

Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology

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Received: 9 November 2016 / Accepted: 10 April 2017
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Abstract Human coronaviruses (hCoVs) can be divided into low pathogenic and highly pathogenic coronaviruses. The low pathogenic CoVs infect the upper respiratory tract and cause mild, cold-like respiratory illness. In contrast, highly pathogenic hCoVs such as severe acute respiratory syndrome CoV (SARS-CoV) and Middle East respiratory syndrome CoV (MERS-CoV) predominantly infect lower airways and cause fatal pneumonia. Severe pneumonia caused by pathogenic hCoVs is often associated with rapid virus replication, massive inflammatory cell infiltration and elevated pro-inflammatory cytokine/chemokine responses resulting in acute lung injury (ALI), and acute respiratory distress syndrome (ARDS). Recent studies in experimentally infected animal strongly suggest a crucial role for virus-induced immunopathological events in causing fatal pneumonia after hCoV infections. Here we review the current understanding of how a dysregulated immune response may cause lung immunopathology leading to deleterious clinical manifestations after pathogenic hCoV infections.

Keywords SARS-CoV · MERS-CoV · Cytokine storm · Immunopathology · Interferon · Monocyte-macrophage

Introduction

Coronaviruses belong to the virus family Coronaviridae and are enveloped, positive-sense RNA viruses. The coronavirus genome is approximately 31 Kb, making these viruses the largest known RNA viruses [1, 2]. Coronaviruses infect a variety of host species, including humans and several other vertebrates. These viruses predominantly cause respiratory and intestinal tract infections and induce a wide range of clinical manifestations [3, 4]. Coronaviruses infecting the respiratory tract have long been recognized as significant pathogens in domestic and companion animals and as the cause of mild and severe respiratory illness in humans [4, 5]. In general, coronaviruses infecting humans can be classified into low pathogenic hCoVs, which include HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU and highly pathogenic CoVs such as severe acute respiratory syndrome CoV (SARS-CoV) and Middle East respiratory syndrome CoV (MERS-CoV) [6, 7]. Low pathogenic hCoV infect upper airways and cause seasonal mild to moderate cold-like respiratory illnesses in healthy individuals. In contrast, the highly pathogenic hCoVs (pathogenic hCoV or hCoV hereafter) infect the lower respiratory tract and cause severe pneumonia, which sometimes leads to fatal acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), resulting in high morbidity and mortality [8–12].

Highly pathogenic hCoVs pose a substantial threat to public health. During the 2002–2003 epidemic, SARS-CoV infected approximately 8400 individuals with a 9.6% overall mortality rate [13, 14]. More recently, MERS-CoV crossed species to infect 1936 individuals resulting in 690 deaths (~36% mortality rate) as of April 5, 2017 [15, 16]. Recent identification of SARS-like coronaviruses in bats and MERS-CoV in domesticated camels makes it likely that these viruses will continue to cross species barriers and cause

This article is a contribution to the special issue on Cytokine Storm in Infectious Diseases - Guest Editor: John Teijaro

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additional outbreaks in human populations [17–20]. These highly pathogenic hCoVs cause a wide spectrum of clinical manifestations in humans, with a large fraction of patients developing short period of moderate clinical illness and a small but a substantial number of patients experiencing severe disease characterized by ALI and ARDS [21–23, 10]. Thus, there are basically two groups of patients, those developing milder disease, which resolved and those with severe disease, which was commonly fatal. The disease severity in pathogenic hCoV infections was also influenced by several factors such as initial viral titers in the airways and age and comorbid conditions of the infected individual. While younger individuals below 18 years experience mild-moderate clinical illness, elderly individuals exhibit worse outcomes after infection with SARS-CoV or MERS-CoV [22, 10, 24]. Additionally, individuals with comorbid conditions such as diabetes, obesity, heart failure, and renal failure among others experience severe disease, particularly after MERS-CoV infection [25, 26].

Despite several years of research, specific factors causing the unusually high morbidity and mortality following pathogenic hCoVs are incompletely understood. Virus-induced direct cytopathic effects and viral evasion of host immune responses are believed to play major roles in disease severity. However, studies from humans who died of SARS and more recent studies in animal models suggested that a dysregulated immune response occurred, resulting in an exuberant inflammation and lethal disease. In this review, we discuss recent advances in our understanding of hCoV pathogenesis, with a special emphasis on cytokine storm and immunopathology as causes for deleterious consequences during hCoV infections.

