

# Molecular pathology of emerging coronavirus infections

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## Abstract

Respiratory viruses can cause a wide spectrum of pulmonary diseases, ranging from mild, upper respiratory tract infections to severe and life-threatening lower respiratory tract infections, including the development of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). Viral clearance and subsequent recovery from infection require activation of an effective host immune response; however, many immune effector cells may also cause injury to host tissues. Severe acute respiratory syndrome (SARS) coronavirus and Middle East respiratory syndrome (MERS) coronavirus cause severe infection of the lower respiratory tract, with 10% and 35% overall mortality rates, respectively; however, >50% mortality rates are seen in the aged and immunosuppressed populations. While these viruses are susceptible to interferon treatment *in vitro*, they both encode numerous genes that allow for successful evasion of the host immune system until after high virus titres have been achieved. In this review, we discuss the importance of the innate immune response and the development of lung pathology following human coronavirus infection.

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## Introduction

Acute lung injury (ALI) and its more severe form, acute respiratory distress syndrome (ARDS), can arise after many types of injury to the lung, including sepsis, mechanical and chemical injury and bacterial and viral infections [1]. In ALI, the mortality rate is in the range 20–30%, with about 55% of the cases progressing to ARDS within a few days. ARDS causes significant morbidity and approximately 40% mortality, resulting in ~75 000 deaths/year in the USA alone [2]. In the past two decades, five emerging viruses have been known to cause significant ARDS-related mortality, including influenza H1N1 2009 and, in particular, the highly pathogenic avian influenza H5N1 and H7N9 viruses and the SARS and MERS coronaviruses. In this review we focus on mechanisms of coronavirus-induced lung pathogenesis and ARDS.

Human coronavirus (CoV) infections have traditionally caused a low percentage of annual upper and lower respiratory infections [3], including severe disease outcomes in the elderly, immunocompromised patients and infants. HCoV-OC43 (OC43) and HCoV-229E (229E) were the first documented human CoVs but, more recently, HCoV-NL63 (NL63) [4] and HCoV-HKU1 (HKU1) [5] were identified as a

consequence of increased viral surveillance efforts in the early twenty-first century (Table 1). These four viruses usually cause acute infection of the upper respiratory tract and less frequently are associated with lower respiratory tract [6,7] diseases as well. Severe disease is both rare and typically associated with co-morbidities and/or immunosenescence. The past 15 years have also seen the emergence of two new human coronaviruses that cause significant disease and mortality. SARS-CoV was identified in 2003 and caused an acute, atypical pneumonia and diffuse alveolar damage (DAD) in roughly 8000 patients [8,9]. Those over 65 years of age often developed ARDS, resulting in mortality rates that exceeded 50%. Overall, SARS-CoV infection caused nearly 800 fatalities, representing a nearly 10% mortality rate. More recently, in 2012, a new human coronavirus, designated MERS-CoV, was identified. MERS-CoV continues to circulate in camels and humans, with over 857 official cases and 334 deaths, representing an approximately 35% case fatality rate to date in humans [10,11]. MERS-CoV-induced disease is particularly severe in aged patients and those with pre-existing co-morbidities. MERS-CoV does not appear to be highly pathogenic or virulent in camels.

SARS-CoV and MERS-CoV have clear zoonotic origins, although their exact paths from animal reservoir to human infection are not yet clear. Viruses with

Table 1. Human coronaviruses, their receptors, emergence, disease and infection data

Virus	Receptor	Discovery and estimated date of divergence	Cell types infected	Disease types caused
OC43	Receptor unknown, sialic acid and HLA class 1 involvement [113,114]	Divergence from BCov in 1890 [115]	Ciliated airway epithelial cells [116], macrophages in culture [117], neuronal cells [118]	Upper respiratory infection, GI infection, pneumonia [119]
229E	Aminopeptidase N [120]	Divergence in 1700–1800 [121], divergence from NL63 in the eleventh century [122]	Non-ciliated airway epithelial cells [116], human monocytes [123], neuronal cells [118]	Upper respiratory infection [124], GI infection, pneumonia [125]
NL63	Ace2 [126]	Discovered in 2004 [4], divergence between 1200 and 1500 [127]	Ciliated airway epithelial cells [116]	Upper and lower respiratory infection [6,128], associated with croup in children [129]
HKU1	Unknown	Discovered in 2005 [5]	Ciliated airway epithelial cells [116]	Upper respiratory infection and pneumonia [130], enteric symptoms [131]
SARS	Ace2 [130], role for DC-sign [132]	Emerged in 2002 [133], divergence estimates from 1986–2002 [134,135]	Epithelial cells [136], ciliated cells, type II pneumocytes [137]	Lower respiratory infection [99,138], pneumonia, DAD, ARDS
MERS	DPP4 [139]	Emerged in 2012 [25], common ancestor from 2011–2012 [140]	Airway epithelial cells [141], renal epithelial cells [142], dendritic cells [143]	Lower respiratory infection [25,144], pneumonia, renal failure

