SARS-CoV-2 Infection: Host Response, Immunity, and Therapeutic Targets

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Received 16 November 2021; accepted 25 February 2022

Abstract—Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has resulted in a global pandemic with severe socioeconomic effects. Immunopathogenesis of COVID-19 leads to acute respiratory distress syndrome (ARDS) and organ failure. Binding of SARS-CoV-2 spike protein to human angiotensin-converting enzyme 2 (hACE2) on bronchiolar and alveolar epithelial cells triggers host inflammatory pathways that lead to pathophysiological changes. Proinflammatory cytokines and type I interferon (IFN) signaling in alveolar epithelial cells counter barrier disruption, modulate host innate immune response to induce chemotaxis, and initiate the resolution of inflammation. Here, we discuss experimental models to study SARS-CoV-2 infection, molecular pathways involved in SARS-CoV-2-induced inflammation, and viral hijacking of anti-inflammatory pathways, such as delayed type-I IFN response. Mechanisms of alveolar adaptation to hypoxia, adenosinergic signaling, and regulatory microRNAs are discussed as potential therapeutic targets for COVID-19.

KEY WORDS: SARS-CoV-2; COVID-19; host responses; inflammation; immunity.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) [1] is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1] in humans. Since the start of the pandemic in 2020, COVID-19 has caused nearly 5 million deaths worldwide, with hundreds of millions of people testing positive for SARS-CoV-2 (https://covid19.who.int) accessed on 10/29/2021. Efficient human-to-human transmission facilitated global spread following initial failures of containment [2–4], and after more than a year of widely variable worldwide attempts to mitigate disease progression, vaccination has offered hope of limiting disease in many parts of the world [5–7]. However,
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resistance towards vaccination efforts in combination with strains that are more easily transmitted has led to increasing rates of hospitalization and mortality during the pandemic. COVID-19 is usually a self-resolving disease and the virus burden can peak as early as day 3 and is usually cleared in one-to-two weeks in healthy individuals [8, 9]. While the symptoms of COVID-19 are mild in the majority of the infected children and young adult population [10], severe disease happens in approximately 13% of the population with over 2% mortality [11]. Serious underlying conditions including immunocompromised individuals, obesity, chronic lung diseases, diabetes, organ transplant, and cardiovascular comorbidities, as well as male sex and older age, are identified as risk factors in increased mortality rates in adults [12–17]. Such patient populations tend to develop more critical complications including ARDS, septic shock, metabolic acidosis, coagulation dysfunction, and multiple organ failure [12, 18–21]. Importantly, liver disease and cirrhosis seem to also contribute to case fatality rates and liver injury is seen in up to 60% of clinical cases [22–24]. Patients with mild symptoms can often recover at home. Hospitalized patients who require supplemental oxygen are recommended remdesivir, dexamethasone, or monoclonal antibodies such as baricitinib or tocilizumab. However, there is currently no cure for COVID-19. SARS-CoV-2 infection not only causes multiple organ involvement in the acute phase of infection, but may also result in a high incidence of post-acute sequelae of COVID (PASC) [25–27]. Approximately, 10–30% of COVID-19 survivors experiences effects beyond 4 weeks of acute onset. The most frequent symptoms are fatigue, dyspnea, anxiety and depression. There are also often detected abnormalities in the renal, cardiovascular, and coagulation systems [26]. Mechanistic insight of the pathogenesis of PASC is needed to improve clinical outcomes.

Coronavirus outbreaks are not novel phenomena, as demonstrated by the severe acute respiratory syndrome epidemic in 2003 (SARS-CoV) and the Middle East respiratory syndrome outbreak in 2012 (MERS-CoV) [28]. Based on characterizations of prior CoVs and rapid worldwide investigations into SARS-CoV-2 [29–31], it was quickly recognized that human angiotensin-converting enzyme 2 (hACE2) is the principal receptor for SARS-CoV-2 spike protein. Among the wide distribution of hACE2-expressing tissues, hACE2 is notably present on the luminal surfaces of respiratory epithelial cells, including type 2 pneumocytes and ciliated bronchial epithelial cells [32]. Engagement of viral spike with hACE2 at these cellular interfaces facilitates viral entry and initiates a cascade of immunological responses that may ultimately result in acute lung injury combined with hypoxemiac respiratory failure [33]. Contributing to the challenge of responding to SARS-CoV-2, thousands of viral mutations have already been described, including many spike point mutations (e.g., N501Y, A570D, D614G, P681R, T716I, S982A, and D1118H) and amino acid deletions (e.g., S Δ69–70 and S Δ144) [34–36]. According to the World Health Organization (WHO), four “Variants of Concern (VOC)” (Alpha to Delta) have been identified with a trend in increased transmissibility and antigenicity [36–38]. First identified in India in October 2020, Delta variant has a more than 40% increase in transmission and since spread to over 110 countries. Sequencing and competition studies suggested that the spike is critical for viral transmission. The accumulated mutation P681R at the furin cleavage site of the spike has been proved to significantly increase the viral replication fitness of the Delta variant [39]. The recently emerged “Delta Plus” variant (B. 1.617.2.1 or AY.1) also contains K417N mutation on the RBD of spike. The Omicron variant, causing a recent case surge, is even more infectious than original Wuhan strain of SARS-CoV-2 virus or known intervening variants. The Omicron variant causes attenuated disease in small animal models of SARS-CoV-2 infection [40, 41]. In this review, we will discuss the pathophysiology and experimental models of COVID-19, and highlight potential clinical interventions to prevent and treat COVID-19.

