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REVIEW

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COVID-19: Current knowledge in clinical features, immunological responses, and vaccine development

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Abstract

The COVID-19 pandemic has unfolded to be the most challenging global health crisis in a century. In 11 months since its first emergence, according to WHO, the causative infectious agent SARS-CoV-2 has infected more than 100 million people and claimed more than 2.15 million lives worldwide. Moreover, the world has raced to understand the virus and natural immunity and to develop vaccines. Thus, within a short 11 months a number of highly promising COVID-19 vaccines were developed at an unprecedented speed and are now being deployed via emergency use authorization for immunization. Although a considerable number of review contributions are being published, all of them attempt to capture only a specific aspect of COVID-19 or its therapeutic approaches based on ever-expanding information. Here, we provide a comprehensive overview to conceptually thread together the latest information on global epidemiology and mitigation strategies, clinical features, viral pathogenesis and immune responses, and the current state of vaccine development.

KEYWORDS

COVID-19, immunity, SARS-CoV-2, therapy, vaccines

1 | INTRODUCTION

In December 2019, a cluster of patients with unexplained viral pneumonia was identified in Wuhan, China.[1] To identify the causative agent of this disease, a large number of tests were conducted, which ruled out several etiological agents that may cause similar symptoms, including the severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and other common respiratory pathogens. Finally, researchers identified the cause being a novel coronavirus termed as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[1] With a rapid increase in the number of infected people, on March 11th the World Health Organization (WHO) declared the coronavirus disease 2019 (COVID-19) as a pandemic[2] (Figure 1). SARS-CoV-2 has infected over 100 million individuals and has

Abbreviations: ACE2, angiotensin-converting enzyme 2; Ad5, human serotype 5 adenovirus; ADE, antibody-dependent enhancement of disease; AMs, alveolar macrophages; ARDS, acute respiratory distress syndrome; ChAd, chimpanzee-derived adenovirus; COVID-19, the coronavirus disease 2019; CT, computed tomography; DCs, dendritic cells; NAb, neutralizing antibodies; RBD, receptor-binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TII, trained innate immunity.

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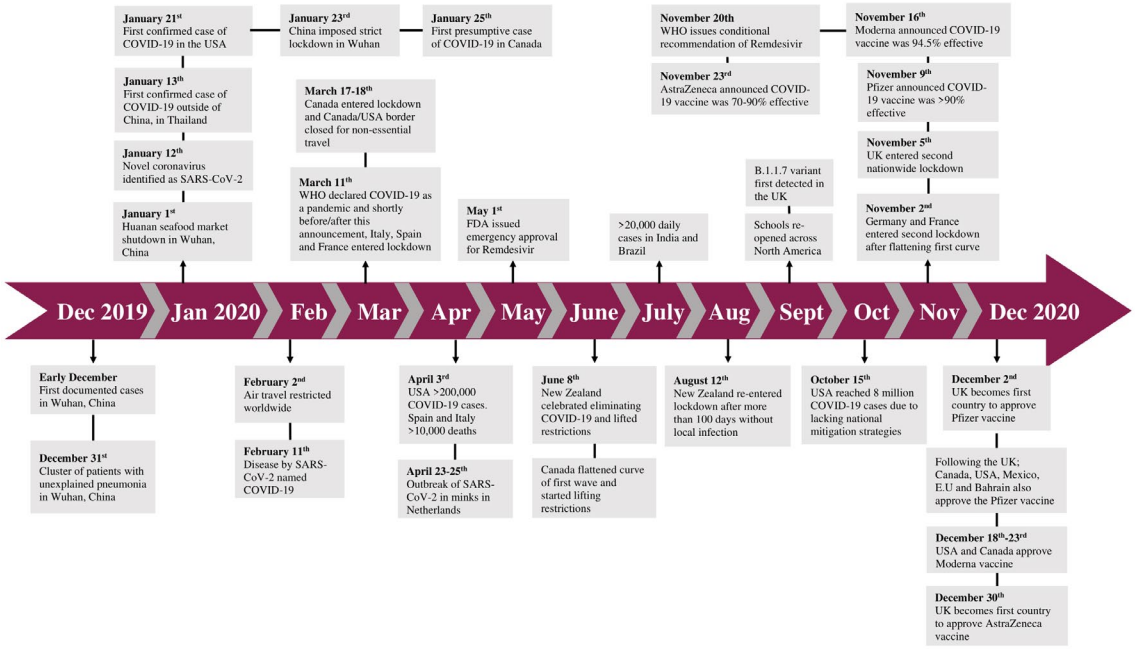

FIGURE 1 A timeline of the major global events of the COVID-19 pandemic. The first reported cases were in December 2019 in Wuhan, China. In the following 12 months, there have been more than 100 million cases and 2.15 million deaths worldwide

killed over 2.15 million people worldwide as of January 28th, 2021 and the numbers continue to grow.[3] Since COVID-19 is new to mankind, it is imperative to develop safe and effective vaccine strategies to successfully control the pandemic and return to normalcy. Although COVID-19-related reviews continue to capture the ever-expanding information, almost all of them focus only on a particular aspect with few conceptually linking together the clinical aspects, immunity, and vaccines. This review intends to provide a general overview of the latest information on epidemiology and mitigation strategies, clinical features, viral pathogenesis and immunological responses, and vaccine development for COVID-19.

