Viral Pathogens and Acute Lung Injury: Investigations Inspired by the SARS Epidemic and the 2009 H1N1 Influenza Pandemic

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Abstract

Acute viral pneumonia is an important cause of acute lung injury (ALI), although not enough is known about the exact incidence of viral infection in ALI. Polymerase chain reaction-based assays, direct fluorescent antigen (DFA) assays, and viral cultures can detect viruses in samples from the human respiratory tract, but the presence of the virus does not prove it to be a pathogen, nor does it give information regarding the interaction of viruses with the host immune response and bacterial flora of the respiratory tract. The severe acute respiratory syndrome (SARS) epidemic and the 2009 H1N1 influenza pandemic provided a better understanding of how viral pathogens mediate lung injury. Although the viruses initially infect the respiratory epithelium, the relative role of epithelial damage and endothelial dysfunction has not been well defined. The inflammatory host immune response to H1N1 infection is a major contributor to lung injury. The SARS coronavirus causes lung injury and inflammation in part through actions on the nonclassical renin angiotensin pathway. The lessons learned from the pandemic outbreaks of SARS coronavirus and H1N1 capture key principles of virally mediated ALI. There are pathogen-specific pathways underlying virally mediated ALI that converge onto a common end pathway resulting in diffuse alveolar damage. In terms of therapy, lung protective ventilation is the cornerstone of supportive care. There is little evidence that corticosteroids are beneficial, and they might be harmful. Future therapeutic strategies may be targeted to specific pathogens, the pathogenetic pathways in the host immune response, or enhancing repair and regeneration of tissue damage.

Keywords

- acute respiratory distress syndrome (ARDS)
- angiotensin-converting enzyme 2 (ACE2)
- pulmonary edema
- alveolar epithelium
- lung endothelium

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are important causes of morbidity and mortality. In the United States, the estimated incidence of ALI is 190,600 cases per year with an in-hospital mortality rate of nearly 40%.[1] Little is known about the incidence of viral infection in adults with ALI. A study of 592 children in Italy with community-acquired pneumonia, without specification of ALI, found that 74% of the enrolled children had a viral infection by direct fluorescent antigen (DFA) or polymerase chain reaction (PCR).[2] In clinical practice, viral infections are diagnosed using DFA assays, PCR-based assays, and viral culture. These methods only assess the most well-known human pathogens, including influenza A and B, parainfluenza, respiratory syncytial virus (RSV), adenovirus, metapneumovirus, rhinovirus, enterovirus, coronavirus, and cytomegalovirus (CMV). Research laboratories have the ability to detect many more viruses with nucleic acid amplification methods, although the clinical significance of these viruses is not well understood. This review focuses on the two viruses that caused major respiratory illness epidemics in

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the last decade: severe acute respiratory syndrome coronavirus (SARS CoV) and the 2009 pandemic H1N1 influenza A virus. In the years following these outbreaks, research using animal models elucidated some of the mechanisms involved in virally mediated ALI. Investigations into other common viral pathogens have also reshaped the way we think about the role of viral infection in ALI. For example, CMV is a common pathogen in severely immunosuppressed populations (especially organ transplant recipients), but was previously thought to be an uncommon pathogen in nosocomial pneumonia. With new diagnostic tools, the importance of detection, prevention, and treatment of CMV pneumonia in a broader population of critically ill patients is being reconsidered. Seroprevalence for CMV in adults ranges from 50 to 90%. CMV has been detected in nonimmunocompromised adults with critical illness and is thought to be reactivation of latent infection. It is associated with longer intensive care unit (ICU) and hospital stays, longer duration of mechanical ventilation, and increased rates of nosocomial infections. CMV infection is associated with increased interleukin (IL)-6 and IL-8 in vitro and in vivo, and these cytokines have been associated with ALI and ARDS.[3] Autopsy studies suggest that CMV may be an important pathogen in ventilator-associated pneumonia (VAP).[4] These observations provide the rationale for a prospective, randomized trial of CMV prevention with an antiviral agent such as ganciclovir as a novel means of improving outcomes in critically ill patients. In the era of sensitive and rapid nucleic acid amplification diagnostic tests, we can detect the presence of infectious agents, but determining when particular viruses are pathogenic and how they interact with the host immune system to cause lung injury or resolution of infection is an area of active research. It will be important to gain a better understanding of molecular pathophysiology of community-acquired and nosocomial viral pneumonias to devise more targeted therapies because current treatments are largely supportive.

The underlying pathophysiology of virally mediated ALI is not well understood, and it is likely that there are unique signature mechanisms to each viral strain that converge onto a common end pathway resulting in diffuse alveolar damage (DAD). It remains to be seen whether epithelial injury is the primary lesion or is coincident to endothelial injury. Most community-acquired respiratory viral pneumonias are inhaled and bind to receptors in the upper respiratory tract. Although the viruses initially infect the respiratory epithelium, it is possible that this is merely a portal of entry, and the important steps in alveolar damage are mediated primarily by endothelial injury resulting in elaboration of cytokines and chemokines and recruitment of both innate and adaptive immune cells. The specific cytokine profiles vary by viral pathogen and may be driven by macrophages, epithelial cells, endothelial cells, or some combination of crosstalk. If lung injury is not primarily mediated by viral infection, but rather is a result of the inflammatory host response, then viral clearance may not be central to the resolution of lung injury. The lessons learned from studying pandemic outbreaks of SARS CoV and H1N1 infection are reviewed in this article.