SARS-COV-2 INFECTION AND HOST RESPONSES

Viral Structure and Host-Cell Interactions

SARS-CoV-2, a betacoronavirus, is a member of the coronaviridae family, along with other human disease-causing coronaviruses including SARS-CoV and MERS [42]. SARS-CoV-2 is enveloped with a single positive-strand RNA genome contained in a capsid (Fig. 1A). Each SARS-CoV-2 particle contains four main structural proteins: spike, membrane, envelope, and nucleocapsid [43]. The spike protein is heavily glycosylated and mediates anchoring to ACE2, and, together with transmembrane serine protease 2 (TMPRSS2), facilitates viral entry [44, 45]. The viral membrane protein helps in virion assembly and the nucleocapsid protein promotes assembly of the nucleocapsid in association with viral genomic RNA.
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[46]. Intracellular SARS-CoV-2 infection is initiated by the binding of viral spike protein to cell surface ACE2 on alveolar epithelial cells, which results in alveolar inflammation and the release of chemokines and cytokines [33, 47, 48]. Furin-like protease is required for the preactivation of spike cleavage to facilitate quick entry into the cells [49–51]. Upon entry, pathogen-associated molecular patterns (PAMPs) initiate Toll-like receptor signal
transduction, including single-stranded RNA (ssRNA) fragments from the SARS-CoV-2 genome directly activating endosomal TLR7/8 and NFκB transcriptional activation of proinflammatory cytokine production and release follows [52–54]. Furthermore, signal transduction through dsRNA-sensing receptors RIG-1/MDA5 stimulates IFN response via IFN regulatory factor 3/7 (IRF3/7) resulting in type I IFN and chemokine production [53, 55, 56]. The accessory viral proteins along with the spike protein consequently develop calcium/potassium ion channels (unlike the SARS-CoV which develops NA+/K+ channels) and trigger NLRP3 inflammasome pathway thereby leading to IL-1β-dependent pyroptosis, a form of cell death induced by inflammation [57]. Alveolar epithelial pyroptosis further releases danger-associated molecular patterns (DAMPs) in the interstitial space that bind to the DAMP-TLRs, TLR2 and TLR4, on the microvascular endothelial cells thereby activating the production and secretion of proinflammatory cytokines, type-I IFNs and chemokines [58]. Proinflammatory cytokines, including TNFα, IL6, and type I IFNs mediate chemotaxis and/or immune cell dysfunction resulting in ARDS and tissue damage (Fig. 1B; left panel) [59, 60]. Overactivated immune responses, in turn, result in further tissue damage and organ dysfunction.

**SARS-CoV-2-Associated Immunopathogenesis**

SARS-CoV-2 infection-driven-immune responses include innate immune sensing, innate immune responses, and adaptive immunity (Fig. 1). Currently, the understanding of SARS-CoV-2 immunopathogenesis relies heavily on human studies and previous knowledge of other coronaviruses, including SARS-CoV and MERS [61]. Innate immune sensing of SARS-CoV-2 is mediated by the recognition of viral RNA by pattern recognition receptors (PRRs) which trigger the release of cytokines and chemokines. An early and sufficient release of cytokines such as IFNs is crucial for the successful control of viral replication and host survival [62]. Many coronaviruses develop strategies to escape innate immune sensing by avoiding PRR activation and by interfering with downstream IFN responses [61]. Recent study indicated that the Alpha variant of SARS-CoV-2 suppresses innate immune responses more efficiently compared to earlier lineages [63]. Mechanistically, increased protein level of Orf9b from the alpha variant interacts with TOM70 and inhibits innate immune responses by dampening RNA sensing in airway epithelial cells [63].