2 | CLINICAL FEATURES

2.1 | Epidemiology

COVID-19 has spread rapidly and becomes a pressing global crisis. It began with the first five documented cases in Wuhan, China between December 18th and 29th of 2019 (Figure 1).[1] By January 22nd of 2020 there were 571 cases in 25 Chinese provinces and by January 30th of 2020 the case numbers grew to be 7734 in China with 90 cases reported in multiple other regions including Taiwan, Thailand, Sri Lanka, Nepal, Japan, Singapore, South Korea, UAE, United States, India, and Canada.[4] Italy was one of the hardest hit countries during the first wave of the pandemic with 110 574 cases and had one of the highest mortality rates from COVID-19 with 13 155 deaths by April 1st, 2020 (Figure 1).[5] The emergence of an infectious disease comprises three vital elements: infectious source, route of transmission, and a susceptible population.[6] SARS-CoV-2-infected individuals are the source of virus transmission as they produce large quantities of virus in the upper respiratory tract during the pre-symptomatic period.[7] The majority of infected individuals remain asymptomatic and continue to carry out routine activities, leading to rapid and undetected spread of infection. The viral load detected in asymptomatic individuals is similar to that of symptomatic patients, indicating that asymptomatic infections have the potential for transmission.[7,8] The basic reproductive number (R0) of SARS-CoV-2, which is used to describe the transmissibility of an infectious agent, was 2.97 at the beginning of the outbreak.[9,10] Person-to-person transmission of SARS-CoV-2 primarily occurs through aerosol droplets, close contact, and potentially fecal-oral transmission.[11] While SARS-CoV-2 viral RNA was found to be stable on the surface of plastic and stainless steel in an experimental setting,[12] real-life studies investigating the infectious potential of inanimate material and patient fomites showed that they were not contaminated by viable virus, suggesting that contact transmission is unlikely to occur via contaminated surfaces.[13,14] The extent of severity and mortality rates among patients with SARS-CoV-2 infection are less than those with SARS and MERS, but the prevalence and transmissibility of SARS-CoV-2 are much greater than SARS and MERS.[15] As a result, the number of COVID-19 cases globally has reached over 100 million in over 200 countries and territories,[3] and at the time of writing, many countries/cities around the globe

are being hard-hit by the second wave of the pandemic with lockdowns and/or curfews re-imposed (Figure 1).

2.2 | Risk factors

All age groups are susceptible to SARS-CoV-2 infection, but the elderly and those with certain pre-existing health conditions are particularly prone to severe disease.[16] A systematic review of current literature has found that children account for 1%-5% of all COVID-19 cases.[17] Death and incidence of severe disease has been extremely rare among children (Figure 2). For instance, incidences of severe pneumonia, lymphocytopenia, and increase of inflammatory markers were found to be scarce in children.[17] While the mechanisms accounting for milder disease and lack of development of pneumonia in children remain unclear, it has been recently found that angiotensin-converting enzyme 2 (ACE2) and/or cellular serine protease TMPRSS2, two key receptors in SARS-CoV-2 pathogenesis, are differentially expressed between adults and children. Recent studies examining the expression of these two receptors found that, children expressed significantly lower levels of ACE2 and/or TMPRSS2 in the upper and lower airways when compared to adults, hinting at a possible explanation for differential disease outcomes in these two age groups[18,19] (Figure 2). Furthermore, adults who smoked or had COPD were found to have significantly higher levels of ACE2 and TMPRSS2 expression in the airways than healthy non-smoking adults, and the patients with hypertension were found to have significantly higher levels of ACE2 and TMPRSS2 expression in PBMCs.[18] Furthermore, patients with asthma had significantly higher levels of TMPRSS2 but not ACE2 in bronchial epithelial tissue.[18] This increased expression may provide some explanation for the worsened disease outcomes among patients with pre-existing chronic cardiovascular and/or respiratory conditions (Figure 2).

The mean incubation period of SARS-CoV-2 is 5-6 days but can reach up to 24 days making screening for infection rather difficult.[15,20] Since the majority of the population is susceptible to SARS-CoV-2 infection, exposure history is one of the most important risk factors. This may represent another reason why fewer children become infected compared to adults as young children are more likely to have regimented schedules and less likely to be at social gatherings. It is important to note that the risk of contracting SARS-CoV-2 is not equally associated with poor disease outcomes. Various factors, such as socio-economic determinants, pre-existing health conditions, and exposure levels play an important role in determining the extent of disease that exposed individuals may experience.[21,22] Other risk factors associated with poor disease outcomes include being >65 years of age, having hypertension and/or obesity (Figure 2).[15,23] Higher circulating levels of LDH, D-dimer,

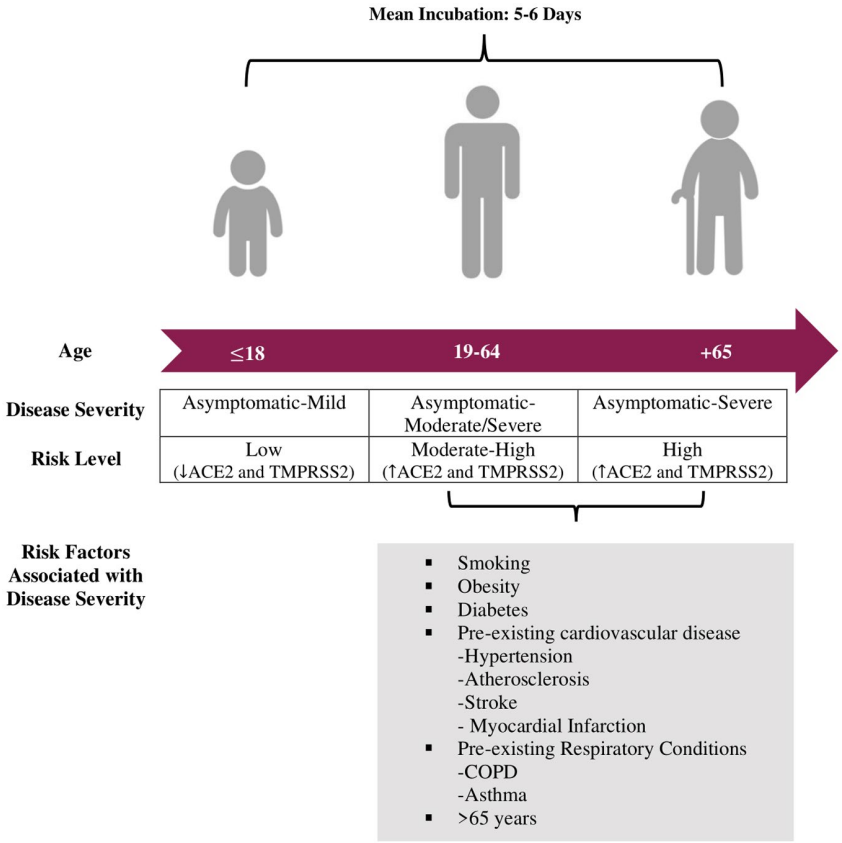

FIGURE 2 Clinical features of COVID-19. Typical risk factors, disease severity, and risk level associated with disease severity in three distinct age groups: under 18, 19-64, 65+. The mean incubation period of SARS-CoV-2 is 5-6 days and can reach up to 24 days. Children tend to be asymptomatic, whereas older people are at higher risk of more severe disease, particularly those with identified co-morbidities