high nucleotide identity to SARS-CoV were found in key amplifying hosts such as palm civets and raccoon dogs in Guangdong Province China during the 2002–2003 SARS epidemic [12]. Later studies identified highly conserved viruses circulating in horseshoe bats, including some strains that are able to bind to, and infect, human cells [13–16]. The existence of novel bat SARS-like coronaviruses that also use bat, civet and human angiotensin 1 converting enzyme 2 (ACE2) receptors for entry, such as SARS-CoV, strongly suggests an opportunity for further zoonotic disease outbreaks in human and animal populations.

SARS causes an atypical pneumonia characterized by cough, fever and infiltrates with a ground-glass appearance on X-ray [17,18]. Early-stage disease was characterized by acute DAD, with oedema, fibrin and hyaline membranes in the alveolar spaces, typical of ALI [19]. Other patients predominantly showed an acute fibrinous and organizing pneumonia pattern or a mixture of the two patterns [20,21]. Longer-term disease courses typically progressed to organizing phase DAD and eventual deposition of fibrous tissue. Autopsy of fatal SARS-CoV cases also revealed denuded airways, haemorrhage and increased macrophage populations in the lung [22,23]. During the SARS epidemic, researchers noted that late-term disease progression was unrelated to viraemia but was more likely to be associated with immunopathological damage [24].

MERS-CoV has caused sporadic infections, along with several local outbreaks throughout the Middle East since its discovery in 2012 [25,26]. Although much remains unknown, closely related viruses have been isolated from camels [27] and highly homologous MERS-like bat CoVs have been identified in African *Neoromicia capensis* bats [28]. Local surveillance efforts have detected high levels of antibodies that

recognize MERS-CoV in dromedary camels [29]; furthermore, sampling of archived camel serum samples has revealed MERS antibodies from as early as 1992 [30]. These data suggest that bat to camel to human transmission routes may have seeded the 2012 outbreak in human populations, perhaps associated with the expanding camel trade that has emerged between equatorial Africa and Saudi Arabia over the past 20 years.

Animal models of human disease should recapitulate many of the pathological and immune outcomes seen in human infections. Numerous models have been established to better enable our understanding of the mechanics of SARS-CoV infection and pathogenesis, although few recapitulate the human disease phenotypes (Table 2). Initial studies utilized late epidemic strains in non-human primates [31–33], where mild to severe disease was observed, depending on the study location and animal age. To date, the differences in disease severity noted in primates have not been reconciled, but may reflect differences in virus strains or infection conditions. Although still under development, MERS-CoV replication and disease have been reported in both rhesus macaques and common marmosets [34,35]. SARS-CoV replication resulted in limited disease in young models of immunocompetent mice [36–38]; however, mild clinical disease was noted in 1 year-old mice [39]. A mouse-adapted SARS (MA-SARS) strain was also developed that provides a model for moderate to lethal disease, depending on infectious dose, animal age and genetic background of the host [40–42] (Table 2). The MA-SARS model faithfully replicates the age-dependent susceptibility observed in human patients, as well as key features of human lung pathology, including virus tropism to airway epithelial cells and type II pneumocytes, pneumonia, hyaline membrane formation, development of

DAD and denudation of airway epithelial cells [40,43]. A limitation may be the rapid clearance of virus titres that is seen in younger and, to a much lesser extent, in aged animals. Development of the MA-SARS model has allowed for in-depth studies of viral pathogenesis and the host immune response, taking advantage of immunological tools and reagents for the mouse as well as the existence of knockout mouse strains. Use of these tools has greatly added to our understanding of SARS-CoV pathogenesis, far beyond what could be learned in *in vitro* experiments or observational studies of human cases. Because of receptor incompatibilities, MERS-CoV does not replicate in mice unless the animals are first transduced with adenovirus vectors encoding the receptor for entry, human dipeptidyl peptidase-4 (DPP4) [44].

In this review we focus solely on hCoV interactions within the context of the respiratory system and infection of relevant cell types. More specifically, we review some CoV–host interactions that alter cell-intrinsic antiviral defence programmes and other host pathways that contribute to pathological findings of ARDS, with its associated exudative and organizing phase diffuse alveolar damage and pulmonary fibrosis.