The infected epithelial cells initiate a robust type-I and III IFN (IFN λ binds to IFNL1R) response and release inflammatory cytokines including IL-6 and IL-1β to recruit and activate granulocytes, DCs, and macrophages to the lung, driving the immunopathogenesis of SARS-CoV-2 (Fig. 1B; right panel) [64, 65]. Furthermore, increased levels of hyaluronan, which can be elicited by TNF-α and IL6 have been detected in patients with COVID-19 ARDS [66, 67]. Besides the direct stimulation of myeloid cells by infected alveolar epithelial cells, several recent studies suggested that SARS-CoV-2 stimulates monocytes from peripheral blood to elicit inflammatory responses by the release of TNF-α, IL-1β and IL-6 [68, 69], while there is a lack of productive viral replication in these cells. In patients with severe COVID-19, single cell RNA sequencing analysis of bronchoalveolar lavage fluid uncovered abundant proinflammatory monocyte-derived macrophages [70]. In addition, a recent single cell RNA sequencing analysis of the bronchial alveolar lavage fluid from ferrets infected with SARS-CoV-2 identified many subpopulations of macrophages at 2 days post-infection. Additional studies are crucial to illustrate how monocytes functionally contribute to viral clearance and late-stage hyperinflammation [71]. RNA velocity analysis indicated complex kinetics in both M1 and M2 macrophages originated from monocyte-derived macrophages [71], indicating the complex nature of the macrophage responses.

Pulmonary innate lymphoid cells, including natural killer (NK) cells, play important roles in controlling viral infection through type-I IFN (IFNα/β)-mediated IFN γ production (in addition to IL-12 and IL-18) to facilitate helper T cell responses [72, 73]. Several studies have demonstrated a decreased NK cell population in the peripheral blood of COVID-19 patients [74, 75]; however, it is still unclear whether it is the result of NK cell recruitment to the pulmonary microenvironment. Functionally, NK cells in the peripheral blood from COVID-19-infected individuals showed less activated status with potential impairment in cytotoxicity and chemokine/cytokine production [76]. So far, there is no evidence suggesting that SARS-CoV-2 directly infects NK cells. Thus, the aberrant activation status could be partially explained by the increased expression of inhibitory or immune checkpoint molecules such as LAG3, TIM3, and NKG2A in NK cells from COVID-19 patients [76]. Other groups of innate lymphoid cells such as ILC1/2/3 are less studied in SARS-CoV-2 infection. However, based on their important role in promoting the rapid innate immune response to
pathogens, more attention should be given to these cells in the immunopathogenesis of SARS-CoV-2 infection.

Adaptive immunity plays an instrumental role in the control and immunopathogenesis of SARS-CoV-2. Lymphopenia is commonly observed in blood from COVID-19 patients and the degree of lymphopenia is correlated with the severity of the disease [61]. Although the cause of COVID-19 lymphopenia is currently unknown, it was speculated that recruitment of lymphocytes to the infected lung might be a contributing factor [70]. SARS-CoV-2-reactive CD4 and CD8 T cells have been identified in bronchial alveolar lavage fluid and in peripheral blood from COVID-19 patients [70, 77, 78]. In patients with severe COVID-19, the population of regulatory T cells and γδ T cells are decreased [77, 79, 80], suggesting the lack of sufficient immune regulation and viral defense. Similarly, peripheral CD8 T cells from severe COVID-19 patients have reduced cytotoxicity marked by lower expression of CD107a and Granzyme B [74]. Of note, the phenotype and function of CD8 T cells in infected lungs are unlike those observed in peripheral blood as bronchial alveolar lavage (BAL) CD8 T cells expressed higher levels of cytotoxic genes [70]. Thus, future efforts are needed to address the functional relevance and mechanism of the discrepancy. Besides T cells, B cells are important for the production of neutralizing antibodies to defend against viral infections. Indeed, SARS-CoV-2-specific antibodies, as well as plasma cells and memory B cells, have been detected in the majority of COVID-19 patients although the correlation of antibody titers with disease severity is inconclusive [81–83]. Due to its recent emergence, it is essentially unknown how long-lasting the B cell immunity against SARS-CoV-2 is in preventing viral reinfection. However, knowledge from the SARS-CoV-1 and MER-CoV suggested that at least 2 to 3 years are the typical time frame for protection [84, 85]. However, with the emergence of multiple variants of the SARS-CoV-2 viruses, it is unlikely that the neutralizing antibodies will prevent reinfection as effectively did the previous CoVs.

LABORATORY APPROACHES TO STUDY SARS-COV-2 PATHOGENESIS AND HOST RESPONSES

To better understand the pathogenesis of COVID-19, it is crucial to develop experimental animal models to help delineate stepwise mechanisms involved in the pathogenesis of ARDS and multiple organ failure in COVID-19 [86, 87]. Figure 2 illustrates a brief account of laboratory animal models in the establishment of SARS-CoV-2 infections and COVID-19 [88–90].