Background and Epidemiology of Severe Acute Respiratory Syndrome

In February 2003 an outbreak of SARS was first reported in the Guangdong province of China. Patients presented with fever, lower respiratory tract symptoms, and infiltrates on chest radiography consistent with pneumonia or ARDS. Within 1 month, cases were reported in Hong Kong, Singapore, Vietnam, and Canada, and the World Health Organization (WHO) launched efforts to investigate the illness and contain the rapid spread. The index case was a physician from Guandong province who had traveled to Hong Kong 5 days after symptom onset. The illness rapidly spread to more than two dozen countries in North America, South America, Europe, and Asia before the SARS global outbreak of 2003 was contained (http://www.who.int/csr/sars/en/). In April 2003, the causative agent was identified as a new member of the order Nidovirale and the coronavirus family (Coronaviridae).[5] The SARS CoV is an enveloped RNA virus that replicates with transcription of discontinuous nested messenger RNA (mRNA). The reservoir for the virus is thought to be civet cats, a nocturnal mammal considered a delicacy in southern China. Horseshoe bats may also be a reservoir. The incubation period is 2 to 7 days before symptom onset, and peak viral shedding in respiratory secretions occurs relatively late, between 6 and 11 days. The virus is spread through respiratory secretion shedding and via contact with fomites. Airborne transmission, particularly on international flights, contributed to superspreader outbreak phenomenon. By July 2003 there were 8,096 cases reported and 774 deaths due to SARS CoV, yielding a case fatality rate of 9.6%. In July 2003 the WHO lifted the travel advisory and the outbreak of SARS ended. Later in 2003 and 2004, there were four small SARS outbreaks. Three of these were laboratory-based outbreaks; one was attributed to exposure to an infected palm civet.

Several epidemiological studies using logistic regression showed that older age and underlying comorbid conditions (diabetes, chronic obstructive pulmonary disease, hepatitis B infection, cancer, and cardiac disease) were associated with worse outcomes including ICU admission, mechanical ventilation, and death.[6,7] The estimated case fatality rate was 13.2% (9.8 to 16.8) for patients younger than 60 years and 43.3% (35.2 to 52.4) for patients aged 60 years or older assuming a parametric gamma distribution.[8,9] The characteristics of patients most susceptible to infection are fairly nonspecific and provided limited insight into the mechanism of ALI that is mediated by SARS CoV infection. There has been some speculation that the severity of infection in older individuals is related to oxidative-stress machinery and an increased innate immunity response and more robust inflammatory response in older individuals. Aged macaques have a stronger host response to viral infection and show an increase in differential expression of genes associated with inflammation. Specifically nuclear factor kappa B (NF-kB) expression was upregulated, and type I interferon (IFN) was downregulated in older macaques and was associated with more severe lung injury.[10] The clinical pathology of patients who die with SARS infection cannot easily distinguish SARS-mediated ALI

from other types of lung injury and ARDS. Early reports from SARS case fatalities from Nan Fang Hospital Guangdong, China, showed pulmonary lesions that included DAD with hyaline membrane formation. Local hemorrhage and necrosis, desquamative pulmonary alveolitis and bronchitis, proliferation and desquamation of alveolar epithelial cells, exudation of protein and monocytes, lymphocytes and plasma cells in alveoli, hyaline membrane formation, and viral inclusion bodies in alveolar epithelial cells were also observed. Electron microscopy demonstrated clusters of viral particles, consistent with CoV, in lung tissue.[11] In 2005 a case control series from the University of Toronto described postmortem studies of pathology specimens from 20 patients who died between March and July 2003 with SARS confirmed by reverse transcription (RT)-PCR. The authors compared these patients to 22 age- and gender-matched SARS CoV–negative patients who presented with lower respiratory tract signs and symptoms and died during the same time period. The SARS patients had a longer mean duration of illness than controls and were more likely to have evidence of organizing pneumonia on hematoxylin and eosin–stained slides. All of the SARS cases exhibited pathological evidence of DAD, whereas 50% of controls had evidence of DAD. The authors reported significant overlap between SARS and non-SARS cases of patients who died with ARDS. They noted that it is difficult, if not impossible, to differentiate alveolar damage from SARS infection from other etiologies.[12] Overall, these findings suggest that the mechanism of ALI initiated by SARS CoV infection has a common pathway with other mechanisms that result in DAD and organizing pneumonia.

Treatment Recommendations Based on Human Studies

The outbreak of SARS infection was brief thanks to the effective containment efforts orchestrated by WHO and the Centers for Disease Control and Prevention (CDC) with the cooperation of governments of affected countries. The short-lived outbreak of SARS CoV infection provided little time for studying effective therapies. As in most cases of ALI, treatment of SARS is largely supportive; there is no known effective targeted therapy, and there is no clear role for corticosteroids. Limited in vitro and clinical trial data from ARDS studies exists for treatment with ribavirin, corticosteroids, lopinavir and ritonavir (LPV/r), type I IFN, intravenous immunoglobulin (IVIG), and SARS convalescent plasma. A systematic review of the literature sponsored by WHO reported that SARS CoV infection in tissue culture models was inhibited by ribavirin, lopinavir, and type I IFN. However, in SARS-infected patient reports on ribavirin, most studies were inconclusive, and four showed possible harm. Seven studies of convalescent plasma or IVIG, three of IFN type I, and two of LPV/r were inconclusive. In 29 studies of steroid use, 25 were inconclusive and 4 were classified as causing possible harm.[13] There have been no cases of SARS reported since 2004. Although the best care strategy for infected individuals is still unclear, the virus provided an opportunity to study pathways involved in ALI that may be common in lung injury mediated by other infectious agents or systemic illnesses.

Experimental Animal Models of SARS Infection

The membrane-bound Spike glycoprotein is critical for receptor binding and membrane fusion of the virus to host cells. In 2003, Li et al[14] published data from immunoprecipitation experiments and mass spectroscopy that identified a novel protein, angiotensin-converting enzyme 2 (ACE2) as the binding target for the Spike protein on the SARS CoV virus. ACE2 is an integral membrane metallopeptidase that cleaves angiotensin II (ATII) to form angiotensin 1–7 (Ang 1–7). Binding of SARS CoV to the ACE2 receptor causes internalization of the virus and downregulation of ACE2 expression. This discovery led to extensive investigation into the role of ACE2 in animal ALI models.

