Molecular Pathology of Emerging Coronavirus Infections

Lisa E. Gralinski[1] and Ralph S. Baric[1,2,#]

[1]Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

[2] Department of Microbiology and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

[#] corresponding author, contact rbaric@email.unc.ed

Conflict of Interest

The authors declare that they have no conflicts of interest.

Keywords:

SARS-CoV, MERS-CoV, coronavirus, acute respiratory distress syndrome, ARDS, acute lung injury, type II pneumocytes

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/path.4454

Abstract

Respiratory viruses can cause a wide spectrum of pulmonary disease ranging from mild, upper respiratory tract infections to severe and life-threatening lower respiratory tract infection including 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.

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 ranges between 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 each in year in the United States alone [2]. In the past two decades, five emerging viruses are 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 and in 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 21st 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 8,000 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, a new human coronavirus designated MERS-CoV was identified in 2012. 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 high nucleotide identity to SARS-CoV were found in key amplifying hosts like 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, like SARS-CoV, strongly suggests an opportunity for further zoonotic disease outbreaks in human and animal populations.

SARS caused 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 showed predominantly 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 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 has 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 on 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 defense 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-κB signaling is an important component of numerous cellular responses including stress, cytokine signaling, response to bacterial or viral infection and apoptosis [45, 46]. The SARS-CoV envelope (E) protein stimulates NF-κB signaling [47] leading to lung cytokine signaling and inflammatory cell recruitment. The SARS-CoV papain-like protease (PLP) has also been shown to antagonize NF-κB signaling [48] *in vitro*. Chemical inhibitors of NF-κB signaling 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 defense 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 hours). Late in infection, ISGs showed nearly universally increased expression following SARS-CoV infection, except for *ACE2* and *Serping1*. 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 downregulation of subsets of ISGs. No consistent pattern in upregulation or downregulation 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 structure, 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 defense 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 in 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.

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 as to whether antagonists of nuclear import are encoded in the viral genome [73]. Transcriptional profiling of SARS-CoV-infected macaques

revealed robust IFN signaling, 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 vs. high MOI *in vitro* studies. Significantly they also highlight the need to examine or at least consider expressing and signaling 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 atype 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 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 *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 IL4 and IL13; they have an anti-inflammatory role and play an important role in wound healing processes [add reference Mantovani A, Biswas SK, Galdiero MR, Sica A, Locati M. Macrophage plasticity and polarization in tissue repair and remodelling. J Pathol 2013; 229: 176–185.]

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 Angiotensin2 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 Angiotensin2, a negative regulator of the renin-angiotensin system [83, 84]. This system controls blood pressure and is involved in 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 [add cross-reference to van den Brand JMA, Smits SL. Haagmans BL. Pathogenesis of Middle East respiratory syndrome coronavirus. J Pathol 2015 - the ARI article on MERS].

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 signaling may be a means to prevent or control development of severe lung disease. Expression of the ISG *Serping1* 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.

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 between 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 hours of infection [43], it ultimately induces a massive signaling response in infected lungs. Proinflammatory cytokines and chemokines including IL-6, TNF-alpha, IL1-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 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 likely is 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 signaling 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 signaling 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 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 signaling are part of the wound healing response along with other extracellular matrix (ECM) remodeling pathways [101]. SARS-CoV infection causes massive tissue remodeling through urokinase and coagulation

pathways activity as discussed above. Other important wound healing pathways and ECM proteins with altered signaling following SARS-CoV infection include matrix metalloproteinases, EGFR and collagens [43]. Successful recovery from ALI requires a delicate balance of proinflammatory, profibrotic and profibrinolytic responses. By altering ISG expression including *ACE2*, *STAT1* and *Serping1* SARS-CoV infection of alveolar epithelial cells sets the stage for 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 breath [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 timepoints 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 21st 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, proinflammatory 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.

Acknowledgements

This work was supported by funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, contract number HHSN272200800060C, U19 AI109761 and U19 AI100625.

Author Contributions

Written and edited by LEG and RSB.

Figures/Tables

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 11th 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

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	Virus replication, mild pneumonia in aged mice [39]	Minimal pathogenesis, especially in young mice [36]	Yes [39]
SARS-MA15	Mouse	6 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]

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) of MA-SARS shows denudation of the epithelial cells. C and 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 of MA-SARS. F. Perivascular cuffing in a B6 mouse 4 days post infection with 10^5 PFU of MA-SARS. G. Hyaline membranes in the parenchyma of the lung of a B6 mouse 7 days post infection with 10^5 PFU of MA-SARS. H. Inflammation in the lung of a B6 mouse 7 days post infection with 10^4 PFU of MA-SARS. I. Haemorrhage in the lung of a *Serpine1* mouse 7 days post infection with 10^4 PFU of MA-SARS.

