mortem", "NETs", "reactive oxygen species", "nitric oxide", "toxic damage", "myeloperoxidase", "NADPH", "peroxynitrite", "acute lung injury", "apoptosis", "ARDS", and "cytokine storm". We reviewed relevant articles that were found in these searches, articles cited by those found, and articles present in our own files. Only English-language articles that were published before July, 2013, were included.

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Contributors
KRS, RAMF, and TK wrote the Review. EJBVK, RAMF, and TK edited the Review. KRS prepared the figures and EJBVK took the histology images.

Conflicts of interest
We declare that we have no conflicts of interest.

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