**Ferret Model of SARS-CoV-2 Infection**

Administration of SARS-COV-2 Wuhan strain SARS-CoV-2 strain BetaCoV/Wuhan/IVDC-HB-01/2019 (CTan-H), or SARS-CoV-2 strain BetaCoV/Wuhan/IVDC-HB-envF13-20/2020 (F13-E), lead to efficient viral replication in ferrets in the nasal turbinates, tonsils, and the soft palate up to day 10 post-inoculation, with 20% mortality by day 10 in CTan-H- and day 11 in F13-E-infected ferrets [88], presumably due to the increase binding affinity of ferret ACE2 to SARS-CoV-2 [91]. A shift in the host response with an increased ratio of production of proinflammatory CCL2, CCL8, and CXCL9 to diminished IFNβ and IFN λ production was observed in SARS-CoV-2-infected ferrets’ nasal washes [90]. These phenotypes were consistent with cytokine profiles in serum samples from COVID-19 patients. However, the viral replication is mostly restricted in the upper airway compared to human COVID-19, which is heavily involved in the lung.

**Hamster Model of SARS-CoV-2 Infection**

Using golden hamster models for human SARS-CoV-2, studies have shown that these animals have increased viral burden in the nasal mucosa and bronchial epithelial cells, associated with lung pathologic changes within day 2 to 5, pneumocyte hyperplasia post-day 7, and regional consolidation and multilobar glass opacity by day 8 followed by viral clearance [92, 93]. However, the hamster model only represents mild-moderate COVID-19, as no mortality is observed in the infected animals.

**Nonhuman Primate Model of SARS-CoV-2 Infection**

Non-human primates have also been explored in preclinical studies of SARS-CoV-2 infection. Several studies reveal that infection of non-human primates including cynomolgus macaques [94], rhesus macaques, and baboons resemble mild to moderate
Host responses during SARS-CoV-2 infection human COVID-19 pathogenesis, indicating the feasibility of using these animals as preclinical models to study pathogenesis of SARS-CoV-2 infection. However, additional nonhuman primate models are needed to resemble severe COVID-19.

Murine-Adapted SARS-CoV-2

Laboratory mouse strains are not at all susceptible to native SARS-CoV-2 infection [95]. Thus, the development of murine-adapted SARS-CoV-2 is crucial to recapitulate the processes of viral entry and pathogenesis without altering the genetic framework of experimental animals (Fig. 2b). SARS-CoV-2 has been adapted into a murine host by passaging the human SARS-CoV-2 in BALB/c mice up to 6 passages (MASCp6), demonstrating increased virulence in both aged and young BALB/c mice [96]. Besides passaging of SARS-CoV-2 in mice, point mutations of the viral genome Q493K, at the RBD, predictably binding to N31 residue of the mouse ACE2 receptor, facilitate the development of murine-adapted (MA) SARS-CoV-2 stain [95]. Further passaging of the MA SARS-CoV-2 strain at passage 10 generated MA10 stain, which exhibited 10% mortality in 10-week young and 80% mortality in 1-year aged BALB/c mice, respectively, at day 7 post-intranasal challenge [97]. These murine-adapted SARS-CoV-2 stains have become instrumental in genetic studies of molecular pathways controlling the pathogenesis of COVID-19.

Human ACE2 Overexpression and SARS-CoV-2 in Mice

Since mACE2 does not efficiently bind to SARS-CoV-2, animal models expressing hACE2 have been investigated. For example, mice with hACE2 overexpression driven by the cytokeratin-18 promoter (K18-hACE2) showed susceptibility to SARS-CoV-2 infection marked by sufficient viral replication, impaired pulmonary function, and infiltration of immune cells [98]. Using reverse genetics, Hfh4 (also known as Foxj1; a lung ciliated epithelial cell promoter)-promoter-driven hACE2 was expressed in the ciliated airway epithelium

Fig. 2 Experimental strategies to study SARS-CoV-2 infection and COVID-19-like disease in vivo. A Non-human primates, ferrets, golden hamsters, and mice, have been employed in pathogenesis studies of SARS-CoV-2. These animals could be infected by native SARS-CoV-2 virus (Blue color viral particles) and exhibit significant pathophysiological changes resembling COVID-19. B Generation of mouse-adapted SARS-CoV-2 virus. Intranasal inoculation of human SARS-CoV-2 isolates is carried out (blue viral particles) in wildtype BALBc/J mice. Mouse lungs are harvested, and the supernatants of the lung tissue homogenates are intranasally administered into naïve mice. The process is repeated for 6 passages minimum to achieve mouse adaptability (green viral particles). Method of passaging and generation of mouse-adapted viral strain has also been successful in C57B6/J mice (brown viral particles). C Human ACE2 expressing adenovirus (Adv) vectors are delivered intranasally to overexpress ACE2 in C57B6/J mouse lungs. After 48 h, intranasal inoculation of human SARS-CoV-2 isolates is carried out (blue viral particles) to establish infectivity.
and in neuronal cells of BALB/c mice thereby increasing SARS-CoV-2 binding in vivo and resulting in 40% mortality [95]. Intragastric and intranasal instillation of SARS-CoV-2 in C57BL/6 mice expressing hACE2 using CRISPR-Cas9 system resulted in similar high viral burden in the lungs, trachea, brain, and intestines in young and aged mice. Moreover, intragastrically infected mice also developed severe pulmonary pathology [99]. The CRISPR-Cas9 system proved to be more effective than the above-discussed methods as it replaced mAce2 gene in the loci GRC m38.p6 on chromosome X, with hACE2 gene, thereby ubiquitously expressing hACE2 in all tissues. Although viral burden was similar in both young and aged mice, inflammatory cell infiltration, alveolar damage, and focal hemorrhage were more pronounced in aged mice infected with SARS-CoV-2 [99]. Besides transgenic overexpression, Han K et al. delivered Ad5-hACE2 via oropharyngeal route and achieved a robust inflammatory response upon SARS-CoV-2 challenge. The authors confirmed that viral protein was localized on the pneumocytes in the septa, and the disease outcome as well as inflammatory response correlated with that in COVID-19 patients [100].