Clinical features of highly pathogenic CoV infection in humans

SARS-CoV infection in humans resulted in an acute respiratory illness that varied from mild febrile illness to ALI and in some cases ARDS and death [27, 10]. The clinical course of SARS presents in three distinct phases. The initial phase was characterized by robust virus replication accompanied by fever, cough, and other symptoms, all of which subsided in a few days. The second clinical phase was associated with high fever, hypoxemia, and progression to pneumonia-like symptoms, despite a progressive decline in virus titers towards the end of this phase [28]. During the third phase, ~20% of patients progressed to ARDS, which often resulted in death [29, 30]. Because of a progressive decline in virus titers, the third phase is thought to have resulted from exuberant host inflammatory responses.

The most common clinical manifestations of MERS include flu-like symptoms such as fever, sore throat, non-productive cough, myalgia, shortness of breath, and dyspnea, which rapidly progress to pneumonia [25, 21]. Other atypical presentations include mild respiratory illness without fever,

chills, wheezing, and palpitations. MERS-CoV in humans also causes gastrointestinal symptoms such as abdominal pain, vomiting, and diarrhea. The majority of MERS patients with dyspnea progress to develop severe pneumonia and require admission to an intensive care unit (ICU). Although most healthy individuals present with mild-moderate respiratory illness, immunocompromised and individuals with comorbid conditions experience severe respiratory illness, which often progressed to ARDS [21]. Overall, MERS-CoV caused severe disease in primary index cases, immunocompromised individuals and in patients with comorbid conditions, but secondary cases of household contacts or healthcare workers were mostly asymptomatic or showed mild respiratory illness.

Lung pathology of hCoV infections

Gross and microscopic pathology of SARS

Typically, analyses of lungs from patients who succumbed to SARS showed lung consolidation and edema with pleural effusions, focal hemorrhages, and mucopurulent material in the tracheobronchial tree. Diffuse alveolar damage (DAD) was a prominent histological feature in SARS lungs [31, 32]. Other changes included hyaline membrane formation, alveolar hemorrhage, and fibrin exudation in alveolar spaces with septal and alveolar fibrosis observed during later stages [32, 33]. Staining for viral antigen revealed infection of airway and alveolar epithelial cells, vascular endothelial cells, and macrophages [31, 32]. Furthermore, SARS-CoV viral particles and viral genome were also detected in monocytes and lymphocytes [31].

In addition to these changes, histological examination of lungs from patients who died of SARS revealed extensive cellular infiltrates in the interstitium and alveoli. These cellular infiltrates included neutrophils and macrophages with macrophages being the predominant cell type [31, 32]. These results correlated with increased numbers of neutrophils and monocytes and lower CD4 and CD8 T cell counts in the peripheral blood samples of patients with fatal SARS [34–36].

Gross and microscopic pathology of MERS

Despite numerous laboratory-confirmed cases and deaths due to MERS-CoV infection in several countries, only one autopsy report of MERS in humans is available. Analysis of lung tissue from this patient showed pleural, pericardial, and abdominal effusions associated with generalized congestion, edema, and consolidation of lungs [37]. Similar to SARS-CoV infection, DAD was a prominent feature in the lungs. Additionally, epithelial cell necrosis, sloughing of bronchiolar epithelium, alveolar edema, and thickening of alveolar septa were also noted. Immunohistochemical examination showed that MERS-CoV predominantly infected airways and alveolar epithelial cells,

and endothelial cells and macrophages. The severity of lung lesions correlated with extensive infiltration of neutrophils and macrophages in the lungs and higher numbers of these cells in the peripheral blood of MERS patients [37].

Cytokine and chemokine responses during pathogenic hCoV infections

Cytokines and chemokines have long been thought to play an important role in immunity and immunopathology during virus infections. A rapid and well-coordinated innate immune response is the first line of defense against viral infections, but dysregulated and excessive immune responses may cause immunopathology [38–40]. Although there is no direct evidence for the involvement of pro-inflammatory cytokines and chemokines in lung pathology during SARS and MERS, correlative evidence from patients with severe disease suggests a role for hyper-inflammatory responses in hCoV pathogenesis.

Cytokine and chemokine responses to SARS-CoV infection

While SARS-CoV productively infects airway and alveolar epithelial cells, infection of hematopoietic cells such as dendritic cells (DCs), monocyte-macrophages, and other PBMC-derived cells is abortive. SARS-CoV infection of DCs induces low-level expression of antiviral cytokines IFN- α β , moderate up-regulation of pro-inflammatory cytokines TNF and IL-6, and a significant up-regulation of inflammatory chemokines CCL3, CCL5, CCL2, and CXCL10 [41, 42]. Similarly, SARS-CoV-infected macrophages show delayed but elevated levels of IFN and other pro-inflammatory cytokines [42]. Additionally, SARS-CoV-infected airway epithelial cells (AECs) also produce large amounts of CCL3, CCL5, CCL2, and CXCL10 [43]. The delayed but excessive production of these cytokines and chemokines is thought to induce a dysregulated innate immune response to SARS-CoV infection.