### Innate immune response

NF- $\kappa$ B signalling is an important component of numerous cellular responses, including stress, cytokine signalling, response to bacterial or viral infection and apoptosis [45,46]. The SARS-CoV envelope (E) protein stimulates NF- $\kappa$ B signalling [47], leading to lung cytokine signalling and inflammatory cell recruitment. The SARS-CoV papain-like protease (PLP) has also been shown to antagonize NF- $\kappa$ B signalling [48] *in vitro*. Chemical inhibitors of NF- $\kappa$ B signalling reduce lung pathology and inflammation following MA-SARS infection, demonstrating the importance of this pathway [47] in pathogenesis. While the SARS-CoV E protein is not required for viral replication, it is important for inhibition of the host cellular stress response, apoptosis and unfolded protein response [49,50]. The E protein, along with the SARS-CoV ORF3a and ORF8a proteins, has ion channel activity [50] and may contribute to vascular permeability and fluid accumulation in the lung following SARS-CoV infection. SARS-CoV lacking E has been shown to be an effective vaccine [51,52] and a MERS-CoV clone lacking E has been generated [53], although replication requires expression of E *in trans*.

SARS-CoV, and to a greater extent MERS-CoV, are highly sensitive to interferon treatment in cell culture. Interestingly, SARS-CoV pathogenesis does not significantly change in various type I interferon (IFN) knockout mouse models, except for a slight increase in overall virus titres [54–56]. Despite this, STAT1- and Myd88-deficient mice are significantly more vulnerable to lethal outcomes following infection [56,57]. Like many viruses, CoVs encode a suite of genes that antagonize cell-intrinsic innate immune defence programmes

in the infected host cell (reviewed in [58]). Numerous *in vitro* studies have demonstrated the IFN antagonist activity of both SARS-CoV and MERS-CoV proteins [59–61], and a detailed review of SARS-CoV evasion of the innate immune response was recently published by Totura and Baric [62].

Analysis of IFN-stimulated gene (ISG) expression in Calu-3 human airway epithelial cells highlighted the ability of SARS-CoV and MERS-CoV to avoid detection by the host [63]. As compared with influenza A viruses, ISG transcripts and proteins are not induced until late after SARS-CoV and MERS-CoV infection, when peak titres have already occurred in culture (~18–24 h). Late in infection, ISGs showed nearly universally increased expression following SARS-CoV infection, except for *ACE2* and *Serpinc1*. However, a much larger subset of ISGs had significantly decreased expression following MERS-CoV infection. Like MERS-CoV, H5N1 VN1203 infection also resulted in significant down-regulation of subsets of ISGs. No consistent pattern in up-regulation or down-regulation of gene expression correlated with transcription factor usage, suggesting that a novel mechanism may be responsible for expression of the ISG subsets. Cells infected with MERS-CoV and H5N1 avian influenza were shown to have specifically altered open and closed chromatin structures, potentially limiting the ability of transcription factors to access and bind certain ISG promoter regions. The mechanism by which MERS-CoV induces this chromatin structural alteration is as yet unknown. In contrast, the NS1 protein of H5N1 was responsible for the chromatin changes in influenza-infected cells. Although speculative, it seems likely that many RNA viruses may encode strategies to epigenetically alter host chromatin structure, influencing host gene expression. This newly identified method of ISG control requires additional study.

SARS-CoV further evades the host immune response by masking its RNA genome. This mechanism may be partially mediated by the production of double-membrane vesicles, which could sequester RNA replication intermediates away from the host-sensing machinery [64,65]. *MDA5* and *IFIT1* are important host antiviral sensor or antiviral defence ISGs that detect viral RNAs. *IFIT1* recognizes unmethylated 2'-O RNA [66] and alters efficient translation/stability of uncapped viral mRNAs [67]. SARS-CoV and other coronavirus RNAs are protected from IFIT recognition because they encode a 2'-O-methyltransferase (2-OMT) activity in the viral replicase protein, nsp16 [68,69]. SARS-CoV is much more sensitive to interferon treatment in the absence of functional nsp16 methyltransferase activity, and mutant viral titres drop rapidly in both infected epithelial cells and mice. Deletion or knockdown of either *MDA5* or *IFIT1* restored mutant SARS-CoV viral loads demonstrated the essential role of these host proteins in detecting pathogen-associated molecular patterns. Ablation of the 2-OMT activity may provide a universal strategy to rationally design live attenuated mutants of contemporary and newly emerging CoV.