**Infectious SARS-CoV-2 Clones and Luciferase Reporter Constructs to Study Pathogenesis and Host Response in Vitro**

An infectious cDNA clone of SARS-CoV-2 has been developed to mimic human disease phenotype, and to engineer reporter viruses [101, 102]. Here, Dr. Pei-Yong Shi’s laboratory employed a reverse-genetic system to clone the infectious SARS-CoV-2 ORFs cDNA, with monomeric NeonGreen (mNeoG) fluorescence tag to obtain mNeoG-fluorescence-tagged infectious clone of SARS-CoV-2 (icSARS-CoV-2-mNG) [101]. Infection of Vero-E6 cells with icSARS-CoV-2-mNG showed sustained infectivity with similar fluorescence for up to five passages (Fig. 3a). Another reporter virus was designed by the same group to use nanoluciferase signal as a measurement of viral load for the testing of anti-viral drugs or neutralization antibodies [103]. The advantage of the built-in infectious clone is resistance to any spontaneous mutations during passaging of SARS-CoV-2 isolates. Besides the reporter viruses, recombinant SARS-CoV-2 proteins expressing luciferase replicons of individual SARS-CoV-2 ORFs have been developed to reduce the risk of accidental exposure to infectious viral particles, and therefore, these replicons can be used in BSL2 facility for potential antiviral studies [55, 104].

**Pseudoviruses and hACE2 Overexpression in SARS-CoV-2 Pathogenesis Studies in Vitro**

Pseudoviruses such as vesicular stomatitis virus (VSV) and polinton-like virus (pLV) have been employed as SARS-CoV-2 spike protein-expressing vectors in understanding the strategies of the spike protein binding to the host receptors, such as heparan sulfate, ACE2, and TMPRSS2 [44, 105, 106]. In the VSV-SARS-CoV-2 Spike expression system in vitro (Fig. 3b), greater infectivity and host response were observed in human lung adenocarcinoma cells, Calu-3, human colorectal epithelial cancer cells, Caco-2, and monkey kidney epithelial cells, Vero-E6, in comparison to ACE-2 overexpressing fibroblast cells HEK293, and BHK-1 [44, 107]. More importantly, neutralization of the VSV-SARS-CoV-2 Spike pseudo vectors with soluble-form of human ACE2 abrogated the infectivity in the tested cells [44, 107]. Several studies employed over-expression of ACE2 (Fig. 3c) in human cell lines including A549, HeLa-cells, and BHK-1 fibroblasts to increase susceptibility to SARS-CoV-2 infection in vitro [33, 90].

**POTENTIAL ANTI-INFLAMMATORY PATHWAYS INVOLVED IN COVID-19**

COVID-19 pathophysiology is accompanied by hypoxic conditions due to pulmonary edema and impaired gas exchange [108]. Hypoxia inducible factors (HIF) are crucial for the adaptation to hypoxic conditions [109]. Figure 4 illustrates the process of stabilization of HIF1α under hypoxic conditions. Several studies, including those from our laboratory, have demonstrated the important roles of hypoxia-inducible factors [110, 111], especially HIF1α, in modulating inflammation during acute organ injury, including acute lung injury [112–122]. HIF have been shown to inhibit SARS-CoV-2 replication. A recent study by Dr. Jane McKeating et al. demonstrated that hypoxia and pharmacologic HIF stabilization inhibits SARS-CoV-2 replication in pulmonary epithelial cells in vitro by reducing the expression of ACE2 and inhibiting viral RNA replication [123]. Collectively, the current knowledge of the role of HIF in ARDS and viral pneumonia points to a lung-protective role of HIF in SARS-CoV-2-associated
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ARDS, and importantly, a clinical trial using vadadustat, a HIF activator, is currently ongoing (NCT04478071).