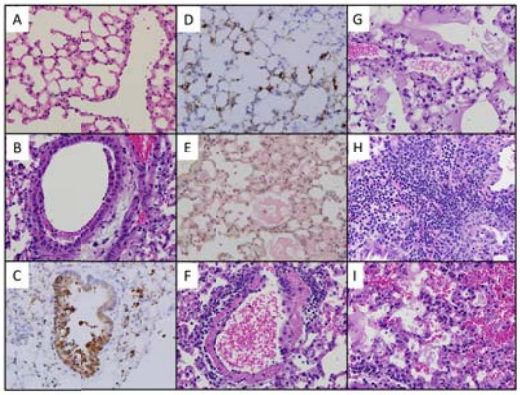
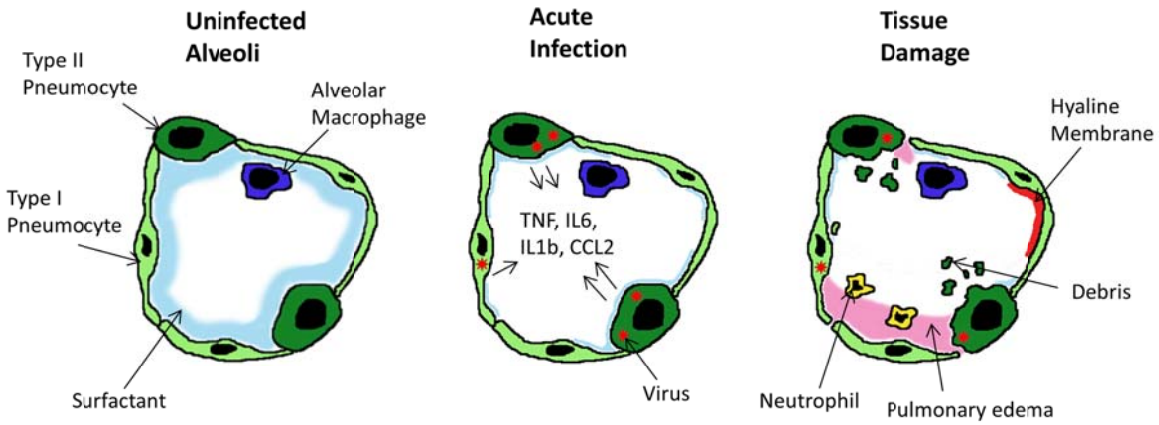


Figure 2. Model of an infected alveolus in the lung. Type I and type II pneumocytes make up the alveolar walls, resident alveolar macrophages and pulmonary surfactant exist in the airspace (A). In the acute phase of SARS-CoV infection (B) type I and type II pneumocytes are infected and secrete inflammatory 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).



NB – Figure 2 says ‘Uninfected alveoli’ but should say ‘Uninfected alveolus’ as it only illustrates a single alveolus. Edema should be oedema.

References

1. Johnson ER, Matthay MA. Acute lung injury: epidemiology, pathogenesis, and treatment. J Aerosol Med Pulm Drug Deliv. Aug;23(4):243-52.
2. Reynolds HN, McCunn M, Borg U, et al. Acute respiratory distress syndrome: estimated incidence and mortality rate in a 5 million-person population base. Crit Care. 1998;2(1):29-34.
3. Cabeca TK, Granato C, Bellei N. Epidemiological and clinical features of human coronavirus infections among different subsets of patients. Influenza Other Respir Viruses. Nov;7(6):1040-7.
4. van der Hoek L, Pyrc K, Jebbink MF, et al. Identification of a new human coronavirus. Nat Med. 2004 Apr;10(4):368-73.
5. Woo PC, Lau SK, Chu CM, et al. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. J Virol. 2005 Jan;79(2):884-95.
6. Reina J, Lopez-Causape C, Rojo-Molinero E, et al. Clinico-epidemiological characteristics of acute respiratory infections caused by coronavirus OC43, NL63 and 229E. Rev Clin Esp. Jun 20.
7. Lau SK, Woo PC, Yip CC, et al. Coronavirus HKU1 and other coronavirus infections in Hong Kong. J Clin Microbiol. 2006 Jun;44(6):2063-71.
8. Drosten C, Gunther S, Preiser W, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med. 2003 May 15;348(20):1967-76.
9. http://www.who.int/csr/sars/country/table2004_04_21/en/.
10. http://www.who.int/csr/don/2014_07_23_mers/en/). .
11. ProMed. Available from: http://www.promedmail.org/direct.php?id=20140912.2770252.
12. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. Science. 2003 Oct 10;302(5643):276-8.
13. Lau SK, Woo PC, Li KS, et al. Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. Proc Natl Acad Sci U S A. 2005 Sep 27;102(39):14040-5.
14. Li W, Shi Z, Yu M, et al. Bats are natural reservoirs of SARS-like coronaviruses. Science. 2005 Oct 28;310(5748):676-9.
15. Ren W, Qu X, Li W, et al. Difference in receptor usage between severe acute respiratory syndrome (SARS) coronavirus and SARS-like coronavirus of bat origin. J Virol. 2008 Feb;82(4):1899-907.
16. Ge XY, Li JL, Yang XL, et al. Isolation and characterization of a bat SARS-like coronavirus that uses the ACE2 receptor. Nature. 2013 Nov 28;503(7477):535-8.
17. Franks T, Chong P, Chui P, et al. Lung pathology of severe acute respiratory syndrome (SARS): a study of 8 autopsy cases from Singapore. Human Pathology. 2003;34(8):743-8.
18. Liu J, Tang X, Jiang S, et al. The chest X-ray image features of patients with severe SRAS: a preliminary study. Chin Med J (Engl). 2003 Jul;116(7):968-71.