The tissue tropism of SARS CoV correlates with ACE2 expression and includes the lung, gastrointestinal tract, kidney, and liver. In humans, ACE2 protein has been detected throughout the respiratory tree, including in the epithelial cells of alveoli, trachea, bronchi, bronchial serous glands, as well as pneumocytes, and alveolar monocytes and macrophages.[11] Because ACE2 is expressed in the alveolar epithelium in both type I and type II alveolar epithelial cells and in the vascular endothelium of the lung in humans, it seems that the initial binding of the virus is in the respiratory tract, but progression of severe illness and lung injury involves endothelial pathology as well.[15] Although the C-type lectin CD209L (also known L-SIGN), and dendritic cell-specific C-type lectin bind SARS CoV, ACE2 appears to be the key functional receptor for the virus.[16]

ACE2 plays a central role in the nonclassical renin angiotensin system (RAS). The activity of classical and nonclassical RAS pathways appears to be balanced under normal physiological conditions. However, in several ALI models activation of AT II by ACE increases signaling through angiotensin Ia receptor (AT1aR), and ACE2 activity is downregulated. ACE2 is a negative regulator of the ACE pathway and cleaves AT II to form Ang 1–7 (►**Fig. 1**). The downstream effects of Ang 1–7 in the kidney are mediated by the G protein-coupled receptor, Mas, which appears to be an antagonist of the AT1aR.[17] ACE2 is necessary for the SARS CoV virus to infect alveolar epithelial cells in mice. SARS CoV-infected cells express proinflammatory cytokines including IL-6, IL-8, transforming growth factor (TGF) α and β, and monocyte chemotactic protein I (MCP-1). Some data suggest that a lack of type 1 IFN response may be important in SARS CoV–mediated lung injury.[16] Administration of type I IFN in aged macaques infected with SARS CoV reduced lung injury.[10] A variety of ALI animal models suggest that increased signaling through ACE and its downstream cytokines and decreased signaling through ACE2 may be important for lung injury.

In 2005, Imai et al[18] published findings from a series of murine knockout and rescue experiments that showed AT II is upregulated by ACE and drives ALI through AT1aR and that ACE2 and angiotensin II receptor 2 protect against lung injury

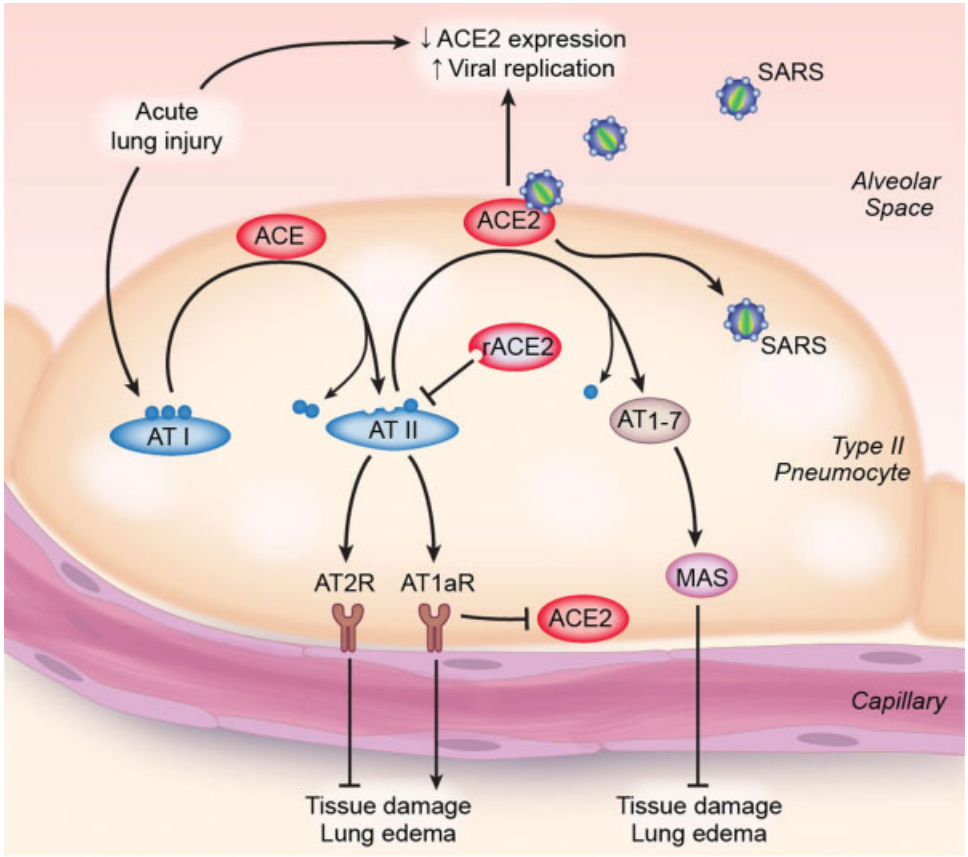

Fig. 1 Schematic representation of severe acute respiratory syndrome coronavirus (SARS CoV) infection mediating acute lung injury through angiotensin-converting enzyme (ACE) and angiotensin-converting enzyme 2 (ACE2) signaling pathways. ACE converts angiotensin I (AT I) to angiotensin II (AT II). AT II binds the angiotensin II receptor 1a (AT1aR), leading to tissue damage and lung edema, or it binds the angiotensin II receptor 2 (AT2R) reducing tissue damage. ACE2 inactivates AT II, generating angiotensin 1–7 (AT 1–7). SARS CoV binds to ACE2 causing downregulation of ACE2 through internalization of this membrane-bound protein and leading to viral replication in the cytoplasm. In this illustration the virus is infecting a type II pneumocyte. Administration of recombinant ACE2 (rACE2) reduces lung damage by inactivating AT II.

in aspiration and sepsis models. The authors showed that loss of ACE2 expression precipitates severe acute lung failure in three separate ALI models. Lung injury in ACE2 knockout mice resulted in increase elastance and worsened hypoxemia compared with wild-type mice subjected to acid aspiration lung injury. The ACE2 knockout lung injury phenotype is rescued by intraperitoneal administration of recombinant human ACE2 protein. ACE knockout is protective against lung injury in a gene dose-dependent fashion with the heterozygote showing an intermediate reduction of lung compliance after acid-induced lung injury as compared with the wild-type and ACE knockout. ACE knockout rescues ACE2 knockout lung injury in a gene dose-dependent fashion and results in improved lung compliance and reduction of pulmonary edema seen in ACE2 knockout mice with acid aspiration lung injury. AT1 receptor blockade rescues ACE2 knockout lung injury. An AT1aR antagonist ameliorates lung injury in wild-type mice with acid aspiration lung injury. Similar results have been reported for bleomycin-induced lung injury models. ACE2 knockout worsened bleomycin-induced lung injury.[19]

The findings from the foregoing series of knockout and rescue experiments showed that increased ACE activity mediates ALI, and ACE2 activity is protective. Investigators inferred that therapeutic strategies that inhibit the RAS would mitigate downstream elaboration of inflammatory cytokines and ameliorate lung injury.[20] A prospective study of a lipopolysaccharide (LPS)-mediated lung injury model in rats showed that pretreatment with enalapril, an ACE inhibitor that inhibits ACE activity and increases serum ACE2 activity, reduced serum expression of proinflammatory cytokines and reduced lung injury by LPS. Pretreatment with enalapril suppressed nuclear factor kappa B (NF-κB), a downstream target of AT II in rats subjected to LPS-mediated lung injury.