C-reactive protein, and IL-6 are also correlated with severe SARS-CoV-2 disease.[23,24] SARS-CoV-2 patients that suffer pre-existing concurrent cardiovascular or cerebrovascular diseases and hyperglycemia are associated with a higher risk of mortality from COVID-19.[25]

2.3 | Mitigation measures

It has been a challenge to governments at all levels to minimize deaths and hospitalizations due to COVID-19.[26] Various mitigation strategies have been undertaken in different countries at different times to prevent hospitals from being overwhelmed and reduce COVID-19 morbidity and mortality while attempting to keep the economy ongoing.[26] Mitigation measures enforced by many countries encompass mask-wearing and hand-washing, physical distancing, cancelations of social and mass gathering events, partial/complete lock-downs including border closures and travel restrictions, and/or testing/contact-tracing/quarantine/isolation.[26,27]

Since no vaccine exists, China announced risk mitigation measures in Wuhan including quarantine requirements, city lockdown, and isolating infected populations which controlled the spread of the virus[27] (Figure 1). Hong Kong, Taiwan, South Korea, and Mongolia implemented even more stringent risk mitigation measures such as travel restrictions, isolation of travelers from Wuhan, closure of schools and hygienic measures.[27] Soon after, two regions in Italy were severely impacted by the virus as there was a huge surge in cases forcing the entire country to enter lockdown (Figure 1).[28] Other European countries also experienced an increase in COVID-19 cases shortly after and implemented similar mitigation strategies to control the spread of the virus.[27]

Around the globe, each country has adopted various measures in response to COVID-19, to slow down transmission and prevent oversaturation of health-care systems. These measures in some cases have drastically varied from country to country, from travel restrictions to social distancing, and complete lockdowns. In early stages of the pandemic there was quite some confusion, particularly in western countries, about the protective effects of facial masks and physical distancing until the established evidence surfaced.[29,30] To this point, several restrictions levied have proven effective in slowing down the spread of COVID-19, but the greatest effect is obtained by applying a combination of measures.[27,31] Interestingly, during the 1918-19 Spanish Flu pandemic similar travel restrictions and quarantine measures were implemented in many countries. It is estimated that implementation of these measures during the 1918-19 pandemic resulted in a reduction of death rates by 50%.[32] A study comparing the hospitalization rates in two Canadian provinces during the first wave of COVID-19 has revealed that the curve of hospitalization rates in the province of British Columbia, but not of Ontario, was flattened with the implementation of social distancing and limitations on social gatherings.[33] At the present time, while the true efficacy of these mitigation measures in controlling the current COVID-19 pandemic remain to be fully established, mathematical modeling provides insight into what is expected to occur or what may have transpired.[34-36] Mathematical modeling of COVID-19 transmission in Wuhan showed that the implementation of quarantine measures resulted in the R0 value decreasing from 2.65 to 1.98. This study also predicted that implementing lockdown 7 days earlier would have resulted in a 72% decrease of infected individuals.[37] Overall, it is apparent that the extent of COVID-19 control has been closely associated with the stringency of mitigation measures undertaken in various regions of the world (Figure 1). For instance, while many Asian and European countries as well as Canada had successfully flattened the curve of the first wave and lifted, to varying extents, the initially imposed mitigation measures to revive the economy until the second wave struck, countries such as the United States lack a national mitigation strategy and have never flattened the first wave, now meeting with huge daily cases and deaths with an over-taxation of health-care systems[38] (Figure 1).

Nonetheless, while mitigation measures are necessary for the control of COVID-19, they bear serious economic consequences.[39-41] Goldman Sachs predicted that the United States gross domestic product would shrink by 5% in the second quarter of 2020 largely due to the pandemic, loss of productivity, and the implementation of control measures.[42] Along with the economic impact, there has been a sentiment of resistance to government imposed COVID-19 restrictions, quite prominently seen in the United States, where people have organized anti-mask and anti-quarantine rallies.[43] This sentiment has spread to other countries as well, with rallies being organized in many cities such as the Canadian cities of Toronto and Montreal.[44,45] The balance between effective mitigation measures to control the spread and protecting national economies is extremely complex. In fact, modeling and investigation into hospital admission rates associated with mitigation methods, varying lockdown scenarios, times, and duration, indicate that lockdown measures outperform less stringent restrictions in reducing cumulative deaths and pressure on health-care system.[46-48] It was also projected that lockdown at the early stage of transmission would have saved more lives. The short-term lockdowns, so-called circuit breaker intervention in the United Kingdom were found to have the biggest impact when the infection rate is low.[48] Imposing mitigation methods at the national or regional levels thus needs to be carefully chosen considering the transmission rate and health-care infrastructure, but the benefit of such measures needs to be weighed against the socioeconomic impacts.

2.4 | Clinical characteristics

A wide range of clinical manifestations are seen in SARS-CoV-2 patients, ranging from mild/moderate to severe, rapidly progressive, and fulminant disease. Symptoms of SARS-CoV-2 are non-specific and disease presentation can range from asymptomatic to severe pneumonia. Incidence of asymptomatic SARS-CoV-2 cases ranges from 1.6% to 51.7% and these people do not present typical clinical symptoms or signs and do not present apparent abnormalities in lung computed tomography (CT).[49-54] The most common symptoms of COVID-19 are fever, cough, myalgia, or fatigue, which are similar to those of SARS and MERS; atypical symptoms include sputum, headache, hemoptysis, vomiting, and diarrhea.[55,56] Some patients may present with sore throat, rhinorrhea, headache, and confusion a few days before the onset of fever, indicating that fever is a critical symptom, but not the initial manifestation of infection.[56] Furthermore, some patients experience loss of smell (hyposmia) or taste (hypogeusia), which are now being considered early warning signs and indications for self-isolation.[57,58] Diagnosed patients may also present with lymphopenia, thrombocytopenia, and leukopenia.[55,59,60] Most of the patients had elevated levels of C-reactive protein; less common were elevated levels of alanine aminotransferase, aspartate aminotransferase, creatine kinase, and D-dimer.[55]

The clinical course of SARS-CoV-2-induced pneumonia displays a broad spectrum of severity and progression patterns. Around 6.5%-31.7% of hospitalized patients required admission to the ICU and roughly 29% of these patients developed acute respiratory distress syndrome (ARDS) 8 days following symptom onset.[21,56,61] Of the hospitalized patients, a small percentage ranging from 2% to 20% required invasive mechanical ventilation[62] which indicates poor course of disease and can lead to progression of multiple organ dysfunction (MODS) and mortality.[21,56] The most common comorbidities were hypertension, obesity, and diabetes, in which severe illness and death were more prevalent among older patients (Figure 2).[21,63] The clinical characteristics presented in these cases represent the more severe end of confirmed SARS-CoV-2 cases with respiratory distress and pneumonia and are not the same as mild or asymptomatic cases.