19. Gu J, Korteweg C. Pathology and pathogenesis of severe acute respiratory syndrome. Am J Pathol. 2007 Apr;170(4):1136-47.
20. Hwang DM, Chamberlain DW, Poutanen SM, et al. Pulmonary pathology of severe acute respiratory syndrome in Toronto. Mod Pathol. 2005 Jan;18(1):1-10.
21. Nicholls J, Dong XP, Jiang G, et al. SARS: clinical virology and pathogenesis. Respirology. 2003 Nov;8 Suppl:S6-8.
22. Ding Y, Wang H, Shen H, et al. The clinical pathology of severe acute respiratory syndrome (SARS): a report from China. J Pathol. 2003 Jul;200(3):282-9.
23. Hsiao CH, Wu MZ, Chen CL, et al. Evolution of pulmonary pathology in severe acute respiratory syndrome. J Formos Med Assoc. 2005 Feb;104(2):75-81.
24. Peiris JS, Chu CM, Cheng VC, et al. Clinical progression and viral load in a community outbreak of coronavirus-associated SARS pneumonia: a prospective study. Lancet. 2003 May 24;361(9371):1767-72.
25. Zaki AM, van Boheemen S, Bestebroer TM, et al. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. N Engl J Med. 2012 Nov 8;367(19):1814-20.
26. Al-Abdallat MM, Payne DC, Alqasrawi S, et al. Hospital-Associated Outbreak of Middle East Respiratory Syndrome Coronavirus: A Serologic, Epidemiologic, and Clinical Description. Clin Infect Dis. 2014 May 14.
27. Raj VS, Farag EA, Reusken CB, et al. Isolation of MERS Coronavirus from a Dromedary Camel, Qatar, 2014. Emerg Infect Dis. 2014 Aug;20(8).
28. Corman VM, Ithete NL, Richards LR, et al. Rooting the phylogenetic tree of MERS-Coronavirus by characterization of a conspecific virus from an African Bat. J Virol. 2014 Jul 16.
29. Reusken CB, Haagmans BL, Muller MA, et al. Middle East respiratory syndrome coronavirus neutralising serum antibodies in dromedary camels: a comparative serological study. Lancet Infect Dis. 2013 Oct;13(10):859-66.
30. Corman VM, Jores J, Meyer B, et al. Antibodies against MERS Coronavirus in Dromedary Camels, Kenya, 1992-2013. Emerg Infect Dis. 2014 Aug;20(8).
31. Fouchier RA, Kuiken T, Schutten M, et al. Aetiology: Koch's postulates fulfilled for SARS virus. Nature. 2003 May 15;423(6937):240.
32. Haagmans BL, Kuiken T, Martina BE, et al. Pegylated interferon-alpha protects type 1 pneumocytes against SARS coronavirus infection in macaques. Nat Med. 2004 Mar;10(3):290-3.
33. McAuliffe J, Vogel L, Roberts A, et al. Replication of SARS coronavirus administered into the respiratory tract of African Green, rhesus and cynomolgus monkeys. Virology. 2004 Dec 5;330(1):8-15.
34. Falzarano D, de Wit E, Feldmann F, et al. Infection with MERS-CoV Causes Lethal Pneumonia in the Common Marmoset. PLoS Pathog. 2014 Aug;10(8):e1004250.
35. de Wit E, Rasmussen AL, Falzarano D, et al. Middle East respiratory syndrome coronavirus (MERS-CoV) causes transient lower respiratory tract infection in rhesus macaques. Proc Natl Acad Sci U S A. 2013 Oct 8;110(41):16598-603.
36. Subbarao K, McAuliffe J, Vogel L, et al. Prior infection and passive transfer of neutralizing antibody prevent replication of severe acute respiratory syndrome coronavirus in the respiratory tract of mice. J Virol. 2004 Apr;78(7):3572-7.
37. Glass WG, Subbarao K, Murphy B, et al. Mechanisms of host defense following severe acute respiratory syndrome-coronavirus (SARS-CoV) pulmonary infection of mice. J Immunol. 2004 Sep 15;173(6):4030-9.