In rats subjected to LPS-induced lung injury and moderate tidal volume mechanical ventilation, oxygenation was improved in animals treated with losartan, an angiotensin receptor blocker. Administration of cyclic Ang 1–7 also significantly improved oxygenation in this animal model of lung injury. Although earlier studies focused on serum levels of the ACE and ACE2, Wösten-van Asperen et al[21] showed that, in bronchoalveolar lavage (BAL) fluid in rats with LPS- and mechanical ventilation–induced ARDS, ACE activity was higher and ACE2 activity was lower than in spontaneously breathing animals. Similarly, AT II levels were higher and Ang

1–7 levels were lower in animals with ARDS than in spontaneously breathing animals. Although it was not statistically significant, total protein in the BAL specimens of animals with LPS-induced lung injury treated with either losartan or cyclic Ang 1–7 tended to be lower. This finding suggests that treatments aimed at blunting ACE signaling pathway activity or enhancing the ACE2 signaling pathway may reduce inflammation, lung injury, and the amount of protein that accumulates in the alveoli.

Treml et al[22] conducted a prospective, randomized, double-blinded study of recombinant human ACE2 treatment in piglets with LPS-induced ARDS. The animals were intubated, mechanically ventilated, and had pulmonary artery catheters placed prior to LPS-induced lung injury. This was a small study with six piglets receiving sham normal saline infusion and six animals receiving infusion of recombinant ACE2. Another three animals served as negative controls and received no LPS and no therapy. The piglets treated with recombinant ACE2 had higher partial PaO_2 and a reduction in the mean pulmonary artery pressure with unchanged wedge pressures. The authors used inert gas elimination to evaluate ventilation and perfusion matching and found that pulmonary blood flow was more homogeneous in animals treated with ACE2. The authors addressed the concern of lowering systemic blood pressure with agents that inhibit the RAS by cleaving AT II. In control animals, not exposed to LPS, treatment with ACE2 induced hypotension and AT II levels were undetectable in these animals. Animals with LPS-induced lung injury were found to have an eightfold elevation in AT II. Treatment with ACE2 did not result in AT II levels below basal levels and did not change the blood pressure significantly.

Cytokine profiles that reflect the downstream signaling effects of the disruption of the ACE and ACE2 balance have been examined in lung samples obtained postmortem from SARS victims, from African green monkeys, from macaques, and from mice. Quantitative PCR analysis of postmortem samples from SARS victims showed a variety of genes were upregulated, including IL-8.[23] In situ hybridization studies of postmortem lung specimens using monoclonal antibodies showed an elaboration of various proinflammatory cytokines by ACE2-positive, SARS CoV–infected cells. MCP-1, TGF B1, tumor necrosis-α (TNF-α), IL-1b and cardiotrophin-like cytokine were upregulated in lungs and other organs of the SARS patients. African green monkeys develop more severe lung injury than macaques after SARS CoV infection and IL-6, IL-8, CXCL1, CXCL2, are induced and upregulated only in African green monkeys. These findings suggest that some of these differentially expressed proinflammatory genes may be critical in the pathogenesis of severe lung injury induced by SARS CoV infection.[24] In summary, downstream signaling pathways activated by SARS CoV infection, mediated by ACE and ACE2 activity, are not well understood but appear to reflect nonspecific elaboration of proinflammatory cytokines.

Beyond postmortem studies of gross pathology and gene expression studies from tissue samples from SARS victims, little is known about the ACE/ACE2 pathway in ALI. Kharofa et al[25] published results from a small retrospective study of 162 patients with stage I through III small cell and non–small cell lung cancer who received radiation therapy within the Veterans Affairs health care system between 2004 and 2009. The authors found that the rate of grade 2 pneumonitis was significantly lower in patients taking ACE inhibitors (2%) than in nonusers (11%). The patients who self-reported ACE inhibitor use were similar to those who did not use this class of medication with respect to types and stages of cancer, radiation dosage, lung volume irradiated, age, and concurrent chemotherapy. This small retrospective cohort study suggests that exposure to ACE inhibitors may reduce the risk of radiation pneumonitis.

Postmortem pathology studies and various in vitro and in vivo model systems of SARS CoV infection suggest that the virus enters through the respiratory tract and binds ACE2 in the alveolar epithelium. Infection is followed by serological evidence of increased ACE activity and decreased ACE2 activity. The signaling pathways that are activated by binding the SARS CoV to ACE2 and the subsequent downstream cytokine elaboration appear to share common features with other mechanisms of ALI and result in a pathological phenotype indistinguishable from other mechanisms of lung injury. The animal models of ALI that are rescued by administration of intravenous or intraperitoneal agents to restore the balance of ACE and ACE2 activity suggest that the vascular endothelium is also involved in lung injury and that therapy may be delivered systemically rather than to the alveolar epithelium, the site of initial viral binding and infection. The epidemiological features of persons at high risk of severe infection and complications do not suggest a particular host defect. It has been proposed that older individuals may elaborate a more robust inflammatory response due to alterations in the oxidative-stress machinery that come with age. However, systemic corticosteroids administered to blunt the downstream inflammatory cytokine elaboration were not effective treatments for patients with SARS CoV infection. The important downstream targets upregulated by ACE and downregulated by ACE2 are not yet well understood. The relative contributions of endothelial and epithelial processes to the development of lung injury and the molecular pathways that link initial binding of the virus to alveolar cells with the development of DAD are under active investigation. The pathways involved in lung injury induced by SARS CoV infection may be common to other mechanisms of injury, including LPS, acid aspiration, and cecal ligation and perforation, and therefore may provide the framework for developing a more universal therapy for lung injury mediated by other infectious and noninfectious processes.