Table 2. Non-human primate and mouse models of SARS-CoV and MERS-CoV infection; less common models include hamster [145], ferret [146] and cat

Virus	Animal model	Virus modifications?	Disease types caused	Drawbacks	Aged model?
SARS-CoV	Rhesus macaque	None	Viral replication, mild pneumonia [147]	Expense, ethical considerations, no severe disease	
	African green monkey	None	Viral replication, pneumonitis [33], hyaline membrane formation [102]	Expense, ethical considerations, no severe disease	
	Cynomolgus macaque	None	Viral replication, upper respiratory symptoms, pneumonia [31,148]	Expense, ethical considerations	Yes [149]
	Ace2 transgenic mice	None	Viral replication, weight loss, inflammatory cell infiltrates [150]	Virus causes encephalitis [150], use of knockout mice requires extensive breeding	
	Mouse	None	Viral replication, mild pneumonia in aged mice [39]	Minimal pathogenesis, especially in young mice [36]	Yes [39]
SARS-MA15	Mouse	Six point mutations from serial mouse passage [40]	Viral replication, weight loss [40], pneumonia, DAD [43], pulmonary fibrosis [56]	Virus has been adapted from human strains	Yes [41]
MERS-CoV	Rhesus macaque	None	Viral replication [35], transient pneumonia	Expense, ethical considerations, no severe disease	
	hAd5-DPP4 mouse	None	Viral replication [44], weight loss in immune knockouts	Requires infection with hAd5 to express human DPP4	Yes [44]

Both *in vivo* and *in vitro* studies have addressed the role of specific proteins in the innate immune system, often ISGs, in SARS-CoV pathogenesis. Transcriptional analysis on autopsy tissue from SARS-CoV-infected patients revealed increased expression of *STAT1* along with other IFN-induced cytokines [70]. The SARS-CoV accessory protein ORF6 was identified as an interferon antagonist important for viral replication in low multiplicity of infection (MOI) *in vitro* infections [71,72]. ORF6 was subsequently found to sequester Karyopherin 2 $\alpha$ , a nuclear import factor, and block the nuclear translocation of *STAT1* after SARS-CoV infection. Interestingly, *STAT1* translocation to the nucleus is not blocked in MERS-CoV-infected cells, so it remains uncertain whether antagonists of nuclear import are encoded in the viral genome [73]. Transcriptional profiling of SARS-CoV-infected macaques revealed robust IFN signalling, including *STAT1* translocation to the nucleus, in the lung but not in the cells that stained positive for viral antigen [74]. These data highlight the importance of *in vivo* studies versus high MOI *in vitro* studies. Significantly, they also highlight the need to examine, or at least consider, expressing and signalling differences in specific cell types instead of global transcriptomic studies in those *in vivo* experiments.

*STAT1* knockout mice have been studied extensively in the context of viral infection, typically showing a heightened susceptibility to disease, due to the lack of a type I IFN response [75]. These knockouts were first tested for SARS-CoV susceptibility using the Tor2 strain in a sublethal model; animals deficient in *STAT1* were unable to clear virus from the lung and developed a more severe and longer-lasting pneumonia than the control mice [76]. Frieman *et al* showed that *STAT1* knockout mice are highly susceptible to infection with MA-SARS in a novel, IFN-independent mechanism [56]. MA-SARS-infection causes massive inflammatory

cell influx in the lungs of *STAT1* knockout mice, including large numbers of macrophages, neutrophils and eosinophils. *STAT1* knockout mice have gross pathological changes in their lungs, including massive haemorrhage as well as increased lung size and stiffness. As seen in some humans, these mice develop severe pulmonary fibrosis and succumb to disease at late time points after infection. Stained lung sections revealed the presence of collagen protein in alveolar exudates in *STAT1* knockouts, indicating development of early-stage pulmonary fibrosis. Subsequent studies demonstrated that *STAT1* knockout animals developed a Th2-skewed immune response and had significant numbers of alternatively activated or M2 macrophages in their lungs [77]. These macrophages were characterized by positive CD11c, arginase and mannose receptor staining. *STAT6* is required for the development of alternatively activated macrophages, and *STAT1/STAT6* double knockout mice do not develop the severe lung disease and pro-fibrotic lesions observed in the *STAT1* single knockout [78], thus demonstrating that these macrophages are essential for development of the pulmonary fibrosis phenotype. Further elegant experiments showed that it is the *STAT1* deficiency in monocyte/macrophage cells, not the infected epithelial cells, that drives alternatively activated macrophage production and induction of fibrotic lung disease following SARS-CoV infection. Alternatively activated macrophages are typically induced by the Th2 cytokines IL-4 and IL-13; they have an anti-inflammatory role and play an important role in wound-healing processes [151].