The role of purinergic signaling has been studied extensively in acute and chronic mucosal inflammation (Fig. 5) [124–137]. The role of ectonucleotidases CD39 (ectonucleoside triphosphate diphosphohydrolase-1) and CD73 (ecto-5′-nucleotidase) in the pathophysiology of COVID-19 remained speculative although their functions in the modulation of extracellular ATP hydrolysis and adenosine signaling in other inflammatory conditions indicate potential involvement. CD73 is decreased on CD8 T cells and NKT cells in peripheral blood and CD73-CD8 T cells and NK cells have higher level of cytotoxicity [137]. A humanized anti-CD73 monoclonal antibody, AK119, has been evaluated in healthy subjects as a potential treatment for COVID-19 (NCT04516564). A recent study by Dr. M. Serena Longhi’s team reveals that ectonucleotidases CD39 (ectonucleoside triphosphate diphosphohydrolase-1) is increased in PBMC of severe COVID-19 cases, indicating potential T cell exhaustion [138]. Additionally, adenosine signaling has been investigated extensively in the setting of acute organ injury and inflammatory diseases including ARDS [124, 125, 139–144]. Indeed, several pilot clinical trials suggested inhaled adenosine as a safe therapeutic intervention for COVID-19 with potential for clinical benefit [145, 146], supporting the feasibility of large-scale randomized clinical trials. Together, the potential impact of purinergic signaling in COVID-19-related ARDS warrants further investigations.

Previous studies have indicated the microRNAs are crucial player during immunopathogenesis [147–154], including acute lung injury and ARDS [154–156]. Earlier studies utilizing computational prediction and in silico analysis identified several microRNA clusters that
are associated with increased mortality of COVID-19 and viral-derived microRNAs targets several host genes [157]. Similarly, several coronavirus genome sequences including SARS-CoV-2 genome have been predicted as potential miRNA binding sites [158–160]. In addition, a recent study identified three circulating microRNAs (miR-423-5p, miR-23a-3p, and miR-195-5p) as signature microRNA predictor for SARS-CoV-2 infection [161]. However, their functional roles in viral replication and host responses are yet to be determined. In summary, future studies of the role of miRNAs in SARS-CoV-2 infection will provide potential therapeutic targeting for COVID-19.

EMERGING PHARMACOLOGIC INTERVENTIONS OF COVID‑19 TREATMENT

Blocking Viral Entry

Administration of neutralization antibodies was proved important in the early phase of the pandemic with convalescent plasma donations. As of now, FDA has approved convalescent plasma treatment with high titer COVID-19 plasma containing Ortho Diagnostic’s Vitros® anti-SARS-CoV-2 IgG, as well as low titer COVID-19 convalescent plasma [162]. However, despite massive NIH investments, convalescent serum has failed to demonstrate evidence of robust protection against severe disease. Besides convalescent plasma, currently, three antibodies have received Emergency USE Authorizations (EUAs) from the FDA to treat COVID-19 as an intravenous (IV) infusion. These antibodies are bamlanivimab plus etesevimab, casirivimab plus imdevimab, and sotrovimab (NIH, COVID-19 treatment guidelines). Bamlanivimab plus etesevimab were developed by Eli Lilly® and targets the spike protein from the SARS-CoV-2. Treatments of bamlanivimab plus etesevimab results in reduced incidence of hospitalization and death in high-risk COVID-19 patients (NCT04427501) [163]. Casirivimab plus imdevimab (REGEN-COV), an antibody cocktail developed by Regeneron®, reduces the risk of hospitalization or death in outpatients with COVID-19 (NCT04425629) prior to the emergence of Omicron.
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variant [164]. Sotrovimab was developed by GlaxoSmithKline targeting a highly conserved epitope of sarbecoviruses including SARS-CoV-1. If given within 5 days after disease onset, sotrovimab halted COVID-19 progression leading to hospitalization or death (NCT04545060) [165].

Reducing Viral Replication-Anti-Viral Drugs in Practice

Due to their fundamentally important roles in host invasion, the key structural proteins of SARS-CoV-2 are actively studied as potential therapeutic targets. Remdesivir (GS-5734, Veklury) has been shown to be safe and effective in reducing the recovery time in hospitalized COVID-19 patients [103, 166]. The small molecule serves as an adenosine analog and stalls RdRp-mediated RNA synthesis [167]. It is the first experimental antiviral drug to be approved by FDA, particularly in young children and the elderly regardless of the severity of the disease.

Recently, molnupiravir, an oral antiviral drug from Merck®, was reported to provide clinical benefit in non-hospitalized COVID-19 patients [168]. Mechanistically, the active form of molnupiravir, β-D-N4-hydroxycytidine triphosphate, induces RNA mutagenesis as an analog of cytidine or uridine triphosphate, thus inducing SARS-CoV-2 mutagenesis during RNA synthesis in viral replication [169]. One potential concern is the associated risk of possibly predisposing to the emergence of new variants, which could be an unpredictable factor for the long-term impact of molnupiravir [170]. On the other hand, molnupiravir’s ability to cause mutations in human cells could be another potential concern for long-term outcomes. The FDA has recently granted EAU for molnupiravir for the treatment of mild to moderate COVID-19.