Background and Epidemiology of 2009 Pandemic H1N1 Influenza

Six years after the SARS global outbreak, the 2009 H1N1 influenza pandemic erupted and with it came a surge of research investigating the mechanisms of lung injury that develop in severe cases of H1N1 infection. Influenza viruses belong to the family Orthomyoviridae, and are enveloped negative-sense RNA viruses with segmented genomes. There are three antigenically distinct subtypes, A, B, and C, which circulate globally among human populations. Influenza A

viruses are subdivided based on antigenic characterization of the surface glycoproteins hemagglutinin (HA) and neuraminidase (NA). There are 16 HA subtypes and nine NA subtypes. Influenza is an acute respiratory disease that presents with sudden onset of high fevers, chills, myalgias, upper respiratory tract symptoms, and diarrhea. Infection rarely induces symptoms of lower respiratory tract infections or severe lung injury. Influenza virus infection has been a global health concern since the 1918 Spanish flu pandemic. Three influenza pandemics occurred in the 20th century. The pandemics of 1918, 1957, and 1968 were caused by different antigenic subtypes of influenza A: H1N1, H2N2, and H3N2, respectively. In March 2009 a novel influenza virus emerged in Mexico and the United States and quickly spread worldwide. The pandemic A (H1N1) virus originated from the triple-reassortment of swine influenza (H1) virus circulating in North American pigs. On June 11, 2009, WHO declared a world pandemic alert.[26] By August 1, 2010, almost every country had reported laboratory-confirmed cases, with over 18,449 deaths http://www.who.int/csr/don/2010_08_06/en/index.html). A study of 642 cases from the early outbreak in the United States found the most common presenting symptoms were fever (94%), cough (92%), and sore throat (66%); 25% of patients had diarrhea, and 25% had vomiting. Hospitalization and mortality data were known for 399 of these cases and showed a 9% hospitalization rate and a case fatality ratio of 0.5%.[27] A study of 863 confirmed cases in Ontario, Canada, showed similar presenting symptoms and a hospitalization rate of 3.6% and a case fatality ratio of 0.2%.[28]

The 2009 pandemic H1N1 virus developed by reassortment among several influenza A strains. Nucleic acid sequencing showed that the HA, nucleoprotein (NP), and nonstructural protein (NS) gene segments were from the classical swine viruses; PB1 gene segment from human seasonal H3N2 influenza viruses; and PB2 and PA genes from avian influenza viruses. NA and M gene segments were genetically different from previously isolated human pathogens and found to originate from Eurasian swine influenza strains. Studies in ferrets and mice showed that, compared with seasonal H1N1, intranasal inoculation with pandemic H1N1 causes a higher morbidity, higher viral titers in lung tissue, and viral shedding in the gastrointestinal tract, suggesting a more invasive pathogen.[29–31] Seasonal influenza causes the highest mortality rates among older persons. In contrast, the 2009 H1N1 pandemic affected young individuals disproportionately.[32]

The majority of H1N1-infected patients were children or adults aged < 60 years; most recovered uneventfully, and the overall mortality was not higher than that of seasonal influenza. Risk factors for more severe infection by pandemic H1N1 include extremes of age, underlying medical illness, obesity, and pregnancy.[33–36] However, some previously healthy patients without comorbidities developed rapidly progressive pneumonia, ARDS, multiorgan failure, and death. ARDS was reported to be the prominent cause of death. Patients with more severe disease presented with fever, cough, respiratory distress, bilateral patchy pneumonia, elevated serum lactate dehydrogenase (LDH) and creatinine kinase levels, and leukopenia. These features were common to cohorts of adults and children studied worldwide.[26,36–40] Findings from several epidemiological studies suggest that pregnant women and obese individuals are more susceptible to severe infection from pandemic H1N1, but the molecular mechanisms underpinning these associations are not clear. The relationship between obesity and the inflammatory cascade responsible for ALI by various mechanisms is an area of active research. Adipocytes and macrophages isolated from obese patients secrete large quantities of proinflammatory cytokines, including IL-6 and TNF-α. The release of these factors into serum has been shown to contribute to the development of both obesity-related metabolic and cardiovascular diseases. It has been hypothesized that this chronic inflammation may also prime lung tissue for ALI.[41] The proinflammatory properties of lectin and anti-inflammatory properties of adiponectin may also influence the risk of developing ALI. Although epidemiological data from the H1N1 pandemic suggest that obese patients are at higher risk for ALI and have elevated levels of various inflammatory cytokines, the cytokine profiles from sera collected from ARDSNet participants showed that obese patients with ALI induced by a variety of mechanisms had lower levels of proinflammatory cytokines IL-6 and IL-8.[42] This finding suggests that the description of serum cytokines profiles varies by disease state, and it is unlikely that cytokine profiles are conserved among mechanisms of ALI from viral infections. Interestingly, the aforementioned study did find elevated levels of surfactant protein D (SP-D) and von Willebrand factor (vWF), markers for endothelial injury, in obese patients with ALI. Epithelial injury may be less important than endothelial disruption in some mechanisms of ALI. Recent studies in obese mice showed that obesity is associated with neutrophil dysfunction and attenuates murine ALI in an LPS model.[43] This study did not specifically investigate the role of the endothelial compartment in murine ALI.

Treatment Recommendations Based on Human Studies

Treatment of H1N1 infection is generally supportive. Most patients infected with the virus experience typical influenza symptoms and fully recover within a week and do not require antiviral therapy. Patients with severe illness or lower respiratory symptoms should be treated with NA inhibitors: oseltamivir, in most cases, or nebulized zanamivir or intravenous peramivir if oseltamivir is not available. In cases of severe illness, higher dosing of oseltamivir and longer duration of treatment may be considered.[28]

Although the authors of a prospective case series of 13 patients in Buenos Aires with ARDS and ALI presenting with flu-like illness suggest that treatment with corticosteroids in addition to NA inhibitors is safe, this is not the recommended treatment strategy for severe H1N1 infection. Only eight patients had PCR-confirmed H1N1, and the small size of this study limits analysis of mortality differences.[44] There are no convincing data suggesting that the use of systemic corticosteroids to treat severe H1N1 infection is beneficial.