High serum levels of pro-inflammatory cytokines (IFN- γ , IL-1, IL-6, IL-12, and TGF β) and chemokines (CCL2, CXCL10, CXCL9, and IL-8) were found in SARS patients with severe disease compared to individuals with uncomplicated SARS [44–47]. Conversely, SARS patients with severe disease had very low levels of the anti-inflammatory cytokine, IL-10 [44]. In addition to pro-inflammatory cytokines and chemokines, individuals with lethal SARS showed elevated levels of IFN (IFN- α and IFN- γ) and IFN-stimulated genes (ISGs) (CXCL10 and CCL-2) compared to healthy controls or individuals with mild-moderate disease [48–51]. These results were the first to suggest a possible role for IFNs and ISGs in the immunopathogenesis of SARS in humans. Thus, it appears from these studies that dysregulated and/or exaggerated cytokine and chemokine responses by SARS-CoV-infected

AECs, DCs, and macrophages could play an important role in SARS pathogenesis.

Cytokine and chemokine responses to MERS-CoV infection

Similar to SARS, MERS-CoV infection of human airway epithelial cells induces significant but delayed IFN and pro-inflammatory cytokine (IL-1 β , IL-6, and IL-8) responses [52]. While MERS-CoV replicates both in naïve and activated human monocyte-macrophages and DCs, only activated T cells support MERS-CoV replication [53–55]. This is in contrast to SARS-CoV, which abortively infected monocyte-macrophages, DCs, and T cells. MERS-CoV infection of THP-1 cells, a monocyte cell line, and human peripheral blood monocyte-derived macrophages and dendritic cells induced delayed but elevated levels of pro-inflammatory cytokines and chemokines such as CCL-2, CCL-3, CCL-5, IL-2, and IL-8 [54, 55]. However, induction of IFN- α / β by monocyte-macrophages and DCs was not substantial except for plasmacytoid dendritic cells, which produced copious amounts of IFNs upon MERS-CoV infection [56]. Recent studies showed elevated levels of serum pro-inflammatory cytokines (IL-6 and IFN- α) and chemokines (IL-8, CXCL-10, and CCL5) in individuals with severe MERS compared to those with mild to moderate disease [57, 58]. High serum cytokine and chemokine levels in MERS patients correlated with increased neutrophil and monocyte numbers in lungs and in the peripheral blood, suggesting a possible role for these cells in lung pathology [57, 58, 37].

Cytokines/chemokines and immunopathology in animal models

Dysregulated inflammatory response in animal models of SARS-CoV infection

Several inbred mouse strains have been evaluated to study SARS-CoV pathogenesis. Mice infected with the human strain of SARS-CoV (SARS-CoV-Urbani) were permissive to virus replication but developed only mild lung pathology and clinical illness [59]. Subsequently, isolation of mouse-adapted strains of SARS-CoV (e.g., SARS-CoV-MA15) allowed studies of lethal SARS [60–62]. MA15 infects airway and alveolar epithelial cells and epithelial cells of other organs [62]. Young mice of many strains (e.g., C57BL/6, 129) support MA15 replication in the lungs but are resistant to developing significant clinical disease [63, 64]. In contrast, young BALB/c mice infected with MA15 develop lethal disease characterized by diffuse alveolar damage, enhanced monocyte/macrophage and neutrophil accumulation, pulmonary edema, and hyaline membrane formation [62].

Furthermore, aged mice of all strains develop lethal clinical disease and succumb to infection [65, 66, 64]. In addition to mouse models, SARS-CoV infection of aged rhesus macaques resulted in significantly more pathology than young adult animals [67]. These animal models replicated several key features of SARS-CoV infection in humans and were thus useful for investigating SARS pathogenesis.