38. Wentworth DE, Gillim-Ross L, Espina N, et al. Mice susceptible to SARS coronavirus. Emerg Infect Dis. 2004 Jul;10(7):1293-6.
39. Roberts A, Paddock C, Vogel L, et al. Aged BALB/c mice as a model for increased severity of severe acute respiratory syndrome in elderly humans. J Virol. 2005 May;79(9):5833-8.
40. Roberts A, Deming D, Paddock CD, et al. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. PLoS Pathog. 2007 Jan;3(1):e5.
41. Sheahan T, Whitmore A, Long K, et al. Successful vaccination strategies that protect aged mice from lethal challenge from influenza virus and heterologous severe acute respiratory syndrome coronavirus. J Virol. Jan;85(1):217-30.
42. Frieman M, Yount B, Agnihothram S, et al. Molecular determinants of severe acute respiratory syndrome coronavirus pathogenesis and virulence in young and aged mouse models of human disease. J Virol. 2012 Jan 2012;86(2):884-97.
43. Gralinski LE, Bankhead A, 3rd, Jeng S, et al. Mechanisms of severe acute respiratory syndrome coronavirus-induced acute lung injury. MBio.4(4).
44. Zhao J, Li K, Wohlford-Lenane C, et al. Rapid generation of a mouse model for Middle East respiratory syndrome. Proc Natl Acad Sci U S A. Apr 1;111(13):4970-5.
45. Collins SE, Mossman KL. Danger, diversity and priming in innate antiviral immunity. Cytokine Growth Factor Rev. 2014 Jul 11.
46. Lancellotti M, Pereira RF, Cury GG, et al. Pathogenic and opportunistic respiratory bacteria-induced apoptosis. Braz J Infect Dis. 2009 Jun;13(3):226-31.
47. DeDiego ML, Nieto-Torres JL, Regla-Nava JA, et al. Inhibition of NF-kappaB-mediated inflammation in severe acute respiratory syndrome coronavirus-infected mice increases survival. J Virol. 2014 Jan;88(2):913-24.
48. Frieman M, Ratia K, Johnston RE, et al. SARS-CoV papain-like protease ubiquitin-like domain and catalytic domain regulate antagonism of IRF3 and NF-kappaB signaling. J Virol. 2009;83(13):6689-705.
49. DeDiego ML, Nieto-Torres JL, Jimenez-Guardeno JM, et al. Severe acute respiratory syndrome coronavirus envelope protein regulates cell stress response and apoptosis. PLoS Pathog. 2011 Oct;7(10):e1002315.
50. DeDiego ML, Nieto-Torres JL, Jimenez-Guardeno JM, et al. Coronavirus virulence genes with main focus on SARS-CoV envelope gene. Virus Res. 2014 Aug 2.
51. Lamirande EW, DeDiego ML, Roberts A, et al. A live attenuated severe acute respiratory syndrome coronavirus is immunogenic and efficacious in golden Syrian hamsters. J Virol. 2008 Aug;82(15):7721-4.
52. Fett C, DeDiego ML, Regla-Nava JA, et al. Complete protection against severe acute respiratory syndrome coronavirus-mediated lethal respiratory disease in aged mice by immunization with a mouse-adapted virus lacking E protein. J Virol. 2013 Jun;87(12):6551-9.
53. Almazan F, DeDiego ML, Sola I, et al. Engineering a replication-competent, propagation-defective Middle East respiratory syndrome coronavirus as a vaccine candidate. MBio. 2013;4(5):e00650-13.
54. Cinatl J, Morgenstern B, Bauer G, et al. Treatment of SARS with human interferons. Lancet. 2003 Jul 26;362(9380):293-4.
55. Strayer DR, Dickey R, Carter WA. Sensitivity of SARS/MERS CoV to Interferons and Other Drugs Based on Achievable Serum Concentrations in Humans. Infect Disord Drug Targets. 2014 Jul 13.