Corticosteroid use has been associated with poor outcomes in other viral respiratory diseases. Early use of corticosteroids in SARS and H3N2 influenza was associated with prolonged periods of viral replication and may have increased the rate of secondary bacterial VAP.[45–47]

Recommendations for respiratory support of patients with ARDS or ALI from H1N1 are similar to the widely accepted use of lung-protective ventilation with lower tidal volume and a fluid conservative strategy.[48,49] Advanced supportive measures for refractory hypoxemia such as extracorporeal membrane oxygenation (ECMO), high-frequency oscillation ventilation, prone positioning, neuromuscular blockade, and inhaled nitric oxide have been effective in ICUs with expertise in these modalities.[38,39,50] IVIG and *N*-acetyl-L-cysteine have been used to treat infections by other influenza subtypes, but their use has not been studied in H1N1 infection.[28,51]

Because the mechanism of ALI in H1N1 infection is not well understood, treatment for patients infected with pandemic influenza A is largely supportive. Unraveling the molecular underpinnings of viral replication and the host immune response to infection may lead to targeted therapies or immunomodulatory strategies that could be more broadly applied to other mechanisms of ALI. Experiments with animal models have added some information about the cytokine cascade and inflammatory response to infection, although extrapolating these data to human infection is challenging because the many competing animal models suggest that there could be significant differences between species. Furthermore, there are many different H1N1 strains used in experimental models, and these strains are all subject to fairly rapid rates of mutations. Epidemiological studies summarized following here provide little information about why some patients develop more severe illness, characterized by an inflammatory response to viral infection leading to ALI with the most severe cases showing nonspecific pathological findings of DAD with or without hemorrhage or necrotizing bronchiolitis. The relationship of epithelial and endothelial injury is not well understood, and experimental models do not always distinguish between direct cytotoxic effects of alveolar epithelial cell infection and host inflammatory responses that arise from endothelial injury and systemic, circulating factors.

Human Studies Performed During the 2009 H1N1 Pandemic

Only a small minority of patients infected with pandemic H1N1 go on to develop serious illness necessitating hospitalization or intensive care. The factors that mitigate the more severe inflammatory host response are still unclear. A study of sera from 57 patients in Hong Kong with H1N1 infection showed that T-helper 1 (Th1) and T-helper 17 (Th17) hypercytokinemia is an early host response in severe 2009 H1N1 cases. A prospective analysis of cytokine and chemokine profiles in sera from 35 patients in Spain with H1N1 infection showed common expression patterns of innate antiviral response proteins across patients with mild illness and those with more severe presentations. All 35 subjects with H1N1 infection showed increased expression of chemokines CXCL10, CCL2, and CCL4. These chemokines are also upregulated in SARS CoV, H5N1, and RSV infection and are markers for innate immune response to viral infections. The authors compared the serum cytokine profiles of infected outpatients with those of patients with noncritical respiratory insufficiency admitted to the hospital, and critically ill patients with respiratory insufficiency. Patients with respiratory compromise had increased levels of Th1 and Th17 cytokines when compared with healthy individuals and outpatient controls. Critically ill patients had elevated serum levels of IL-15, IL-12p70, and IL-6. These proinflammatory cytokines stimulate the adaptive immune response and may represent the pathological difference between the host response to infection that causes mild illness and those that result in severe systemic inflammatory response.[52]

A study of 32 patients in Romania with H1N1, 21 with ARDS and 11 with mild disease, showed that severe influenza A (H1N1) virus infection was characterized by IL-6, IL-15, IL-8, and TNF-α.[53] These cytokines, except TNF-α, had a positive correlation with the admission delay and C-reactive protein, and a negative correlation with the PaO_2: FiO_2 ratio. Of note, in this study IL-17 levels were not elevated in patients with severe disease. Interestingly, obese patients with pandemic H1N1 infection have significantly higher levels of IL-8 than infected nonobese patients. This finding differs from the previously described results from Stapleton et al[42] using sera from ARDSNet patients, collected before the H1N1 pandemic.

Three distinct pulmonary histological patterns have been described in autopsy studies of patients infected with pandemic H1N1; DAD, necrotizing bronchiolitis, and DAD with intense pulmonary hemorrhage.[54] Several case series found a significant proportion of patients with severe H1N1 infection had secondary bacterial pneumonia with *Streptococcus*, *Staphylococcus*, and *Haemophilus* species, and a few cases with multiple bacterial pathogens isolated.[55,56] These autopsy studies show H1N1 results in a nonspecific final pathological pattern and may predispose to superimposed bacterial infections. A recent cohort study by Rice et al[57] found that bacterial coinfection was common in patients infected with pandemic H1N1 admitted to ARDSNet ICUs in North America. Thirty percent of patients in this study had evidence of bacterial coinfection, and of the patients with bacterial infections, 11% had *Staphylococcus aureus* in blood or respiratory cultures and 8% had *Streptococcus pneumoniae*. Human studies from serum, BAL fluid, and autopsy specimens have not shed much light on the molecular mechanisms involved in H1N1-induced ALI. The clinical response to influenza infection ranges from mild disease to severe pneumonia, and it remains unclear whether the inflammatory response to infection is protective or pathogenic. Experiments using a variety of animal models have provided more detailed information about the inflammatory response to infection and some areas for investigations of potential therapeutic strategies.