Studies in animal models have been particularly useful in elucidating the role of cytokines and chemokines in mediating lung immunopathology following hCoV infections. Infection of non-human primates (NHPs) with SARS-CoV induced a dysregulated immune response resulting in increased disease severity in aged but not young NHPs, despite similar viral titers in the airways [67]. Since enhanced expression of genes regulating inflammation but not virus titers correlated with disease severity, an exaggerated immune response is thought to induce lethal disease in aged NHPs [67]. Similarly, in SARS-CoV-infected BALB/c mice, disease severity in aged mice correlated with early and disproportionately strong up-regulation of ARDS-associated inflammatory gene signatures [66]. In a recent study, we identified a pathogenic role for IFN-I in mice infected with MA15. Our results showed that rapid SARS-CoV replication in BALB/c mice induced a delayed IFN- α/β response accompanied by an excessive influx of pathogenic inflammatory monocyte-macrophages (IMMs) [38]. The accumulating IMMs themselves produced additional levels of monocyte chemo-attractants such as CCL2, CCL7, and CCL12 (through IFN- α/β receptor stimulation), resulting in further accumulation of pathogenic IMMs, which in turn enhanced disease severity. These infiltrating IMMs produced elevated levels of pro-inflammatory cytokines such as TNF, IL-6, IL1- β , and iNOS. Blocking IFN signaling, depleting IMMs, or neutralizing a single inflammatory cytokine, TNF, protected mice from lethal SARS-CoV infection. Additionally, IFN- α/β or IMM-derived pro-inflammatory cytokines sensitized T cells to undergo apoptosis, further impeding virus clearance [38]. In another study of SARS-CoV infection, loss of TIR-domain-containing adapter-inducing interferon- β (TRIF), an adapter molecule for TLR3 and TLR4 signaling, resulted in a distinct inflammatory signature characterized by neutrophil and other inflammatory cell infiltration [68]. A dysregulated immune response to SARS-CoV in TRIF-deficient mice was associated with aberrant antiviral IFN (IFN- α and IFN β), pro-inflammatory cytokine and chemokine (IL-6, TNF, IFN- γ , and CCL5), and interferon-stimulated gene (RSAD2, IFIT1, and CXCL10) responses. Notably, virus titers were significantly higher in TLR3^{-/-} and TRIF^{-/-} mice compared to their WT controls [68]. Although the viral factors regulating the pro-inflammatory response of neutrophils and monocyte-macrophages remain to be identified, the E protein of SARS-CoV has been shown to enhance pro-inflammatory cytokine and chemokine and inflammasome activity via its ion channel activity [69–71].

These results support the notion that higher virus titers and dysregulated cytokine/chemokine responses cause a “cytokine storm” with lung immunopathological changes following SARS-CoV infection.

Animal models of MERS-CoV infection and lethal disease

Animal models employed to study MERS include rhesus macaques, rabbits, marmosets, and mice among others. MERS-CoV challenged rhesus macaques developed mild to moderate disease [72]. Similarly, MERS-CoV-infected rabbits displayed mild clinical disease with mild-moderate perivascular, peribronchiolar infiltration, and to a lesser extent lung interstitial inflammation [73, 74]. In contrast, marmosets displayed moderate-severe respiratory disease characterized by broncho-interstitial pneumonia, alveolar edema, and fibrin deposition [75]. Marmosets with severe disease showed increased neutrophil and macrophage infiltration in alveoli and interstitial septa, although whether marmosets develop severe disease remains controversial [75, 76]. Although gross and histological lesions and inflammatory cell infiltration in MERS-CoV infected marmosets resemble human disease, there are no data available describing cytokine and chemokine responses in these animals.

Small laboratory animals, particularly rodents, do not support MERS-CoV replication due to inability of MERS-CoV-spike protein to bind to human DPP4 (hDPP4) orthologs in these animals [77]. The first mouse model to study MERS was generated by intranasal transduction of adenovirus encoding hDPP4. These mice developed mild to moderate pneumonia, especially in immunodeficient mice [78]. Several hDPP4 transgenic mouse models developed thereafter exhibited variable organ tropism and disease severity, depending on the promoter driving the hDPP4 expression [79, 80]. More recently, hDPP4 knock-in mice in which hDPP4 is expressed under the mouse hDPP4 promoter have also been described. These mice also developed moderate clinical disease after infection with human isolates of MERS-CoV [81]. We and others recently developed a similar mouse model and showed that serial passage of human isolate of MERS-CoV resulted in mouse adaptation. Mice infected with this adapted virus caused lethal respiratory illness and will be useful for studies of pathogenesis [82, 83].

Overall, delayed and aberrant antiviral and pro-inflammatory cytokine production in MERS-CoV-infected human macrophages and dendritic cells and high serum pro-inflammatory cytokine levels in patients with severe disease compared to mild-moderate clinical disease suggesting that possible dysregulated and enhanced cytokine responses promote lung pathology following MERS-CoV infection.