Furthermore, the persistence of symptoms in recovered patients is an emerging and pressing issue. A study of 143 recovered patients found that 87.4% of them were still experiencing at least one symptom of COVID-19 up to a month following discharge. The most common symptoms among these patients were fatigue 53.1%, dyspnea 43.4%, joint pain 27.3%, and chest pain 21.7%.[64] Recovered patients with persistent symptoms even months after the initial infection have been described as "long-haulers" or "long COVID," and due to the lack of comprehensive and large scale studies the incidence rate of these long-haulers ranging from 10% to 35% remains to be fully established.[65-70] While the underlying mechanisms for these long-term effects still remain incompletely understood, persistent lung injuries have been seen in COVID-19-recovered patients (detailed in Section 4 below). Some of the long-term symptoms may also be related to invasive treatments used and lasting pathologies at tissue sites other than the lung[65,69] and mitochondrial pathways.[71] As our knowledge in long COVID-19 cases continues to emerge, it is likely that the care of such patients entails a multidisciplinary approach.

Timely and accurate diagnostic SARS-CoV-2 testing is a crucial step in managing the pandemic. Currently, diagnostic testing for COVID-19 is undertaken via a two-pronged approach: direct detection of the viral RNA or immune-based tests to detect viral antigens or antibodies.[72] The most common test detects viral RNA following reverse transcription and DNA amplification by PCR accompanied with real-time results (rRT-PCR).[72] The genes S, N, and E are used as targets in the rRT-PCR assay in combination with the open reading frame 1 (ORF1) and the RNA-dependent RNA polymerase.[73] There are now many rapid antigen detection tests available for SARS-CoV-2 detection. Both WHO and FDA have granted emergency use listings and authorization, respectively, for rapid antigen tests. WHO has listed two and FDA seven rapid antigen tests.[74,75] Antibody testing can provide a complementary role along with RT-PCR in the diagnosis of COVID-19, and these tests also allow for the characterization of individual humoral immune responses to current and previous infections. Hundreds of these tests exist, targeting IgG, IgA, and IgM responses, and are currently being used in clinical settings.[72] While rRT-PCR is performed on nasopharyngeal and throat swabs, antigen-antibody based assays use blood or serum samples. Thus, antigen-antibody tests are suggested to be used as a complementary diagnostic tool for rRT-PCR.

2.5 | Treatment

At present, active symptomatic support remains the key treatment for mildly to moderately ill patients, such as maintaining hydration, nutrition, and controlling fever and cough. For patients with severe infection or those critically ill with respiratory failure, hospitalization is required and oxygen inhalation through a mask, high nasal oxygen flow inhalation, non-invasive ventilation, or mechanical ventilation is indicated.[76,77] Extracorporeal membrane oxygenation (ECMO) may be implemented if all the above methods do not work.[78] Additionally, antibiotics and antifungals may also be required. A study demonstrated the potential benefits accruing from low-dose corticosteroid treatment in a subset of critically ill patients with SARS-CoV-2 infection.[79] Many potential approaches have been suggested based on the progress of SARS-CoV-2 research,

including inhibition of SARS-CoV-2 fusion/entry, disruption of SARS-CoV-2 replication, suppression of excessive inflammatory responses, convalescent plasma (CP) treatment, and the use of vaccines.[80-82]

Arbidol and chloroquine phosphate have been added to the list of potential treatment options for COVID-19. Arbidol was shown to prevent multiple enveloped viruses by inhibiting virus entry/fusion of viral membranes with cellular membranes.[83] Chloroquine, a traditional antimalarial drug, was shown to be effective against SARS-CoV-2 infection in vitro and was more effective in inhibiting exacerbation of pneumonia than control treatment.[84] Although the specific mechanisms of hydroxychloroquine and chloroquine phosphate in the treatment of SARS-CoV-2 infection remain unclear, it is likely to increase the pH of endosomes, which are required for viral cell entry, and impair the glycosylation of ACE2.[85] A recent large-scale trial study of 1561 hospitalized patients treated with hydroxychloroquine and 3155 hospitalized patients receiving usual care found that those receiving hydroxychloroquine did not exhibit a lower death incidence-rate 28 days following treatment.[85] A third update published on December 1 of a previously published living systematic review that focused on treatment of COVID-19 with hydroxychloroquine has concluded that it is becoming increasingly unlikely that in-hospital use of hydroxychloroquine will yield beneficial effects.[86] However, it reports that the outpatient use of hydroxychloroquine is promising.[86]

Many antiviral agents have been developed against viral proteases, polymerases, MTases, and entry proteins. Remdesivir (GS-5734) is a mono-phosphoramidate prodrug of an adenosine analog, which can incorporate nascent viral RNA and inhibit the RNA-dependent RNA polymerase.[87] Remdesivir effectively inhibited SARS-CoV-2 infection in vitro, and improved the first confirmed case of COVID-19 in the United States without any noticeable adverse effects.[87,88] Recent findings from a double-blind, randomized, placebo control trial of 1062 patients found that those treated with Remdesivir had shortened recovery times compared to those given the placebo. Patients given Remdesivir also exhibited a lower incidence of lower respiratory tract infection and on May 1st 2020 the FDA authorized emergency approval for usage of Remdesivir against COVID-19 (Figure 1).[89] On November 20th, however, as part of a living guideline on clinical care for COVID-19, the WHO announced a conditional recommendation against the use of Remdesivir in hospitalized patients, based on a comprehensive analysis of all available data.[90] With the global spread of SARS-CoV-2, vaccination is the most efficient and cost-effective means to prevent and control COVID-19.[91] In order to better understand COVID-19 and develop safe, effective vaccination strategies, it is of utmost importance to increase our knowledge in the natural immunological responses to SARS-CoV-2.