ACE2 is expressed on well-differentiated airway epithelial cells [79] and its expression increases following type I interferon treatment [63]. Both ACE2 protein levels and RNA expression are down-regulated after either *in vitro* or *in vivo* SARS-CoV infection [63,80] and NL63 also down-regulates ACE2 expression

following *in vitro* infection [81]. It has previously been reported that ACE2 and angiotensin-2 protect mice from sepsis- and acid aspiration-induced ALI [82]. Additionally, histopathological lung disease worsens when spike-Fc is inoculated into mice with ALI [80]. The normal function of ACE2 is to inactivate angiotensin-2, a negative regulator of the renin–angiotensin system [83,84]. This system controls blood pressure and is involved in the development of pulmonary hypertension and pulmonary fibrosis. The renin–angiotensin system is involved in lipopolysaccharide (LPS)-induced neutrophil recruitment to the lung [85]. Multiple genome-wide association studies have investigated an association between genetic variation in ACE and susceptibility to ARDS, with mixed results [86]. The role of DPP4 in MERS-CoV infection is discussed in detail by Haagmans *et al* in a separate review in this issue [152].

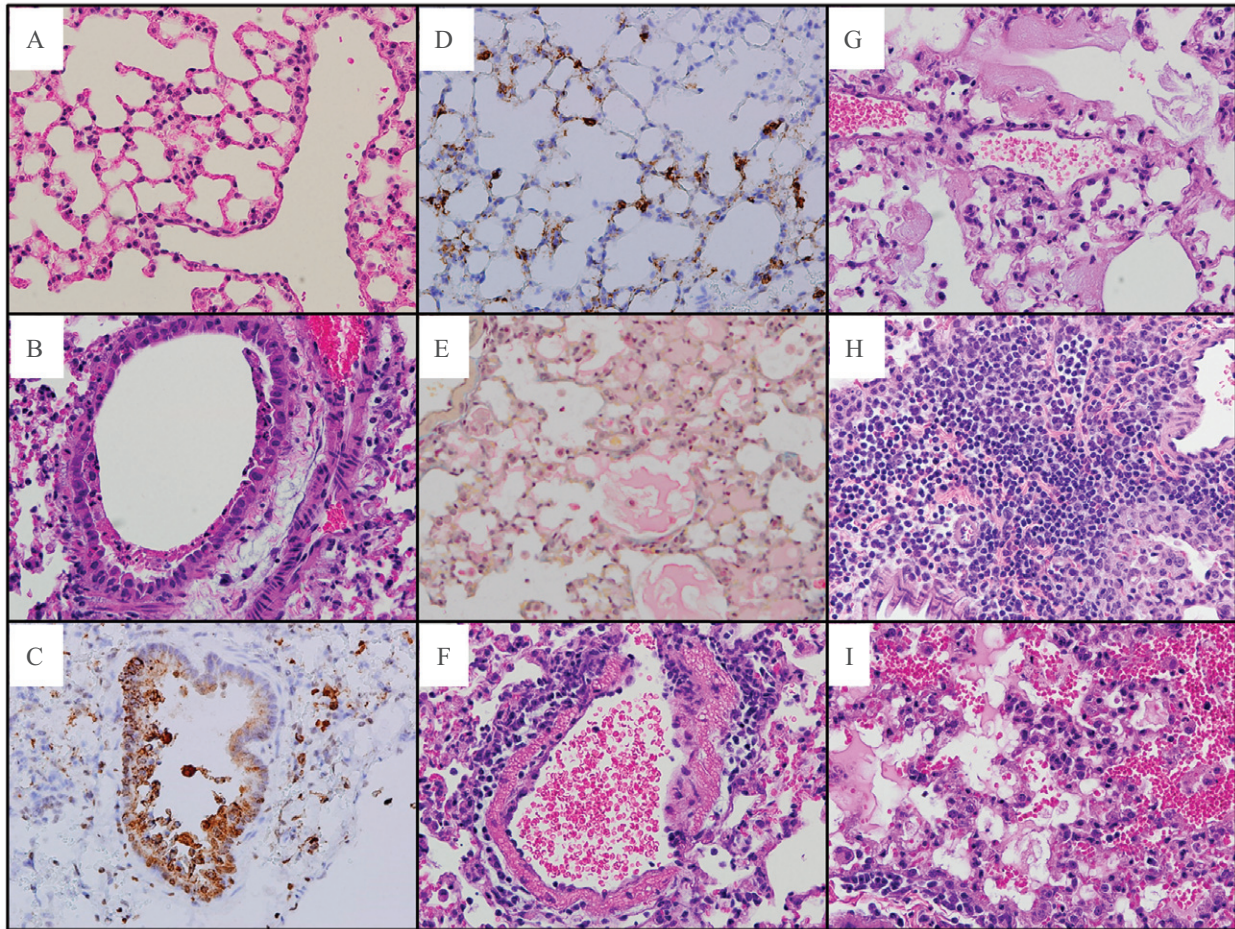
High ISG expression has been linked to development of ARDS [87]. Furthermore, it has been suggested that unregulated IFN responses contributed to development of immunopathology and severe disease following SARS-CoV infection [88]. The data discussed above support this hypothesis and suggest that early control of ISG signalling may be a means of preventing or controlling the development of severe lung disease. Expression of the ISG *Serpine1* is also decreased following SARS-CoV infection of epithelial cells; it functions by inhibiting the complement system as well as several proteases in the coagulation pathway. The role of the coagulation, fibrinolysis and wound healing in ARDS development are discussed below.