CoV antagonism of IFN responses and disease severity

To counter innate antiviral cytokine responses, SARS-CoV and MERS-CoV encode several structural and non-structural proteins (nsps) that antagonize antiviral immune response. SARS-CoV encoded nsp1, nsp3-macrodomein, nsp3-deubiquitinase (DUB), and ORF3b, ORF6, and ORF9b subvert antiviral response by antagonizing IFN and ISG responses [84–89]. While nsp3 impairs IFN responses by unknown mechanism, nsp1 inhibits IFN responses by blocking STAT1 phosphorylation [90, 91]. Additionally, structural proteins such as the membrane (M) and nucleocapsid (N) proteins dampen IFN signaling by inhibiting TBK1/IKKe and by unknown mechanisms, respectively [92–95]. Similarly, MERS-CoV structural proteins M and N and accessory proteins orf3, orf4a, and orf4b antagonize IFN responses [85, 96, 97]. It should be noted that most if not all of these putative antiviral mechanisms were demonstrated in transient expression assays and whether they are actually important in the context of infectious virus remains to be determined. Structural and non-structural protein antagonism of IFN responses further amplifies inflammatory responses by promoting unrestrained virus replication resulting in increased viral PAMPs that further dampen IFN signaling and stimulate PRRs to induce an aberrant inflammatory response. Lack of IFN signaling also leads to an excessive accumulation of Ly6C low monocytes and neutrophils.

Causes of exuberant inflammatory response

Despite several years of research studying SARS and MERS pathogenesis, specific host factors that drive lung pathology following hCoV infections are relatively unknown. However, a careful review of the literature related to SARS-CoV and MERS-CoV pathogenesis in humans and animal models highlights several key factors that may play a crucial role in the initiation and progression of an exacerbated inflammatory responses.

1. *Rapid virus replication*: A notable feature of pathogenic human coronaviruses such as SARS-CoV and MERS-CoV is that both viruses replicate to high titers very early after infection both in vitro and in vivo [38, 98–100, 28]. This high replication could lead to enhanced cytopathic effects and production of higher levels of pro-inflammatory cytokines and chemokines by infected epithelial cells [99, 68, 12]. These cytokines and chemokines in turn orchestrate massive infiltration of inflammatory cells into the lungs [38]. Studies from hCoV infections in humans and experimental animals demonstrated a

strong correlation between high SARS-CoV and MERS-CoV titers and disease severity.

2. *hCoV infection of airway and/or alveolar epithelial cells*: Studies from animal models, especially mouse models, provide correlative evidence for differential disease outcome if the viruses predominantly infect airway epithelial cells versus both airway and alveolar epithelial (type I and type II pneumocytes) cells. In B6 and 129 strains, both of which are permissive to virus replication but resistant to developing clinical disease, viral antigen is predominantly located in airway epithelial cells early after infection. In contrast, in highly susceptible BALB/c mice, virus antigen is detected in the lung airways and in alveolar type I and II pneumocytes (Fig. 1). These results suggest a critical role for hCoV-infected type I and II pneumocytes in mediating lung pathology and host susceptibility.
3. *Delayed IFN responses*: As mentioned in previous sections, both SARS-CoV and MERS-CoV encode multiple structural and non-structural proteins that antagonize IFN responses. hCoV reach high titers very early after infection and harbor multiple proteins that inhibit the IFN response, suggesting that an early antagonism of the IFN response might delay or evade the innate immune response. The delayed IFN signaling further orchestrates IMM responses and sensitizes T cells to apoptosis resulting in dysregulated inflammatory response [38].
4. *Monocyte-macrophages and neutrophil accumulation*: Both human and animal studies demonstrate accumulation of inflammatory monocyte-macrophages and neutrophils in the lungs following hCoV infection. These cells are the predominant source of cytokines and chemokines associated with hCoV lethal disease observed both in humans and animal models [38, 32].

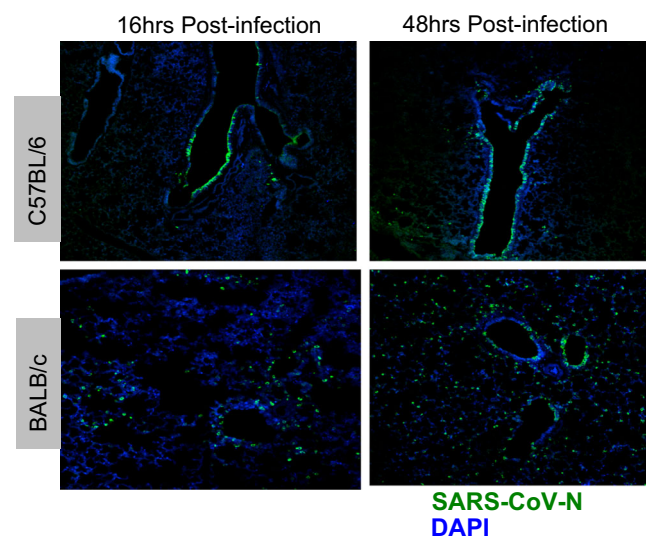


Fig. 1 Staining for SARS-CoV-N antigen in lungs of C57BL/6 and BALB/c mice at 16 and 48 h post-infection