3 | IMMUNOLOGICAL RESPONSES

3.1 | Origin and genomics of SARS-CoV-2

SARS-CoV-2 is a beta-coronavirus in the *Coronaviridae* family and the order of *Nidovirales*. Coronaviruses have the largest genome of all RNA viruses and typically contain six open reading frames (ORFs).[92] SARS-CoV-2 has a unique N-terminal fragment with a spike protein and the genes occur in a 5′ to 3′ order with key structural proteins encoded in ORFs 10 and 11, at the 3′ terminus end.[92] SARS-CoV-2 shares 79% and 50% genome sequence identity with SARS-CoV and MERS-CoV, respectively.[93] SARS-CoV-2 shows higher sequence identity with other bat coronaviruses, including RaTG13, bat-SL-CoVZC45, and bat-SL-CoVZXC21.[93] Interestingly, the virus also has a high genome sequence identity with pangolin coronavirus, especially in the receptor-binding domain (RBD), where SARS-CoV-2 shows some differences from RaTG13.[93,94] This sequence identity at the RBD is a key factor for some groups postulating a recombination event that may have occurred in pangolins, or other animal species as an intermediate species, before jumping to humans.[94] Information surrounding the genome has allowed for the characterization of the SARS-CoV-2 structure and has revealed mechanisms of cell entry and pathogenesis of the virus. It has also allowed rapid development of COVID-19 diagnostics.

Since the emergence of the pandemic, surfacing of SARS-CoV-2 mutants has been a concern. As early as February 2020, a substitution in the spike protein of SARS-CoV-2 was detected and named D614G variant.[95] By June of 2020, this variant had become prominent globally and was suggested to exhibit increased infectivity with comparable disease severity to wild-type strain D614.[95] More recently, another variant of a greater global concern has surfaced in United Kingdom, recorded on September 20th and sequenced in early October and named as B.1.1.7. (Figure 1), encompassing 17 mutations, 8 of which are in the spike protein. One of these eight mutations, N501Y, has also been found on another variant of the virus isolated in South Africa.[96] While much more remains to be discovered about these new variants, the B.1.1.7. variant is 0.4 to 0.7 more transmissible than its parental SARS-CoV-2 strains. At the time of writing, the emerging evidence suggests that some of these recent variants can evade both natural and vaccine-induced humoral immunity in testing tubes while it remains to be fully established whether and to which extent they may lead to reduced vaccine efficacy.[97]

3.2 | Structure of SARS-CoV-2

SARS-CoV-2 is a spherical, enveloped, positive sense single-stranded RNA virus that consists of four main

structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N).[92] The E protein is expressed during replication and although its specific role for SARS-CoV-2 is not clear, recombinant viruses lacking the E protein have exhibited reduced viral titers and abrogated viral maturation, suggesting that this protein is important for viral replication and maturation.[98] The M glycoprotein is the most abundant structural protein, spanning the membrane bilayer three times and leaving a NH2-terminal domain outside the virus as well as a long COOH-terminus inside the virion.[92] The N protein is important for RNA packaging and viral release following infection of host cells.[99] The S protein is of great importance as it mediates the cell entry and initiation of pathogenesis. S is a type I membrane trimer glycoprotein with an S1 domain comprising the RBD, which is required for receptor-binding and an S2 domains responsible for cell membrane fusion.[100] S is further cleaved by host proteases including furin and TMPRSS2, at the S2′ site activating proteins necessary for membrane fusion.[101] Overall, there seems to be key similarities in regard to the S protein and RBD between SARS-CoV-2 and SARS-CoV suggesting similar mechanisms of viral cell entry.

3.3 | Viral cell entry and life cycle

Current evidence indicates that SARS-CoV-2 utilizes S to recognize the hACE2 receptor to facilitate viral entry into host cells. Importantly, it has been shown that after engaging the cell membrane, either viral RNA enters the cytosol or the ACE2/SARS-CoV-2 complex is endocytosed with the entire virus and the viral membrane fuses with the luminal side of the endosome allowing for viral RNA transfer to the cytosol.[102] Various groups have demonstrated that SARS-CoV-2 is able to infect cell lines expressing ACE2 more effectively than those lacking ACE2 expression.[103,104] Another protein that plays a role in SARS-CoV-2 entry is the cellular serine protease, TMPRSS2, which primes the S protein and is essential for viral spread and pathogenesis.[104] Recent cell culture work has provided evidence that heparan sulfate is a necessary cofactor for SARS-CoV-2 infection and that it interacts directly with the RBD changing it to an open confirmation to facilitate binding with ACE2.[105] ACE2 is present in various tissues including the lung, heart, kidney, brain, and testes, which speaks to the ability of SARS-CoV-2 to cause pathology at these anatomical sites in some patients. ACE2 expression was recently analyzed via sc-RNA-seq comparative data between humans, non-human primates, and mouse models showing that ACE2 and TMPRSS2 are co-expressed in lung type II pneumocytes, gut enterocytes, and nasal goblet cells.[106]

Once the S protein binds the ACE2 receptor, it undergoes conformational changes which stimulate viral envelope fusion with the cell membrane. SARS-CoV-2 then releases viral RNA into the host cell and begins the process of replication.[107] Viral genomic RNA first becomes translated into pp1a and pp1b, which are key viral replicase polyproteins, and these polyproteins are then cleaved into small products by viral proteinases. Through discontinuous transcription via polymerase activity a series of subgenomic mRNAs are produced and ultimately translated into key viral proteins. Subsequently, in the host endoplasmic reticulum and Golgi, viral protein, and genomic RNA are assembled into virions and transported out of the cell in vesicles and released into the cytoplasm.[107]