56. Frieman MB, Chen J, Morrison TE, et al. SARS-CoV pathogenesis is regulated by a STAT1 dependent but a type I, II and III interferon receptor independent mechanism. Plos Pathog. 2010;6(4):e1000849.
57. Sheahan T, Morrison TE, Funkhouser W, et al. MyD88 is required for protection from lethal infection with a mouse-adapted SARS-CoV. PLoS Pathog. 2008 Dec;4(12):e1000240.
58. Frieman M, Heise M, Baric R. SARS coronavirus and innate immunity. Virus Res. 2008 Apr;133(1):101-12.
59. Kopecky-Bromberg SA, Martínez-Sobrido L, Frieman M, et al. Severe acute respiratory syndrome coronavirus open reading frame (ORF) 3b, ORF 6, and nucleocapsid proteins function as interferon antagonists.
. J Virol. 2007;81:548-57.
60. Yang Y, Zhang L, Geng H, et al. The structural and accessory proteins M, ORF 4a, ORF 4b, and ORF 5 of Middle East respiratory syndrome coronavirus (MERS-CoV) are potent interferon antagonists. Protein Cell. 2013 Dec;4(12):951-61.
61. Matthews KL, Coleman CM, van der Meer Y, et al. The ORF4b-encoded accessory proteins of Middle East respiratory syndrome coronavirus and two related bat coronaviruses localize to the nucleus and inhibit innate immune signalling. J Gen Virol. 2014 Apr;95(Pt 4):874-82.
62. Totura AL, Baric RS. SARS coronavirus pathogenesis: host innate immune responses and viral antagonism of interferon. Curr Opin Virol. 2012 Jun;2(3):264-75.
63. Menachery VD, Eisfeld AJ, Schafer A, et al. Pathogenic influenza viruses and coronaviruses utilize similar and contrasting approaches to control interferon-stimulated gene responses. MBio. 2014;5(3):e01174-14.
64. van Hemert MJ, van den Worm SH, Knoops K, et al. SARS-coronavirus replication/transcription complexes are membrane-protected and need a host factor for activity in vitro. Plos Pathogen. 2008;4(5):e1000054.
65. Knoops K, Kikkert M, Worm SH, et al. SARS-coronavirus replication is supported by a reticulovesicular network of modified endoplasmic reticulum. PLoS Biol. 2008 Sep 16;6(9):e226.
66. Kato H, Takeuchi O, Mikamo-Satoh E, et al. Length-dependent recognition of double-stranded ribonucleic acids by retinoic acid-inducible gene-I and melanoma differentiation-associated gene 5. J Exp Med. 2008 Jul 7;205(7):1601-10.
67. Diamond MS, Farzan M. The broad-spectrum antiviral functions of IFIT and IFITM proteins. Nat Rev Immunol. 2013 Jan;13(1):46-57.
68. Daffis S, Szretter KJ, Schriewer J, et al. 2'-O methylation of the viral mRNA cap evades host restriction by IFIT family members. Nature. 2010 Nov 18;468(7322):452-6.
69. Menachery VD, Yount BL, Jr., Josset L, et al. Attenuation and restoration of severe acute respiratory syndrome coronavirus mutant lacking 2'-o-methyltransferase activity. J Virol. 2014 Apr;88(8):4251-64.
70. Baas T, Taubenberger JK, Chong PY, et al. SARS-CoV virus-host interactions and comparative etiologies of acute respiratory distress syndrome as determined by transcriptional and cytokine profiling of formalin-fixed paraffin-embedded tissues. J Interferon Cytokine Res. 2006 May;26(5):309-17.
71. Frieman M, Yount B, Heise M, et al. Severe acute respiratory syndrome coronavirus ORF6 antagonizes STAT1 function by sequestering nuclear import factors on the rough endoplasmic reticulum/Golgi membrane. J Virol. 2007 Sep;81(18):9812-24.