Consequences of cytokine storm and immunopathology

1. *Epithelial and endothelial cell apoptosis and vascular leakage*: One of the earliest consequences of rapid virus replication and exuberant pro-inflammatory cytokine/chemokine responses is lung epithelial and endothelial cell apoptosis. IFN- $\alpha\beta$ and IFN- γ induce inflammatory cell infiltration and cause airway and alveolar epithelial cell apoptosis via Fas-FasL- or TRAIL-DR5-dependent mechanisms [101–103]. Additionally, TNF released by IMMs also promotes the apoptosis of both lung epithelial cells and endothelial cells (unpublished observation). Apoptosis of epithelial and endothelial cells compromises lung microvascular and alveolar epithelial cell barrier resulting in vascular leakage and alveolar edema ultimately resulting in hypoxia.
2. *Suboptimal T cell response*: CoV-specific T cells are crucial for virus clearance and limit further damage to host [64, 104]. Additionally, T cell responses also dampen overactive innate immune responses [105, 106]. Exuberant inflammatory responses caused by pathogenic hCoV diminish the T cell response, in the case of SARS-CoV infection via TNF-mediated T cell apoptosis, thus resulting in uncontrolled inflammatory response.
3. *Accumulation of alternatively activated macrophages and altered tissue homeostasis*: In some SARS patients with extended duration of disease, DAD was accompanied by fibrosis of interstitial and alveolar spaces and hyperplasia of pneumocytes. Similar histological features were noticed in lungs of SARS-CoV-challenged *STAT^{-/-}* mice on B6 and 129 backgrounds. Lungs from these mice revealed an enhanced perivascular infiltration of alternatively activated macrophages, neutrophils, and fibroblasts accompanied by extensive fibrin deposition and alveolar collapse, features observed during end stage ALI and ARDS in humans [63, 107]. Further studies revealed that abrogation of *STAT1* signaling, specifically in myeloid cells, resulted in alternative activation of macrophages [108]. In addition, a delicate balance between host coagulation and fibrinolysis processes regulates tissue remodeling and ALI [109].
4. *ARDS*: Inflammatory mediators play a key role in the pathogenesis of ARDS, a primary cause of death in patients infected with SARS-CoV or MERS-CoV [110, 111]. Several pro-inflammatory cytokines, including IL-6, IL-8, IL-1 β , and GM-CSF, reactive oxygen species, and chemokines such as CCL2, CCL-5, IP-10, and CCL3 contribute to ARDS [48, 112, 113]. Additionally, uncontrolled epithelial cell proliferation and impaired tissue remodeling during later stages induce ARDS leading to pulmonary fibrosis and death.

A summary of causes and consequences of cytokine storm and immunopathology to hCoV pathogenesis is demonstrated in Fig. 2.