3.4 | Innate immune response

Some of the key innate immune cell types involved in the innate immune response to SARS-CoV-2 are alveolar macrophages (AMs), neutrophils, monocytes, and dendritic cells (DCs).[108] Mounting evidence suggests that SARS-CoV-2 can suppress the innate immune activation in early stages of infection (Figure 3). AMs are the most abundant cell type located in the airways and are the first line of defense against most pathogens invading the respiratory mucosa.[109] Detection of damage-associated molecular patterns (DAMPS) and pathogen-associated molecular pattern (PAMPs) by AMs leads to the induction of an inflammatory cascade vital for viral control but may also contribute to tissue injury.[109] Detection of the virus is an important step required for the activation of an immune response. Toll-like receptors (TLRs) and RIG-I-like receptors (RLRs) are the two main classes of receptors that recognize viral PAMPs.[110] TLRs 3,7, and 8 located on the endosome of host cells are capable of recognizing the single-stranded RNA genome of SARS-CoV-2, activating TRIF or MyD88, leading to downstream upregulation of the NF-κB pathway and inflammatory genes pertinent to the immune response.[110] RLRs are cytosolic RNA receptors that primarily recognize nucleic acids of RNA viruses, leading to a similar downstream pathway feeding into the activation of NF-κB and IFN production.[111] Following initial recognition and upregulation of interferon-stimulated genes (ISGs), they are able to act in an autocrine and paracrine manner to stimulate IFN signaling through the JAK-STAT pathway.[110] Type I and III IFN production by AMs signal for intracellular antiviral defense in proximate epithelial cells and secretion of IL-6 and IL-1β prompts the recruitment of neutrophils and cytotoxic T cells to the site of infection.[112] Chronic exposure to type I or III IFNs can lead to various interferonopathies. Recent work in various mouse models has suggested that these IFNs disrupt tissue repair by reducing the epithelial cell proliferation and may be an important consideration for immunotherapies

in the treatment and management of severe COVID-19 disease.[113,114]

Monocytes and macrophages express ACE2 and macrophages also express furin and TMPRSS2 which play a role in SARS-CoV-2 cell entry and pathogenesis.[115] Recent ex vivo evidence has suggested that elevated glucose levels in diabetic individuals enhance viral replication and cytokine expression in monocytes.[116] This is deemed to be mediated by a change in the metabolic activity of the cell to favor glycolysis via the HIF-1α axis and co-culture work has revealed that SARS-CoV-2-infected monocytes can directly reduce the T cell response and inhibit epithelial cell survival, potentially contributing to immunopathology.[116] While previous studies have shown that human macrophages and DCs are susceptible to infection by SARS-CoV, the virus was unable to productively replicate within these cells but triggered the production of pro-inflammatory cytokines.[117,118] It remains unclear whether the same holds true for SARS-CoV-2.[115] Although monocytes and macrophages play an important role in the initial immune response, it has been suggested that overactivation or sustained pro-inflammatory state in these cells may contribute to a dysregulated immune response leading to disease severity in COVID-19 patients.[115]

NK cells are able to induce lysis of virus-infected cells which is vital to control viral infection and reduce tissue damage.[119] Peripheral blood from COVID-19 patients has exhibited decreased NK cell counts which is associated with increased disease severity.[119] Ex vivo analysis of NK cells from the peripheral blood of COVID-19 patients showed reduced intracellular expression of granzyme B, CD107a,