Experimental Animal Models of 2009 Pandemic H1N1 Infection

Findings from animal models should be extrapolated to human disease with caution. In the case of pandemic H1N1 influenza infection, it is important to recognize that research groups use a variety of animal models and different viral strains. Several viral isolates have been used by various research groups to study H1N1 infection in mouse models, including pandemic 2009 A/California/07/2009 (CA07), influenza A/Puerto Rico/8/34 (PR8), and A/Beijing/501/2009 (BJ501).[29,58,59] Furthermore, these strains are subject to mutations, which may have important implications for data interpretation. Several researchers have demonstrated viral mutations in clinical and experimental strains that confer higher virulence. For example, a substitution of glutamate by glycine at position 222 of the viral hemagglutinin was found to be significantly more frequent in patients with severe pandemic influenza H1N1; furthermore other mutations have been shown to confer higher virulence in mice.[59–62] In addition to mutations within a given strain, subtype variations also influence experimental findings. Although it is tempting to extrapolate data from other viral subtypes to pandemic H1N1 infection, this approach is misleading. However, Garigliany et al[63] found that the two different influenza A virus subtypes, H1N1 and H5N1, both evoked DAD as a final pathological finding, and they were able to describe different features within the DAD pattern. Interestingly, mice infected with these viruses showed differences in courses of ARDS, and blinded examination of histopathologic findings could distinguish different signature features. These findings suggest that, although infection by these two viral subtypes ultimately results in the nonspecific finding of DAD, there may not be a conserved inflammatory pathway underlying all severe influenza infections. Selecting an appropriate animal model that accurately reflects the human immune response to H1N1 infection and the development of lung injury has been challenging. Models of the 2009 H1N1 pandemic flu have been developed in mice, ferrets, guinea pigs, and nonhuman primates. H1N1 strains have been adapted to infect the host of interest. Mouse models have generated the bulk of available data on H1N1 infection.

Cytokine Signaling

Crowe et al observed that IL-17RA knockout mice recruited fewer neutrophils to the airway in response to challenge with either influenza A virus or hydrochloric acid and that this decrease in neutrophils results in lower amounts of oxidized phospholipids.[64] IL-17 regulates neutrophil and Th1 cell recruitment ligands. Furthermore, the IL-17RA knockout mice had lower morbidity and mortality despite longer latency to viral clearance and higher viral titers. The IL-17RA knockout mice had less lung injury, as indicated by less total protein and LDH activity in the BAL fluid and less severe inflammation on histological examination. The differences in inflammation between wild-type and IL-17RA-deficient mice correlated well with differences in many inflammatory cytokines. Importantly, TNF-α, IL-1β, and IL-6, three proinflammatory cytokines that are generally elevated in severe influenza infection as part of a cytokine storm, all either trended lower or were significantly reduced in the IL-17RA −/− animals. These data support the potential therapeutic manipulation of IL-17 or IL-17RA in ALI.

BAL fluids from 4-week-old B6 mice infected with BJ501 had similar cytokine and chemokine profiles to the human sera samples in the studies of patients from Spain and Hong Kong described earlier. Compared with wild-type B6 mice, IL-17-deficient mice infected with BJ501 had improved survival, less weight loss, less leukocyte infiltration and therefore lower lung injury scores, and decreased lung edema. Intravenously administered anti-IL-17 antibodies also reduced disease severity.[65] Similarly, Crowe et al showed that IL-17RA-deficient mice had reduced weight loss and improved survival after influenza infection.[64] These data support the hypothesis that IL-17 plays a critical role in mediating lung injury. IL-17 is secreted by many cells, including Th17 cells, gamma delta T cells, and natural killer (NK) cells. It acts as a proinflammatory cytokine and links the innate and adaptive immune system by recruiting neutrophils, it has a role in a variety of tissue injury models, and it has been implicated in several disease processes.[66,67] A fully human monoclonal IL-17 antibody has been developed by Novartis and clinical trials in Crohn disease and rheumatoid arthritis are under way.

Airway Macrophages

Following intranasal infection of mice, influenza virus replicates in type II epithelial cells lining the respiratory tract. Alveolar macrophages, located at the interface between air and lung, are mediators of the innate immune response and elaborate cytokine responses that limit further replication of influenza virus.[68] It is not clear whether influenza infection of macrophages is abortive and viral progeny are not released, or if there is perhaps limited release of H1N1 and H5N1 viruses from infected mouse macrophages.[69] Despite similar abilities to infect urine epithelial cells, virus strain BJx109 (H3N2) causes mild disease while virus strain PR8 (H1N1) causes severe disease. BJx109 infects murine airway macrophages with high efficiency, whereas PR8 does not. PR8 causes more severe disease than BJx109. In macrophage-depleted mice, BJx109 causes severe disease, but the disease severity of PR8 infection in macrophage-depleted mice is similar to untreated mice. Expression of the BJx109 HA on a PR8 backbone ameliorates severe disease.[70] Collectively, these findings suggest that internalization and nonproductive infection of airway macrophages could be a critical factor in limiting severe disease caused by influenza infection in mice.

Neutrophils

Data suggest that neutrophils play a key role in the development of ALI after H1N1 infection and that the result of the previously described cytokine elaboration is neutrophil recruitment to the alveolar space. Several mouse models of infection show results that are similar to the epidemiological findings from the 2009 pandemic. Influenza A/swine/Shandong/731/2009 (SD/09) is H1N1 with G222D mutation in the HA. Mice infected with SD/09 developed ARDS and had a 60% mortality rate between days 8 and 10 after infection. Mice show neutrophil-predominant infiltrates in the BAL fluid.

Serum IL-6 and TNF-α were elevated in SD/09 infected mice, as were Il-10, IFN-gamma, and MCP-1.[71]

As already described, pregnant women are at higher risk of developing severe H1N1 infection. Pregnant BALB/c mice have a higher mortality, more severe pneumonitis, higher pulmonary viral load, lower peripheral blood T lymphocytes and antibody responses, higher levels of proinflammatory cytokines and chemokines, and worse fetal development than occurred in nonpregnant controls infected by either wild-type (clinical isolate) or mouse-adapted mutant virus with D222G substitution in HA. Compared with nonpregnant mice, significantly higher levels of IL-1β, macrophage-inflammatory protein (MIP)-1α, and MIP-2 were detected in pregnant mice infected with wild-type virus ($P < 0.03$), whereas levels of IL-6, MIP-1α, and MIP-2 were elevated significantly in pregnant mice infected by mutant virus ($P < 0.02$). Notably, level of IFN-γ was significantly lower in pregnant mice than in nonpregnant mice ($P < 0.05$), whereas TNF-α level was similar between pregnant and nonpregnant mice, whether they were infected by wild-type or mutant virus.[59]

Neutrophilic infiltrates have been observed in BAL fluid and on pathology specimens from patients infected with severe H1N1 infection leading to ARDS, but the mechanism of alveolar damage has not been definitively worked out. Neutrophils have a primary role in the innate immune system through phagocytosis, secretion of reactive oxygen intermediates, and formation of neutrophil extracellular traps (NETs). Neutrophils emit their DNA fibers that carry nuclear and cytoplasmic proteins and trap extracellular pathogens. NETs attach to the capillary endothelium and are associated with vasculitis and vascular damage in sepsis.