Therapeutic approaches

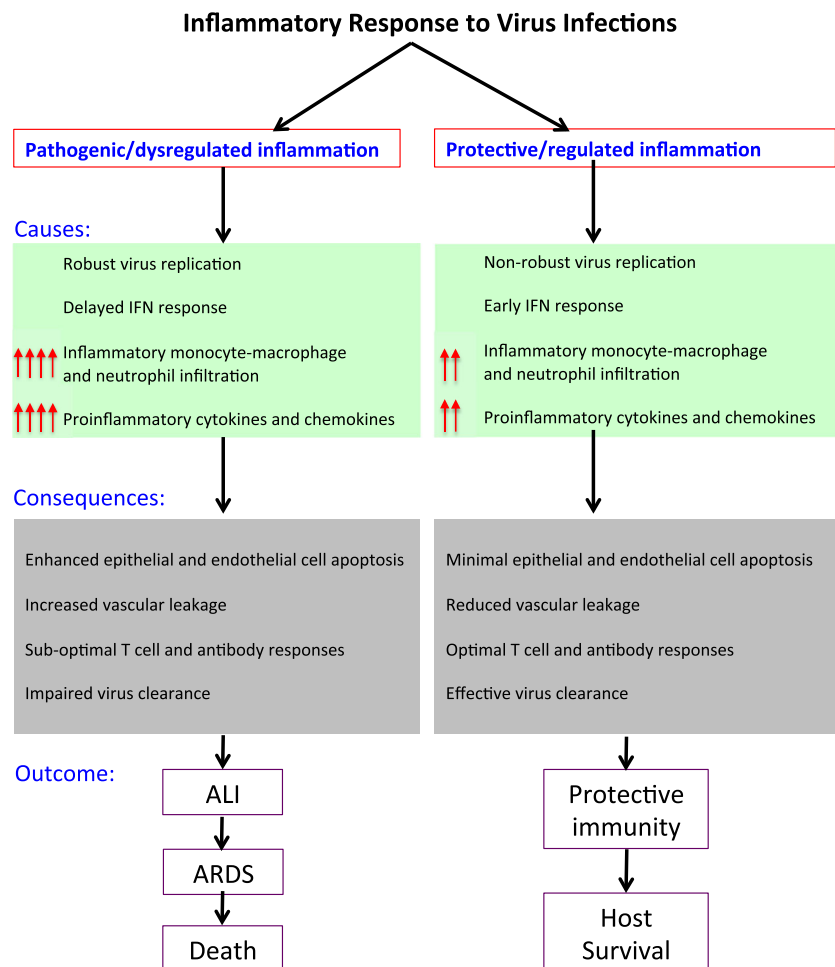
High virus titers and subsequent exuberant inflammatory cytokine and chemokine responses correlate with high morbidity and mortality observed during pathogenic hCoV infections. A systematic review of therapeutic effects of several commonly used antiviral and immunomodulatory agents used during SARS outbreak showed inconclusive results [114]. Similarly, therapeutic interventions aimed towards reducing viral load were somewhat beneficial when administered early but not during later stages of MERS-CoV infection [115–117]. These results suggest that besides controlling viral load, novel strategies directed at attenuating inflammatory responses will likely improve clinical outcomes. Here, we describe agents that have the potential to mitigate hCoV-induced inflammation.

Commonly used therapeutics

Corticosteroid therapy Corticosteroids are a class of steroidal hormones that exert anti-inflammatory functions and are generally used to suppress inflammatory conditions. During the 2003 SARS epidemic, corticosteroids were the mainstay of immunomodulatory therapy. The timely administration of corticosteroids often leads to early improvement in terms of reduced fever, resolution of radiographic lung infiltrates, and better oxygenation [118–120]. However, while some studies showed no beneficial effect, other demonstrated adverse outcomes following corticosteroid therapy during SARS-CoV infection in humans. Early treatment of corticosteroids in SARS patients enhanced plasma viral load in non-ICU patients, thus leading to exacerbated disease [118]. Overall, these results show that the timing, dosage, and duration of corticosteroid therapy are critical if this intervention is to be beneficial in hCoV infections. In general, corticosteroid therapy is not recommended for treatment of hCoV respiratory infections.

Interferons Pegylated and non-pegylated interferons have been under investigation for therapeutic purposes in hCoV-infected individuals. However, therapeutic use of these agents produced mixed results both in humans and animal models of hCoV infections. Early administration of IFN was marginally beneficial in reducing viral load and resulted in moderate improvement in clinical manifestations. In contrast, delayed administration of IFN did not have any advantage compared to placebo controls. Similarly, early administration of

Fig. 2 Schematic representation of protective versus pathogenic inflammatory responses to pathogenic hCoV infections



combination of IFN and ribavirin modestly ameliorated disease severity but did not affect mortality [115, 121, 117, 122].

Other possible therapeutics

IFN- $\alpha\beta$ inhibitors and IFN- λ IFN- $\alpha\beta$ restrict virus replication through induction of ISGs. However, IFN- $\alpha\beta$ can also exacerbate disease by enhancing recruitment and function of IMMs and other innate immune cells. While an early interferon response was protective in SARS-CoV-infected mice, delayed IFN- $\alpha\beta$ signaling dysregulated the anti-SARS-CoV immune response suggesting that timing of IFN therapy is critical in determining the disease outcome. Based on these results, the administration of IFN- $\alpha\beta$ receptor blockers or antagonists should be considered as an option to prevent exuberant inflammatory responses during later stages of severe disease, particularly during SARS [38]. In contrast to IFN- $\alpha\beta$, IFN- λ mainly activates epithelial cells and lacks monocyte-macrophage-mediated pro-inflammatory activity of IFN- $\alpha\beta$ [123]. Additionally, IFN- λ suppresses neutrophil recruitment to the site of inflammation [124]. Since SARS-CoV and MERS-CoV predominantly infect AECs and IFN- λ stimulates

antiviral gene in epithelial cells without over-stimulating the immune system, use of IFN- λ may be an ideal therapeutic option.