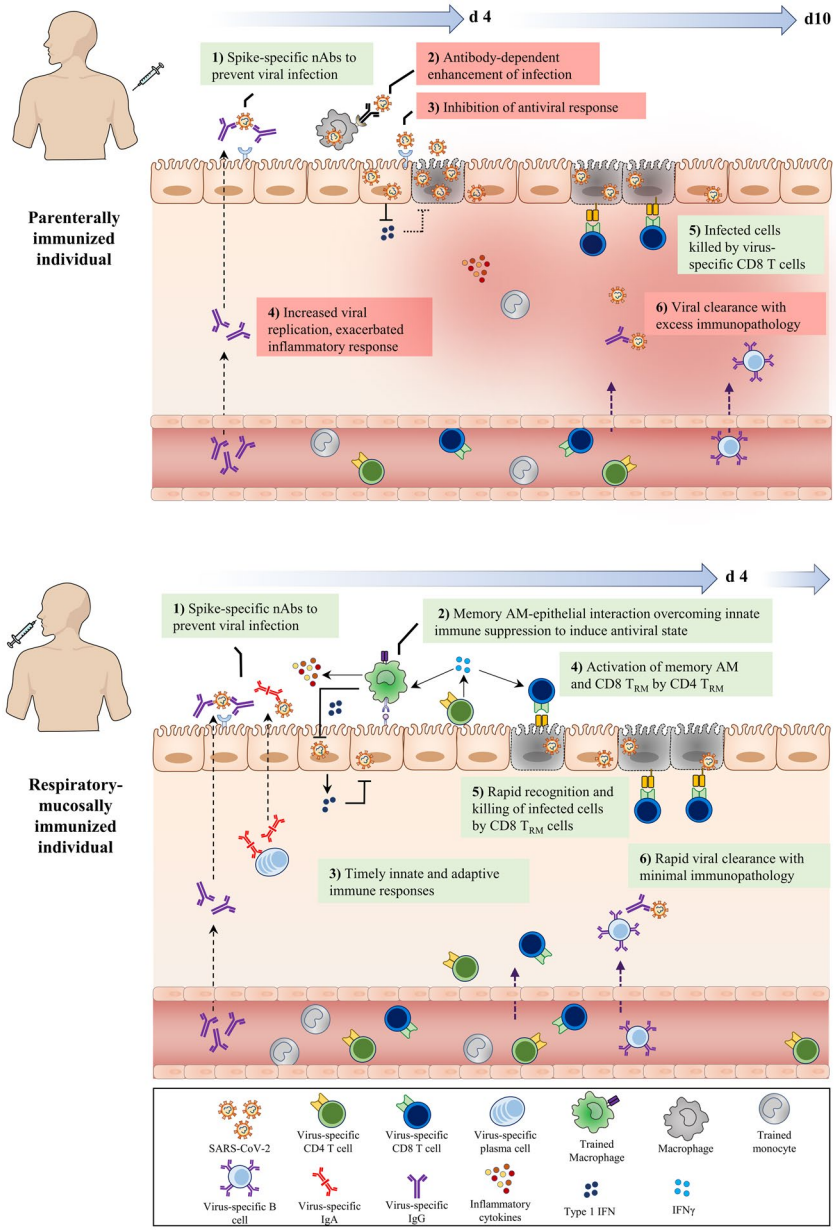

FIGURE 3 Deduced early immunological events at respiratory mucosa of parenterally or mucosally vaccinated hosts upon SARS-CoV-2 exposure. Following the parenteral route of immunization (top panel), circulating monocytes undergo systemic innate immune training through the priming of hematopietic monocyte progenitors in the bone marrow. Furthermore, SARS-CoV-2-specific neutralizing IgG antibodies and T cells are produced and present in the circulation. However, often only the IgG antibodies are transported to the respiratory mucosal surfaces, whereas T cells and trained monocytes remain trapped within the pulmonary vasculature, resulting in an incomplete establishment of respiratory mucosal immunity. Upon SARS-CoV-2 infection, although the neutralizing IgG Abs (nAbs) on the surface of the respiratory mucosa bind to the incoming viruses and block its interaction with ACE2 receptor, it can be insufficient due to suboptimal levels of nAbs and weakly nAbs. However, in some hosts this mechanism may be adequate for protection. On the one hand, escaping virions may gain entry to alveolar macrophages (AM) via Ab and FcγR interaction and on the other hand, the virus infects airway epithelial cells, suppresses innate immune responses by inhibiting antiviral pathways, and delays adaptive $CD8^+$ T cell responses. During this critical time gap (d4-d10), poorly controlled viral replication leads to viral dissemination, dysregulated inflammatory cytokine, and inflammatory monocyte responses, resulting in excessive tissue immunopathology. In comparison, the respiratory mucosal route of immunization (bottom panel), particularly with live attenuated or viral-vectored vaccines amenable for respiratory mucosal (RM) immunization, induces a holistic RM immunity consisting of trained alveolar macrophages (AM), mucosal IgA and IgG Abs, and lung tissue-resident memory T (T_{RM}) cells. RM immunization also induces a level of systemic immune protection by inducing circulating virus-specific Abs and T cells. Upon SARS-CoV-2 infection, the holistic mucosal immunity overcomes the initial virus-mediated innate immune suppression and quickly clears viral infection within the first few days (d4) via a coordinated mucosal immune response by trained (memory) AM, neutralizing mucosal Abs and T_{RM}. nAbs (IgA/IgG) block viral entry to the epithelial cells. In dealing with the viruses that escaped from neutralization, memory AM interact with epithelial cells to overcome viral-imposed innate immune suppression, enhancing antiviral state to inhibit viral replication. Moreover, $CD4^+$ T_{RM} further activate memory AM and $CD8^+$ T_{RM} which kill infected epithelial cells and probably infected AM. Together, such concerted immune responses lead to not only a timely control of viral infection but also prevention of excessive immunopathology and pneumonia

IFN-γ, TNF-α, and other important markers of functionality.[120] Furthermore, NK cells and $CD8^+$ T cells from COVID-19-infected patients have exhibited increased expression of the inhibitory receptor NKG2A, suggesting a decrease in functionality.[120] A recent study looking at the acute phase immune cell profile in PBMCs of COVID-19 patients found that patients exhibited reduced frequencies of DCs, monocytes, NK cells, and T cells.[121] An impairment in the functionality and reduction in the frequency of DCs in the acute phase may delay T cell responses leading to heightened infection and worsened disease outcomes (Figure 3).[121] Overall, the mechanisms by which PRRs and innate immune cells act are vital for recognition of SARS-CoV-2 and induction of an innate immune response to infection.

3.5 | Adaptive immune response

Due to innate immune suppression in early stages of SARS-CoV-2 infection, the activation of adaptive immune responses is impeded particularly in those who developed severe disease (Figure 3). Current reports on the T cell compartment of COVID-19 patients suggests that moderate and severe disease patients exhibit lymphopenia with lower levels of $CD4^+$ and $CD8^+$ T cells.[122] Patients with mild disease outcome have reported increases in T cell numbers, but overall the mechanisms of lymphopenia in moderate to severe patients remains elusive.[119] The overreactive cytokine milieu seen in severe patients may have an effect on lymphopenia in the peripheral blood, given that lymphopenia seems to be correlated with serum levels of IL-6, IL-10, and TNF-α.[119,123] A timely Th1 response induced by $CD4^+$ T cells and direct killing of virally infected cells by $CD8^+$ T cells is a key part of antiviral immunity (Figure 3). $CD4^+$ T cells contribute to the induction of humoral immunity which is also important for antiviral immunity.[119] Zheng et al, have proposed that under conditions of severe COVID-19 disease, $CD8^+$ T cells primarily secrete IFN-γ while virus-specific $CD4^+$ T cells secrete anticipated levels of Th1 cytokines IFN-γ, TNF-α, and IL-2. Severe COVID-19 patients also displayed a reduced proportion of multifunctional $CD4^+$ T cells, which were positive for more than two of IFN-γ, TNF-α, and IL-2, when compared to patients with mild or moderate disease.[120] Given these circumstances, it is evident that patients with severe COVID-19 disease exhibit a dysregulated and insufficient T cell response. Furthermore, T cells from peripheral blood of COVID-19 patients tend to express high levels of PD-1 and Tim-3, markers of exhausted T cells, and has been positively associated with disease severity.[123]

However, a recent study examining T cell responses to S, M, and N proteins has suggested that robust T cell compartment responses are not associated with ameliorated disease outcomes, and in fact a robust T cell response in critical patients may contribute to hyperreactivity and immunopathogenesis.[124] This speaks further to the importance of the timing of both natural immune responses and vaccine-induced immunity at the site of infection[125] (Figure 3). Another important consideration regarding the T cell compartment is pre-existing cross-reactive memory $CD4^+$ T cells in humans unexposed to SARS-CoV-2. Approximately 20%-50% of people possess pre-existing cross-reactive $CD4^+$ T cells although the source of these cell subsets remains speculated.[126] A group mapped 142 T cell epitopes revealing that many of the $CD4^+$ T cells reacting to SARS-CoV-2 epitopes

also cross-react with corresponding homologous sequences from circulating common coronaviruses.[126] This pre-existing memory in some individuals may provide a potential explanation for the varying COVID-19 disease outcomes in different populations.[126] Current knowledge regarding phenotype and functional alterations in the T cell compartment following SARS-CoV-2 infection is still limited and further investigation will provide critical information for vaccine design.[125]