Macrophage-depleted mice infected with PR8 influenza A H1N1 displayed enhanced viral replication, excessive neutrophilic infiltration, alveolar damage, lung edema, and progression to ARDS.[72] Neutrophil-depleted mice showed only mild lung pathology after H1N1 infection. The authors noted prominent NETs formation in lung tissue from the macrophage-depleted animals after H1N1 infection. NETs were also present in normal mice treated with lethal doses of H1N1. In vitro studies suggested that influenza infection enhances NETs formation. Neutrophils incubated with influenza-primed alveolar epithelial cells formed NETs. This particular interaction between NETs and the endothelium could help explain how epithelial and endothelial damage are related in the development of ARDS in response to H1N1 infection.

As previously mentioned, there does not seem to be a conserved pathological mechanism for lung injury induced by different influenza subtypes. In an H3N2 murine model, Tate et al showed that reduced numbers or impaired neutrophil function facilitates progression of mild influenza to severe clinical disease. Neutrophil depletion with monoclonal antibodies was associated with more severe illness and lung injury and enhanced viral replication in a murine model of H3N2 infection.[73,74]

Endothelium

Many of the aforementioned studies do not specifically distinguish between the mechanism of injury to alveolar epithelium and the capillary endothelium. Much of the data are based on serum cytokine levels that are not always correlated with alveolar lavage samples. NETs provide one possible explanation for how the inflammatory response in both compartments may be related. Interestingly, most of the rescue therapies in murine models are administered intraperitoneally or intravenously. Effective systemic therapy delivered hematogenously to the alveolus suggests that the endothelium has an important role in cytokine and chemokine signaling and generating the inflammatory host response in severe influenza. Studies of H5N1 virus in human microvascular endothelial cell cultures show viral tropism for the apical surface and high infectivity.[75] Similar studies of H1N1 infection of monolayer cell culture models of human pulmonary microvascular endothelial cells showed increased expression of inflammatory cytokines Il-6, IL-8, TNF-α, and interferon gamma-induced protein-10. This cytokine expression was reduced by inhibiting pre–B cell colony enhancing factor (a potential biomarker for ALI in animal models).[76] The sphingosine-1-phosphate (S1P) receptor is expressed in endothelial cells and lymphocytes within the lung tissue. In mice, an S1P receptor antagonist suppresses chemokine and cytokine production, including IFN-α, CCL2, EL-6, TNF-α, and IFN-α after influenza infection. The reduction in cytokine elaboration by S1P is associated with decreased extravasation of leukocytes into the alveolar space, but it does not decrease the recruitment of macrophages and NK cells after H1N1 infection.[77] Suppressing the adaptive immune response was associated with improved mortality. Collectively, these data suggest that endothelial cells orchestrate the distinct events of immune cell infiltration and cytokine production following H1N1 infection in mice.

Current and Potential Therapeutic Strategies

Supportive measures including lung protective ventilation and NA inhibitors are the mainstay of therapy for severe influenza infection, resulting in respiratory failure and ALI. Monoclonal antibodies and vaccines directed at conserved regions between influenza strains are areas of ongoing investigation. IL-17 is another target for potential monoclonal antibody therapy. Corticosteroids are not currently recommended and may be harmful.[78,79] Investigations of generalized anti-inflammatory therapy are under way. For example, a combination of caffeine and a statin ameliorated lung injury and viral replication in BALB/c mice infected with H5N1, H3N2, and H1N1.[79] Targeted nucleic acid therapy is also being studied. Fang et al explored a microsatellite DNA mimicking oligodeoxynucleotides (MS ODN) for the treatment of H1N1 infection. On days 2 and 4 postinfection with influenza A/FM/1/47 virus (H1N1), mice were injected with MS ODN named SAT05f. This MS ODN is capable of inhibiting Toll-like receptors 7 and 9. The MS ODN treatment improved survival, less histopathological changes with fewer neutrophils per high-power field, and reduced levels of TNF-α in lung homogenates.

When investigating therapeutic strategies, it is important to consider if it is practical to expect an intervention can be

made before significant lung injury develops in patients infected with H1N1, or any viral pneumonia for that matter. Although it is possible that earlier identification of patients before they require positive pressure ventilation in what is now termed early ALI might facilitate efficacy of future therapies.[80]

In addition, future strategies will need to focus on mechanisms that might enhance repair. The use of stem cells and stem cell–derived growth factors may become an important therapeutic intervention for patients with ALI and ARDS, independent of the mechanisms of initial injury.[81]

Conclusions

The lessons learned from studying pandemic outbreaks of SARS CoV and H1N1 capture some key principles of virally mediated ALI. There appear to be several pathogen-specific pathways underlying virally mediated ALI that converge onto a common end pathway resulting in DAD. It remains to be determined whether alveolar epithelial injury is the primary lesion or is coincident to lung endothelial injury. There also seems to be an interaction of viral infection with the microbiome of the lung that might predispose patients with viral pneumonia to secondary bacterial and fungal pneumonia. Therapeutic strategies may be targeted to specific pathogens or common pathways in the host immune response. Alternatively, perhaps it would be most advantageous to investigate lung repair strategies that rely less on a complete understanding of the complicated virus–host interaction and more on repairing the final common pathway of DAD. These repair strategies may provide treatments for all virally mediated lung injury as well as ALI sustained from a variety of other insults.[82] Scientists speculate that many influenza viruses, including H5N1, are capable of causing a pandemic in the near future.[83] The looming threat of the next outbreak of a pandemic respiratory viral infection makes a compelling argument for the continuation of investigations into the host immune response to viral infection in the lung, better antiviral therapies, and research targeting the fundamental strategies to enhance lung repair from virally mediated ALI.

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