### Acute respiratory distress syndrome (ARDS), coagulation, fibrinolysis and respiratory function

The alveoli of the lung are where gas exchange occurs, providing oxygen to blood flowing through capillaries in the alveolar membrane. The alveolar walls are composed of type I and type II pneumocytes, along with alveolar macrophages [89]. Type I pneumocytes cover 95% of the alveolar surface area and allow for gas exchange with blood in the capillaries of the lung. Type II pneumocytes are the progenitors of type I pneumocytes and are also responsible for generating pulmonary surfactant [90], a mixture of lipids and surfactant proteins that is crucial in reducing surface tension in the lung. SARS-CoV infection causes desquamation of pneumocytes in humans and mice, contributing to alveolar dysfunction, oedema and haemorrhage. Alveolar macrophages play an essential role in surveillance of the local environment and inhibit an excessive immune response, although this inhibition can also block an effective response to SARS-CoV infection [91]. The functions of these cell types are critical in maintaining balance between inflammation, coagulation and wound repair, especially following lung injuries such as viral infection [92].

In ARDS patients, uncontrolled inflammation, fluid accumulation and developing fibrosis severely compromise gas exchange and lead to respiratory failure. SARS-CoV and influenza infect type I and type II pneumocytes in the lung [93,94]. ARDS patients exhibit decreased surfactant levels [95] and MA-SARS infection results in decreased surfactant transcript and protein levels [43]. Decreased surfactant, and the consequent increase in surface tension, reduces the ability of the lung to expand and contract during normal respiration; it also heightens the risk of lung collapse during expiration. Respiratory dysfunction occurs when the alveolar membranes are obstructed, or when the ability of the lung to expand and contract, circulating oxygenated air, is compromised. Lethal SARS-CoV infection in the mouse and human is characterized by a breakdown of alveolar membrane integrity, resulting in accumulation of fluid exudates in the alveolar spaces. Virus infection also results in an overwhelming cytokine response, severe lung tissue damage and respiratory failure [96–99]. The progression from initial disease to diffuse alveolar damage and the exudative and organizing stage of DAD is often independent of high-titre viral replication [24], indicating that this severe disease outcome is primarily driven by an immunopathological response, including inflammatory cell recruitment and viral damage to type II pneumocytes. This conclusion is further supported by non-human primate and mouse models of SARS-CoV infection, where lethal disease is more often associated with severe pulmonary lesions, alveolar exudates and respiratory dysfunction than with high viral load [43,100]. MA-SARS infection results in peak viral titres at 1–2 days post-infection, along with airway denudation and resulting debris, which can occlude the small airways. Severe lung disease, including inflammatory cell infiltrates, haemorrhage, alveolar oedema and hyaline membrane formation typical of the exudative stage of DAD (Figure 1), occurs at days 4–7 post-infection, when virus loads in the lung are dropping rapidly and/or are below the limit of detection. Many ISGs stimulated by SARS-CoV infection are involved in wound-healing responses and thus may contribute to SARS-induced ALI and ARDS.

While SARS-CoV evades detection by the host immune system and causes minimal changes in transcript and protein levels for the first 24 h of infection [43], it ultimately induces a massive signalling response in infected lungs. Pro-inflammatory cytokines and chemokines, including IL-6, TNF $\alpha$ , IL-1 $\beta$  and CCL2 [57], recruit inflammatory cells to the site of infection. Neutrophils and cytotoxic T cells, along with these cytokines, can induce tissue damage, including vascular leakage, and stimulate pulmonary fibrosis [101]. Pro-fibrotic genes, including *Tgfb1*, *Ctgf* and *Pdgfa* and numerous collagen transcripts, have increased expression following MA-SARS infection. Fluid exudates, haemorrhage and fibrin are all observed in the alveolar spaces of SARS patients, as well as in animal models of disease [17,43,102], increasing in severity as a function of age. In response, the coagulation cascade is activated,