72. Zhao J, Falcon A, Zhou H, et al. Severe acute respiratory syndrome coronavirus protein 6 is required for optimal replication. J Virol. 2009 Mar;83(5):2368-73.
73. de Wilde AH, Raj VS, Oudshoorn D, et al. MERS-coronavirus replication induces severe in vitro cytopathology and is strongly inhibited by cyclosporin A or interferon-alpha treatment. J Gen Virol. 2013 Aug;94(Pt 8):1749-60.
74. de Lang A, Baas T, Teal T, et al. Functional genomics highlights differential induction of antiviral pathways in the lungs of SARS-CoV-infected macaques. PLoS Pathog. 2007 Aug 10;3(8):e112.
75. Meraz MA, White JM, Sheehan KC, et al. Targeted disruption of the Stat1 gene in mice reveals unexpected physiologic specificity in the JAK-STAT signaling pathway. Cell. 1996 Feb 9;84(3):431-42.
76. Hogan RJ, Gao G, Rowe T, et al. Resolution of primary severe acute respiratory syndrome-associated coronavirus infection requires Stat1. J Virol. 2004 Oct;78(20):11416-21.
77. Zornetzer GA, Frieman MB, Rosenzweig E, et al. Transcriptomic analysis reveals a mechanism for a prefibrotic phenotype in STAT1 knockout mice during severe acute respiratory syndrome coronavirus infection. J Virol. 2010 Nov;84(21):11297-309.
78. Page C, Goicochea L, Matthews K, et al. Induction of alternatively activated macrophages enhances pathogenesis during severe acute respiratory syndrome coronavirus infection. J Virol. 2012 Dec;86(24):13334-49.
79. Hamming I, Timens W, Bulthuis ML, et al. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol. 2004 Jun;203(2):631-7.
80. Kuba K, Imai Y, Rao S, et al. A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. Nat Med. 2005 Aug;11(8):875-9.
81. Dijkman R, Jebbink MF, Deijs M, et al. Replication-dependent downregulation of cellular angiotensin-converting enzyme 2 protein expression by human coronavirus NL63. J Gen Virol. 2012 Sep;93(Pt 9):1924-9.
82. Imai Y, Kuba K, Rao S, et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. Nature. 2005 Jul 7;436(7047):112-6.
83. Guy JL, Lambert DW, Warner FJ, et al. Membrane-associated zinc peptidase families: comparing ACE and ACE2. Biochim Biophys Acta. 2005 Aug 1;1751(1):2-8.
84. Kuba K, Imai Y, Penninger JM. Angiotensin-converting enzyme 2 in lung diseases. Curr Opin Pharmacol. 2006 Jun;6(3):271-6.
85. Arndt PG, Young SK, Poch KR, et al. Systemic inhibition of the angiotensin-converting enzyme limits lipopolysaccharide-induced lung neutrophil recruitment through both bradykinin and angiotensin II-regulated pathways. J Immunol. 2006 Nov 15;177(10):7233-41.
86. Gong MN. Genetic epidemiology of acute respiratory distress syndrome: implications for future prevention and treatment. Clin Chest Med. 2006 Dec;27(4):705-24; abstract x.
87. Malcolm KC, Kret JE, Young RL, et al. Bacteria-specific neutrophil dysfunction associated with interferon-stimulated gene expression in the acute respiratory distress syndrome. PLoS One. 2011;6(7):e21958.
88. Cameron MJ, Ran L, Xu L, et al. Interferon-mediated immunopathological events are associated with atypical innate and adaptive immune responses in patients with severe acute respiratory syndrome. J Virol. 2007 Aug;81(16):8692-706.

89. Verma GP. Fundamentals of Histology: New Age International Pvt Ltd Publishers; 2001.
90. Fehrenbach H. Alveolar epithelial type II cell: defender of the alveolus revisited. Respir Res. 2001;2(1):33-46.
91. Zhao J, Zhao J, Van Rooijen N, et al. Evasion by stealth. inefficient immune activation underlies poor T cell response and severe disease in SARS-CoV infected mice. Plos Pathog. 2009;5(10):e1000636.
92. Chambers RC, Scotton CJ. Coagulation cascade proteinases in lung injury and fibrosis. Proc Am Thorac Soc. 2012 Jul;9(3):96-101.
93. To KF, Tong JH, Chan PK, et al. Tissue and cellular tropism of the coronavirus associated with severe acute respiratory syndrome: an in-situ hybridization study of fatal cases. J Pathol. 2004 Feb;202(2):157-63.
94. Weinheimer VK, Becher A, Tonnies M, et al. Influenza A viruses target type II pneumocytes in the human lung. J Infect Dis. 2012 Dec 1;206(11):1685-94.
95. Gunther A, Ruppert C, Schmidt R, et al. Surfactant alteration and replacement in acute respiratory distress syndrome. Respir Res. 2001;2(6):353-64.
96. Wong CK, Lam CW, Wu AK, et al. Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. Clin Exp Immunol. 2004 Apr;136(1):95-103.
97. Ng PC, Lam CW, Li AM, et al. Inflammatory cytokine profile in children with severe acute respiratory syndrome. Pediatrics. 2004 Jan;113(1 Pt 1):e7-14.
98. Cheung OY, Chan JW, Ng CK, et al. The spectrum of pathological changes in severe acute respiratory syndrome (SARS). Histopathology. 2004 Aug;45(2):119-24.
99. Booth CM, Matukas LM, Tomlinson GA, et al. Clinical features and short-term outcomes of 144 patients with SARS in the greater Toronto area. JAMA. 2003 Jun 4;289(21):2801-9.
100. Haagmans BL, Osterhaus AD. Nonhuman primate models for SARS. PLoS Med. 2006 May;3(5):e194.
101. Wygrecka M, Jablonska E, Guenther A, et al. Current view on alveolar coagulation and fibrinolysis in acute inflammatory and chronic interstitial lung diseases. Thromb Haemost. 2008 Mar;99(3):494-501.
102. Smits SL, van den Brand JM, de Lang A, et al. Distinct severe acute respiratory syndrome coronavirus-induced acute lung injury pathways in two different nonhuman primate species. J Virol. May;85(9):4234-45.
103. Adams RL, Bird RJ. Review article: Coagulation cascade and therapeutics update: relevance to nephrology. Part 1: Overview of coagulation, thrombophilias and history of anticoagulants. Nephrology (Carlton). 2009 Aug;14(5):462-70.
104. Cesarman-Maus G, Hajjar KA. Molecular mechanisms of fibrinolysis. Br J Haematol. 2005 May;129(3):307-21.
105. Zhao X, Nicholls JM, Chen YG. Severe acute respiratory syndrome-associated coronavirus nucleocapsid protein interacts with Smad3 and modulates transforming growth factor-beta signaling. J Biol Chem. 2008 Feb 8;283(6):3272-80.
106. Wygrecka M, Markart P, Ruppert C, et al. Compartment- and cell-specific expression of coagulation and fibrinolysis factors in the murine lung undergoing inhalational versus intravenous endotoxin application. Thromb Haemost. 2004 Sep;92(3):529-40.
107. Senoo T, Hattori N, Tanimoto T, et al. Suppression of plasminogen activator inhibitor-1 by RNA interference attenuates pulmonary fibrosis. Thorax. 2010 Apr;65(4):334-40.