Another small molecule antiviral drug that recently received FDA EUA is PAXLOVID from Pfizer®. PAXLOVID is an oral medication composed of PF-07321332

Fig. 5 Purinergic signaling and regulation of inflammation during acute lung injury. (1) Normal alveolus (left panel) shows extracellular ATP signaling through the purinergic receptors P2X7, whereas ADP binds to P2Y2 receptor. (2) Ectonucleotidase CD39 metabolizes ATP to generate AMP, and ectonucleotidase CD73 metabolizes AMP to release adenosine (A). Adenosine signaling occurs by binding to its receptors A1AR, A2aAR, ADORA2B, and A3AR; (only A2aAR and ADORA2B are shown for comparison to pathological conditions). (3) Under normal physiological conditions, ATP metabolism and adenosine signaling are sequestered by ENT1 and ENT2 that mobilize free adenosine from the extracellular compartment into the cytosol, and surfactant is released by type II pneumocytes to help the process of gas exchange and maintain alveolar homeostasis. During acute lung injury (right panel), excessive ATP is released into the extracellular space. Increased metabolism and adenosinergic signaling occur in the type II pneumocytes as well as in the immune cells (4) such as macrophages (M), neutrophils (N), natural killer cells (NK), and lymphocytes (T), controlling the dysregulated inflammation, delayed interferon (IFN) response, and extravasation of neutrophils.
and low-dose ritonavir, a CYP3A4 isoenzyme inhibitor to prolong the half-life of PF-07321332 [171]. PF-07321332 targets the main protease of SARS-CoV-2 to hinder viral replication and pre-clinical studies suggested that oral PF-07321332 protects against SARS-CoV-2 infection in a mouse model [172]. The use of ritonavir in PAXLOVID will require further attention during COVID-19 management due to potentially enhancing the bioavailability of other prescribed or over the counter medications [171]. Thus, physicians should use caution and be aware of the interaction with other medications.

**Targeting Endogenous Inflammatory Pathways**

Reducing exacerbated inflammation by anti-inflammatory drugs, such as steroids, and blocking the clotting pathway activation by anticoagulants, such as heparins, are part of the standard practice in COVID-19 patients. For instance, dexamethasone (glucocorticoid) has been demonstrated to reduce mortality in hospitalized COVID-19 patients requiring respiratory support at randomization [173]. Tocilizumab (Actemra), an anti-IL6 receptor monoclonal antibody, has received EUA from FDA after a large-scale clinical trial indicated efficacy (NCT04372186) [174]. Sarilumab (Kevzara) is another monoclonal antibody targeting the IL-6 receptor that has not shown clinical benefit in a phase 3 trial [175]. However, additional studies are still ongoing in patients with severe COVID-19 pneumonia (NCT04386239). Janus Kinase inhibitors, baricitinib (Olumiant), have also received EUA from the FDA as treatment of COVID-19, as combined use of remdesivir and baricitinib confers clinical benefit in

| Potential Therapeutics | Targets/pathways | Cellular networks involved | PMID/Clinical trial.gov Identifier |
|------------------------|------------------|---------------------------|-----------------------------------|
| **Adenosinergic signaling** | | | |
| Adenosine | ADOR | Inflammation | NCT04588441 |
| Dipridamole | ENT1/ENT2 CD39 | Lymphocytes and platelet recovery NK cell infiltration | 32318327/NCT04391179 |
| IPH52-mAb | Anti-CD73 | T effector cell function | 27622077 |
| MED9447-mAb | A2aAR | Myeloid and lymphoid infiltration | 30131376 |
| BMS-986179-mAb | A2BAR | T cell activation | 32727810 |
| CPI-444-mAb |  |  |  |
| AZD4635 |  |  |  |
| PBF-509-mAb |  |  |  |
| MK-3814 small molecule |  |  |  |
| **Hypoxia** | PHDs inhibition |  | 3283981 |
| Vadadustat |  |  | 30805897 |
| Roxadustat |  |  | 2880805 |
| Daprodustat |  |  |  |
| **MicroRNAs** | Decrease autophagy, apoptosis, inflammation | Overexpression of miRNAs | 27146208 |
| miR126 |  |  | 30794808 |
| miR146 |  |  | 31398659 |
| miR150 |  |  | 28931657 |
| miR223 |  |  | 2935794 |
| miR181 |  |  | 28765901 |
| miR127 | Increase lung injury, inflammation | Repression of microRNAs | 28125520 |
| miR155 |  |  | 32321279 |
| miR887 |  |  | 22189082 |
| miR200 |  |  |  |
hospitalized patients with COVID-19 (NCT04401579) [176]. Regarding the modulation of coagulation pathways, the use of unfractionated heparin (UFH) and low molecular weight (LMWH) heparin is included to inhibit clotting factors Xa and thrombin by binding to the antithrombin [177]. In addition, antiplatelet agents such as nafamostat have been proposed to reduce SARS-CoV-2 fusion and TMPRSS2 mediated cleavage of the viral spike, resulting in lower viral burdens in vitro [178]. Administration of tissue-type plasminogen activator may also help in degrading fibrin clots and reduce the risk of micro clotting [179]. Finally, chronotherapy by precisely adjust the timing of the medication might be another interesting approach for the treatment of COVID-19 [180].