Understanding Disease

Figure 1. MA-SARS lung immunopathology. (A) Mock-infected lung stained with haematoxylin and eosin. (B) Large airway of a C57BL/6J (B6) mouse, 7 days post-infection, with  $10^5$  plaque-forming units (PFU) MA-SARS, shows denudation of the epithelial cells. (C, D) Immunohistochemical staining of the SARS-CoV N protein at 2 days post-infection shows staining consistent with infection of airway epithelial cells and type II pneumocytes, respectively. (E) MSB staining highlights fibrin in the parenchyma of the lung (red staining) in B6 mice, 7 days post-infection with  $10^5$  PFU MA-SARS. (F) Perivascular cuffing in a B6 mouse, 4 days post-infection with  $10^5$  PFU MA-SARS. (G) Hyaline membranes in the parenchyma of the lung of a B6 mouse, 7 days post-infection with  $10^5$  PFU MA-SARS. (H) Inflammation in the lung of a B6 mouse, 7 days post-infection with  $10^4$  PFU MA-SARS. (I) Haemorrhage in the lung of a *Serpine1* mouse, 7 days post-infection with  $10^4$  PFU MA-SARS.

including increased *factor 10 (FX)*, *F2*, *F3 (tissue factor)*, *F11*, *F12* and *F7* transcript levels. Activation of the coagulation cascade results in F10 cleavage of prothrombin into thrombin and subsequent thrombin cleavage of fibrinogen into fibrin [103]. Fibrin clots in the alveoli are a prominent feature of SARS-CoV infection in humans and mice. The goal of this coagulation response is likely to protect the host by sealing the alveoli, preventing alveolar flooding and haemorrhage, which limit oxygen exchange and endanger patient survival. Collagen expression is also increased following SARS-CoV infection [43]. Collagen accumulation, fibrin and fibrin clots all contribute to a developing fibrotic lung state, while at the same time stimulating the infected host to up-regulate fibrinolytic pathways [92].

Profibrinolytic genes include members of the urokinase pathway, such as *urokinase (plau)*, *tPA* and *plasmin (plg)* [104]. The urokinase signalling pathway leads to cleavage and activation of plasmin into plasminogen; this protease then cleaves fibrin clots. *Serpine1* and *Serpine2* are negative regulators of urokinase pathway and inhibit urokinase and tissue plasminogen activator (tPA) activity. Urokinase signalling is highly active in the absence of *Serpine1*, and this imbalance often results in haemorrhage in knockout mice. *Serpine1* is highly expressed in SARS patients, non-human primates and small animal models [43,74,105]. ARDS studies, independent of coronavirus infection, have attributed this *Serpine1* expression to alveolar macrophages and type II pneumocytes [106,107]. MA-SARS infection