108. Sung JJ, Wu A, Joynt GM, et al. Severe acute respiratory syndrome: report of treatment and outcome after a major outbreak. Thorax. 2004 May;59(5):414-20.
109. Guery B, Poissy J, el Mansouf L, et al. Clinical features and viral diagnosis of two cases of infection with Middle East Respiratory Syndrome coronavirus: a report of nosocomial transmission. Lancet. 2013 Jun 29;381(9885):2265-72.
110. Zhang Q, Lai K, Xie J, et al. Does unrestrained single-chamber plethysmography provide a valid assessment of airway responsiveness in allergic BALB/c mice? Respir Res. 2009;10:61.
111. Criee CP, Sorichter S, Smith HJ, et al. Body plethysmography--its principles and clinical use. Respir Med. 2011 Jul;105(7):959-71.
112. Thompson BT, Bernard GR. ARDS Network (NHLBI) studies: successes and challenges in ARDS clinical research. Crit Care Clin. 2011 Jul;27(3):459-68.
113. Vlasak R, Luytjes W, Spaan W, et al. Human and bovine coronaviruses recognize sialic acid-containing receptors similar to those of influenza C viruses. Proc Natl Acad Sci U S A. 1988 Jun;85(12):4526-9.
114. Collins AR. HLA class I antigen serves as a receptor for human coronavirus OC43. Immunol Invest. 1993 Mar;22(2):95-103.
115. Vijgen L, Keyaerts E, Moes E, et al. Complete genomic sequence of human coronavirus OC43: molecular clock analysis suggests a relatively recent zoonotic coronavirus transmission event. J Virol. 2005 Feb;79(3):1595-604.
116. Dijkman R, Jebbink MF, Koekkoek SM, et al. Isolation and characterization of current human coronavirus strains in primary human epithelial cell cultures reveal differences in target cell tropism. J Virol. Jun;87(11):6081-90.
117. Collins AR. Human macrophages are susceptible to coronavirus OC43. Adv Exp Med Biol. 1998;440:635-9.
118. Bonavia A, Arbour N, Yong VW, et al. Infection of primary cultures of human neural cells by human coronaviruses 229E and OC43. J Virol. 1997 Jan;71(1):800-6.
119. Vabret A, Mourez T, Gouarin S, et al. An outbreak of coronavirus OC43 respiratory infection in Normandy, France. Clin Infect Dis. 2003 Apr 15;36(8):985-9.
120. Yeager CL, Ashmun RA, Williams RK, et al. Human aminopeptidase N is a receptor for human coronavirus 229E. Nature. 1992 Jun 4;357(6377):420-2.
121. Pfefferle S, Oppong S, Drexler JF, et al. Distant relatives of severe acute respiratory syndrome coronavirus and close relatives of human coronavirus 229E in bats, Ghana. Emerg Infect Dis. 2009 Sep;15(9):1377-84.
122. Pyrc K, Dijkman R, Deng L, et al. Mosaic structure of human coronavirus NL63, one thousand years of evolution. J Mol Biol. 2006 Dec 15;364(5):964-73.
123. Patterson S, Macnaughton MR. Replication of human respiratory coronavirus strain 229E in human macrophages. J Gen Virol. 1982 Jun;60(Pt 2):307-14.
124. Reed SE. The behaviour of recent isolates of human respiratory coronavirus in vitro and in volunteers: evidence of heterogeneity among 229E-related strains. J Med Virol. 1984;13(2):179-92.
125. Pene F, Merlat A, Vabret A, et al. Coronavirus 229E-related pneumonia in immunocompromised patients. Clin Infect Dis. 2003 Oct 1;37(7):929-32.
126. Hofmann H, Pyrc K, van der Hoek L, et al. Human coronavirus NL63 employs the severe acute respiratory syndrome coronavirus receptor for cellular entry. Proc Natl Acad Sci U S A. 2005 May 31;102(22):7988-93.