**Vaccine Development- Prophylactics DNA, RNA, and Protein Vaccines**

Vaccine development is crucial as a preventative measure in the control of respiratory pathogen infections such as influenza virus infection [181, 182]. As of February 2022, FDA has approved SARS-CoV-2 RNA vaccine from Pfizer-BioNTech and Moderna that have shown 90–95% protective efficacy in phase III clinical trials [183, 184]. The Pfizer-BioNTech mRNA vaccine, along with the Moderna vaccines, is already on the roll for global distribution and have been administered in the USA, the UK, and other countries. Besides mRNA vaccines, adenovirus vectors-based vaccines are also developed and studied for the prevention of COVID-19. A non-replicating viral vector (Adenovirus) encoding SARS-CoV-2 spike protein was developed in the UK (ChAdOx1 nCoV-19, AstraZeneca) that showed safety and efficacy from multiple trials [185, 186], and was approved in the UK and EU. Another adenovirus-based vaccines have been developed by Johnson and Johnson and confers protection of COVID-19, especially against the progression to severe diseases [187]. At the time that this review was drafted, 39.2% population worldwide and 58% population of US residents were fully vaccinated. Although the quick evolution of the SARS-CoV-2 virus may affect the effectiveness of vaccines, vaccines from companies like AstraZeneca, Pfizer/BioNTech, and Moderna have proved to provide protection against severe disease and death in the context of the Delta variant with only a slightly reduced efficacy [188–190]. The efficacy of COVID-19 vaccines need to be further evaluated against the Omicron variant. Of note, antibody-dependent enhancement (ADE) has been noticed in SARS-CoV-1 and MERS-CoV infection when a non-neutralizing antibody facilitates viral infection [191, 192], indicating a potential concern for vaccines against SARS-CoV-2. However, there is no evidence to support ADE in COVID-19 at this time [193, 194].

**CONCLUSIONS AND CHALLENGES TO THE FIELD**

Proper regulation of immune responses to environmental or pathogenic stimuli is crucial for the control and the resolution of tissue/organ injury. Previous studies have highlighted sophisticated pathways orchestrating immune responses in inflammatory conditions [121, 141, 195–207]. As the pathophysiology of COVID-19 started unraveling, the importance of inflammation has also been demonstrated in the pathogenesis of multiple organ injuries related to SARS-CoV-2 infection. Substantial progress has been made in the development of safe and efficacious vaccines against SARS-CoV-2. However, the emergence of multiple SARS-CoV-2 variants around the world and potential insufficient coverage of vaccinations in the overall population call for the development of effective therapeutic treatment options. As listed in Table 1, pharmacologic interventions on adenosinergic signaling and HIF signaling could be key steps to reduce acute lung injury in COVID-19 patients. Thus, therapeutic targeting of endogenous hypoxia-dependent anti-inflammatory pathways could potentially alleviate COVID-19-associated organ inflammation.

**ACKNOWLEDGEMENTS**

Figure 2 was created with BioRender.com.

**AUTHOR CONTRIBUTION**

P. Shivshankar drafted the manuscript and figures; H. Karmouty-Quintana, T. Mills, M-F. Doursout, Y. Wang, A. K. Czopik, S. E. Evans, and H. K. Eltzschig edited the manuscript; X. Yuan revised and finalized the manuscript.

**FUNDING**

This work is supported by the National Institute of Health Grants R01HL155950, American Thoracic Society Unrestricted
Grant, American Heart Association Career Development Award (19CD3A4660279), American Lung Association Catalyst Award (CA-20220265), the Center for Clinical and Translational Sciences, McGovern Medical School Pilot Award (1UL1TR003167-01), and Parker B. Francis Fellowship (to X. Yuan); and National Institute of Health Grants R01HL154720, R01DK122796, R01DK109574, R01HL133900, and Department of Defense (DoD) Grant W81XWH2110032 (to H. K. Eltzschig). NHLBI grants R01HL138510, R01HL100157, DoD grant W81XWH-19–1007, and American Heart Association Grant 18IPA34170220 (to HKQ).

DECLARATIONS

Ethics Approval and Consent to Participate Not applicable.

Consent for Publication Not applicable.

Competing Interests The authors declare no competing interests.

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