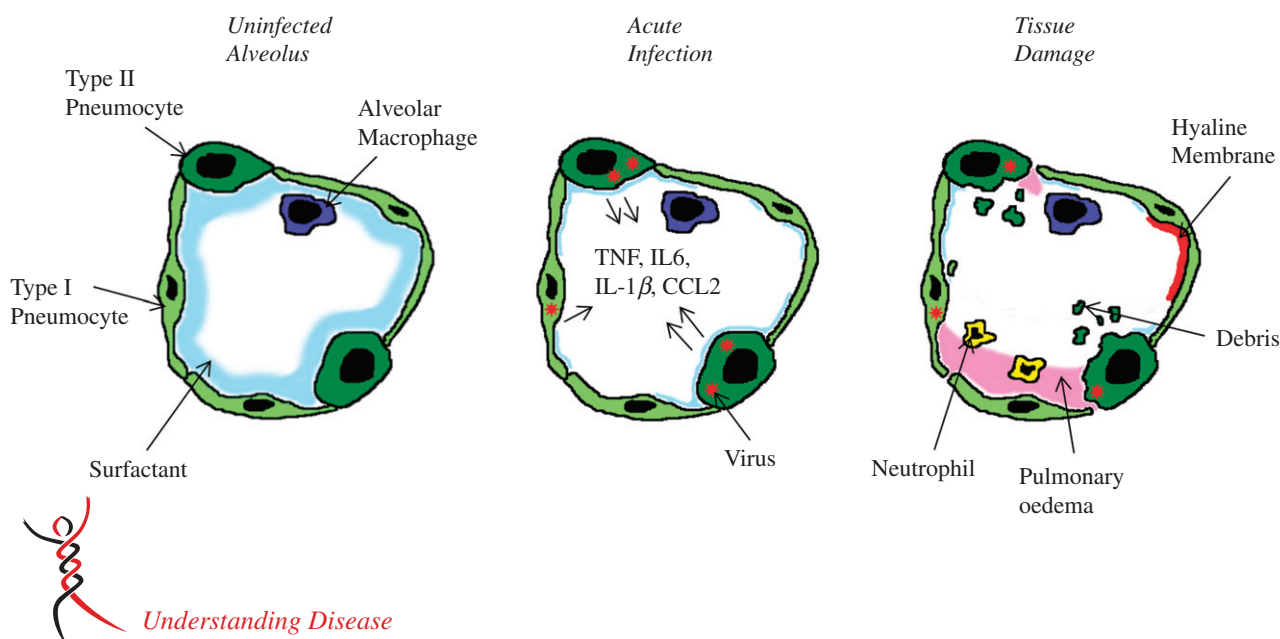


Figure 2. Model of an infected alveolus in the lung. Type I and type II pneumocytes make up the alveolar walls and resident alveolar macrophages and pulmonary surfactant exist in the airspace (A). In the acute phase of SARS-CoV infection (B), type I and type II pneumocytes are infected and secrete inflammatory cytokines, while surfactant levels decrease. During the late stage/tissue damage portion of viral infection, viral titres decrease, while airway debris, pulmonary oedema and hyaline membrane formation all impede respiration (C).

in a *Serpine1* knockout mouse model results in lethal disease with extreme lung pathology [43]. Conversely, MA-SARS-infected mice with a genetic deficiency in *tPA* have increased exudates in the lung. The dysregulation of these coagulation/anti-coagulation cascades can result in worsening end-stage lung disease conditions, resulting in death.

Profibrotic and profibrinolytic signalling are part of the wound-healing response, along with other extracellular matrix (ECM) remodelling pathways [101]. SARS-CoV infection causes massive tissue remodelling through urokinase and coagulation pathways activity, as discussed above. Other important wound-healing pathways and ECM proteins with altered signalling following SARS-CoV infection include matrix metalloproteinases, EGFR and collagens [43]. Successful recovery from ALI requires a delicate balance of pro-inflammatory, profibrotic and profibrinolytic responses. By altering ISG expression, including *ACE2*, *STAT1* and *Serpine1*, SARS-CoV infection of alveolar epithelial cells sets the stage for the development of severe lung disease, including ARDS.

SARS and MERS patients with severe lung disease exhibited lung consolidation, decreased blood oxygen saturation and often required intubation and ventilation [99,108,109]. Small animal models of severe lung disease typically lack physiological readouts of respiratory function that can be directly correlated back to human signs of disease. Whole-body plethysmography captures respiratory data in unrestrained animals, allowing for longitudinal measurement of pulmonary function; these data can also be directly related to some human respiratory metrics [110,111]. SARS-CoV infection causes

increased *Penh*, a calculated measure of airway resistance and increased EF50 (mid-breath exhalation force), indicating that respiratory function is compromised and animals must do more work to breathe [69]. Unpublished data (Gralinski and Menachery) indicate that it is the exhalation portion of each breath that is impacted by SARS-CoV infection, likely due to extensive debris clogging the conducting airways. Further experiments have shown that *Stat1* knockout mice have increased *Penh* levels early after infection, and that at late time-points they have reduced lung capacity, corresponding with the profibrotic histopathological changes observed in the lung (Gralinski, unpublished data).

### Concluding thoughts

ARDS is a devastating end-stage lung disease with no cure. Despite numerous clinical trials, improved clinical outcomes have remained marginal at best [112]. Several highly pathogenic emerging virus infections cause ARDS with high frequency, underscoring the critical public health importance of understanding the virological components and molecular mechanisms that drive this devastating end-stage lung disease. Furthermore, a portion of ARDS cases may progress to pulmonary fibrosis, another clinically devastating end-stage lung disease with few treatment options. Consequently, understanding the development of ARDS following viral infection remains a high-priority research topic that is germane to global health and pandemic disease control. The twenty-first century has demonstrated that zoonotic events will continue to introduce coronaviruses

and other viruses into the human population, and that these viruses have the potential to spread rapidly, cause significant disease in communities and disrupt the global economy. An emerging theme is the connectivity between virus infection, complement and coagulation cascade activation, pro-inflammatory and profibrotic cytokine responses and disease severity. More studies are needed to unravel the complex interactions between these pathways that can interact to promote or dysregulate wound recovery after life-threatening respiratory virus infection. In particular there is a need for including well-articulated animal models that faithfully recapitulate disease processes across species. Only through a better understanding of the interplay between a dysregulated host immune response and ALI and ARDS can more effective treatments and therapeutics be developed.

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### Author contributions

LEG and RSB wrote the paper.

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