127. Huynh J, Li S, Yount B, et al. Evidence supporting a zoonotic origin of human coronavirus strain NL63. J Virol. Dec;86(23):12816-25.
128. Arden KE, Nissen MD, Sloots TP, et al. New human coronavirus, HCoV-NL63, associated with severe lower respiratory tract disease in Australia. J Med Virol. 2005 Mar;75(3):455-62.
129. van der Hoek L, Sure K, Ihorst G, et al. Croup is associated with the novel coronavirus NL63. PLoS Med. 2005 Aug;2(8):e240.
130. Vabret A, Dina J, Gouarin S, et al. Detection of the new human coronavirus HKU1: a report of 6 cases. Clin Infect Dis. 2006 Mar 1;42(5):634-9.
131. Risku M, Lappalainen S, Rasanen S, et al. Detection of human coronaviruses in children with acute gastroenteritis. J Clin Virol. May;48(1):27-30.
132. Yang ZY, Huang Y, Ganesh L, et al. pH-dependent entry of severe acute respiratory syndrome coronavirus is mediated by the spike glycoprotein and enhanced by dendritic cell transfer through DC-SIGN. J Virol. 2004 Jun;78(11):5642-50.
133. Ksiazek TG, Erdman D, Goldsmith CS, et al. A novel coronavirus associated with severe acute respiratory syndrome. N Engl J Med. 2003 May 15;348(20):1953-66.
134. Vijaykrishna D, Smith GJ, Zhang JX, et al. Evolutionary insights into the ecology of coronaviruses. J Virol. 2007 Apr;81(8):4012-20.
135. Hon CC, Lam TY, Shi ZL, et al. Evidence of the recombinant origin of a bat severe acute respiratory syndrome (SARS)-like coronavirus and its implications on the direct ancestor of SARS coronavirus. J Virol. 2008 Feb;82(4):1819-26.
136. Sims AC, Baric RS, Yount B, et al. Severe acute respiratory syndrome coronavirus infection of human ciliated airway epithelia: role of ciliated cells in viral spread in the conducting airways of the lungs. J Virol. 2005 Dec;79(24):15511-24.
137. Nicholls JM, Butany J, Poon LL, et al. Time course and cellular localization of SARS-CoV nucleoprotein and RNA in lungs from fatal cases of SARS. PLoS Med. 2006 Feb;3(2):e27.
138. Nicholls JM, Poon LL, Lee KC, et al. Lung pathology of fatal severe acute respiratory syndrome. Lancet. 2003 May 24;361(9371):1773-8.
139. Raj VS, Mou H, Smits SL, et al. Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. Nature. Mar 14;495(7440):251-4.
140. Cotten M, Watson SJ, Zumla AI, et al. Spread, circulation, and evolution of the Middle East respiratory syndrome coronavirus. MBio.5(1).
141. Zielecki F, Weber M, Eickmann M, et al. Human cell tropism and innate immune system interactions of human respiratory coronavirus EMC compared to those of severe acute respiratory syndrome coronavirus. J Virol. May;87(9):5300-4.
142. Eckerle I, Muller MA, Kallies S, et al. In-vitro renal epithelial cell infection reveals a viral kidney tropism as a potential mechanism for acute renal failure during Middle East Respiratory Syndrome (MERS) Coronavirus infection. Virol J.10:359.
143. Chu H, Zhou J, Wong BH, et al. Productive replication of Middle East respiratory syndrome coronavirus in monocyte-derived dendritic cells modulates innate immune response. Virology. Apr;454-455:197-205.
144. Memish ZA, Zumla AI, Al-Hakeem RF, et al. Family cluster of Middle East respiratory syndrome coronavirus infections. N Engl J Med. Jun 27;368(26):2487-94.
145. Roberts A, Vogel L, Guarner J, et al. Severe acute respiratory syndrome coronavirus infection of golden Syrian hamsters. J Virol. 2005 Jan;79(1):503-11.
146. Martina BE, Haagmans BL, Kuiken T, et al. Virology: SARS virus infection of cats and ferrets. Nature. 2003 Oct 30;425(6961):915.

147. Rowe T, Gao G, Hogan RJ, et al. Macaque model for severe acute respiratory syndrome. J Virol. 2004 Oct;78(20):11401-4.
148. Lawler JV, Endy TP, Hensley LE, et al. Cynomolgus macaque as an animal model for severe acute respiratory syndrome. PLoS Med. 2006 May;3(5):e149.
149. Smits SL, de Lang A, van den Brand JM, et al. Exacerbated innate host response to SARS-CoV in aged non-human primates. PLoS Pathog. Feb;6(2):e1000756.
150. McCray PB, Jr., Pewe L, Wohlford-Lenane C, et al. Lethal infection of K18-hACE2 mice infected with severe acute respiratory syndrome coronavirus. J Virol. 2007 Jan;81(2):813-21.