Suppression of oxidized phospholipids Oxidized phospholipids (OxPL) have been shown to promote ALI by increasing lung macrophage cytokine/chemokine production via TLR4-TRIF signaling in influenza A virus (IAV)-infected mice [125]. In a recent study, therapeutic administration of the TLR4 antagonist, Eritoran, protected mice from lethal IAV infection by reducing the levels of OxPL and inflammatory cytokines and chemokines [126]. Despite potent immunomodulatory functions, Eritoran has no direct antiviral activity, suggesting its use in the amelioration of inflammatory responses. Since pathogenic human coronaviruses cause acute lung injury and promote OxPL production in the lungs [125], strategies to suppress OxPL either by using Eritoran or other similar compounds could be of value in dampening hCoV-induced inflammation.

Sphingosine-1-phosphate receptor 1 agonist therapy In mice infected with IAV, sphingosine-1-phosphate receptor 1

(S1P1) signaling in endothelial cells was shown to orchestrate pathogenic inflammatory responses [127]. Targeted S1P1 agonism restrained excessive inflammatory cell recruitment, suppressed pro-inflammatory cytokines and chemokines, and reduced IAV induced morbidity and mortality [127, 128]. SARS-CoV infects lung epithelial cells and endothelial cells in humans and NHPs [29], so that SARS-CoV infection of endothelial cells may drive S1P1-mediated inflammatory cytokine/chemokine responses and neutrophil and macrophage accumulation. Therefore, S1P1 agonism could be a potential therapeutic agent in hCoV patients to dampen pathogenic cytokine and chemokine responses, if a role for an excessive immune response by these cells is demonstrated.

Inhibitors of monocyte recruitment and function Studies in animal models demonstrate pathogenic roles for IMM during lethal hCoV infections. In a mouse model of cardiac inflammation, systemic delivery of optimized lipid nanoparticles containing a CCR2-silencing short interfering RNA (siRNA) efficiently degraded CCR2 mRNA and impaired IMM recruitment to sites of inflammation thus resulting in improved disease outcome [129, 130]. Since hCoVs are single-stranded RNA (ssRNA) viruses and stimulation of IMM with the TLR7 agonist, R837 (a synthetic ssRNA mimic), induces strong inflammatory responses, it is possible that IMM-specific TLR-7 signaling promotes excessive inflammation in response to hCoV infection. Thus, a TLR7 antagonist-targeted approach to mitigate inflammation could prove beneficial.

Other immunomodulatory agents Several other immunomodulatory agents that could ameliorate inflammatory responses following pathogenic hCoV infections include cytokine/chemokine inhibitors and danger-associated molecular pattern (DAMP) antagonists [131]. Studies from animal models show a significant contribution of TNF to acute lung injury and impaired T cell responses in SARS-CoV-challenged mice. In vivo neutralization of TNF activity or infection of mice lacking TNFR provides protection against SARS-CoV-induced morbidity and mortality [38, 132]. However, it is to be noted that TNF was not detected in the serum of SARS patients at least during later stages of infection.

Conclusion

Inflammation is an indispensable part of an effective immune response, without which successful elimination of an infectious agent is difficult. The inflammatory response begins with the initial recognition of a pathogen, which then mediates immune cell recruitment, eliminates pathogens, and ultimately results in tissue repair and return to homeostasis. However,

certain viruses such as highly pathogenic CoVs, IAV, and ebola viruses induce excessive and prolonged cytokine/chemokine response known as “cytokine storms,” which results in high morbidity and mortality due to immunopathology. Although studies reviewed in this manuscript provide evidence that “cytokine storms” and immunopathology can occur during pathogenic hCoV infections, we do not yet have a sufficient understanding of the specific factor/s responsible for exuberant inflammatory responses. Studies from human autopsies and animal models strongly suggest a pathogenic role for inflammatory cytokines/chemokines derived from IMM and neutrophils. Therefore, therapeutic interventions targeting these pro-inflammatory cytokines and chemokines could prove beneficial in ameliorating undesirable inflammatory responses. Additionally, since high virus titers at early and later stages of infection strongly correlate with disease severity in humans, strategies directed at controlling viral load as well as attenuating the inflammatory response might prove beneficial. Therefore, future studies should focus on identification of specific signaling pathways that mediate inflammatory responses in hCoV-infected patients and animals.

Acknowledgements We thank Dr. Anthony Fehr for careful review of this manuscript. This work was supported in part by grants from the N.I.H. (PO1 AI060699, RO1 AI091322).

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