As seen in the T cell compartment, B cells in peripheral blood of patients with moderate/severe disease were markedly decreased and the number of B cells was negatively correlated with viral burden.[127] B cells in circulation are restored to normal levels in recovered COVID-19 patients and the infusion of plasma from these convalescent patients has exhibited the potential for effective therapeutic treatment in severe COVID-19 cases.[128] These results support that virus-specific antibodies produced by the humoral immune response to SARS-CoV-2 are important for resolving the infection. In a study of 58 convalescent patients with mild disease, only 30% generated relatively low titers of neutralizing antibodies, suggesting an increased risk of reinfection.[129] The authors found that severity of disease correlated with the induction of a stronger immune response and those recovered from severe infection may be more likely to be protected against reinfection.[129] Patients have shown antibody seroconversion as early as day 7, with most seroconverting by day 14.[130] By day 7-10, many patients display an increase in IgG and IgM specific for N and RBD which was followed by a gradual decrease in viral load, further supporting the protective effects of humoral immunity against the virus.[130] In some patients S-specific IgA was detected as early as 6-8 days following disease onset and was maintained over a 6-week observational period. Overall the IgA response in this longitudinal study was more robust than IgM.[131]

Various studies have been looking into the longevity of these anti-SARS-CoV-2 antibodies in recovered patients. This is of great importance as considerations for the potential of developing herd immunity against SARS-CoV-2 greatly depends on the capacity of neutralizing antibodies to reduce transmission.[132] Furthermore, persistence of these antibodies can provide insight into the likelihood and extent of reinfection in convalescent individuals. A study conducted by Isho et al, examining S-specific antibody responses in saliva and serum of convalescent patients found antigen-specific IgG in both biofluids in most samples 16-30 days post-symptom onset and these levels did not drastically decline at 110-115 days post-symptom onset in most samples.[133] Although these results indicate antigen-specific IgG may be long-lasting following SARS-CoV-2 infection, other studies have found contradicting results. A study consisting of 343 North American convalescent COVID-19 patients found that although RBD-specific IgG titers did not decrease 75 days post-symptom onset, RBD-specific IgA and IgM titers significantly decreased 71 and 49 days post-symptom onset.[134] A large-scale community study of 365 000 adults in the United Kingdom found a significant decline in the proportion of the population with detectable antibodies over three-rounds of testing 12, 18, and 24 weeks following the peak of COVID-19 cases.[135] The results of this study suggest that antibody-immunity against SARS-CoV-2 may be waning within the first 6-12 months. However, it is important to note that as this large-scale study was conducted via participants self-pricking and reporting, the results remain to be verified via further investigation. A case study has reported re-infection by a genetically different SARS-CoV-2 which led to more severe disease than the first, indicating that first exposure might not guarantee immunity in all cases.[136]

In an infectious model study of rhesus macaques, two studies have shown that primary infection with SARS-CoV-2 provides protection against a subsequent re-challenge.[137,138] More studies into the potential re-infection, protective capacity and longevity of immunity are critical to furthering our understanding of the pathophysiology of this disease and natural immunity.

While generation of antiviral antibodies is generally thought of as a positive, there is some concern about antibodies worsening disease outcome via antibody-dependent enhancement (ADE).[129] Antibody-dependent enhancement is when antibodies interact with the immune system to promote pathology. The ADE phenomenon has been documented for dengue, SARS-CoV, and other viruses. Neutralizing antibodies in the case of SARS-CoV engage Fc receptors on immune cells, particularly macrophages, which may then promote inflammation and result in tissue injury due to overactivation of these cells.[139] Many important details have yet to be established regarding the role of humoral immunity following SARS-CoV-2 infection.

4 | IMMUNOPATHOLOGICAL PROCESSES

4.1 | Inflammatory cytokine overdrive

Dysregulation of the immune system has been identified as one of the major mechanisms for the worsening of disease outcome in severe COVID-19 patients. The role of $CD8^+$ T cells and NK cells in the antiviral response is of utmost importance in order to mediate effective killing of virally infected cells.[120] As previously mentioned, CTLs and NK cells isolated from the peripheral blood of severe COVID-19 patients were significantly reduced and exhibited increased expression of PD-1, Tim-3, or NKG2A, suggesting functional exhaustion. Functional exhaustion of antiviral lymphocytes may contribute to worsened conditions and immunopathology following SARS-CoV-2 infection.[120] Furthermore,

severe COVID-19 patients exhibited increased levels of neutrophils compared to those with moderate/mild disease.[120] Although neutrophils are an important cell type in the innate immune response, they can exacerbate immunopathology. Unbalanced neutrophil extracellular trap (NET) production, increased production of pro-inflammatory cytokines, and the release of reactive oxygen species by neutrophils can worsen inflammation in the lung tissue, further exacerbating the cytokine storm and contributing to the development of ARDS.[140]

Timing of antiviral immune responses, particularly when viral load is low and patients are still in an asymptomatic/pre-symptomatic stage is key[125] (Figure 3). Swift activation of type I and III IFN responses as well as timely non-excessive production of key pro-inflammatory cytokines allows the host an opportunity to reduce viral load and limit tissue injury.[125] However, SARS-CoV-2, just like MERS-CoV and SARS-CoV, is armed with mechanisms to dampen these key initial responses. This results in a dysregulation of the host's immune response, characterized by excessive infiltration of inflammatory immune cells and overproduction of pro-inflammatory cytokines.[125] Dysregulation of the immune response paired with an increasing viral burden can lead to detrimental viral and immune-mediated pathology (Figure 3).

Overproduction of pro-inflammatory cytokines is a major contributor to the immunopathology seen in severe COVID-19 patients. Elevated serum levels of cytokines including IL-1β, IL-2, IL-7, IL-8, IL-9, IL-10, IL-17, G-CSF, GM-CSF, IFN-γ, TNF-α, IP-10, MCP-1, MIP-1α, and MIP-1β have been associated with the development of ARDS leading to pulmonary edema, lung failure and in some cases cardiac, hepatic, and renal injury.[56,122,141] Patients in the ICU have exhibited higher levels of IL-2, IL-7, IL-10, G-CSF, IP-10, MCP-1, MIP-1α, and TNF-α when compared to non-ICU patients.[122,141] Both IL-1β and TNF-α promote vascular leakage and permeability as well as Th17 responses.[141] Skewing of T cell responses toward Th17 was reported to worsen immunopathology and inflammation. Th17 cells produce IL-17 which has broad pro-inflammatory effects, G-CSF contributing to granulopoiesis and neutrophil recruitment, and IL-22 which may contribute to life-threatening edema by upregulation of mucins and fibrinogens.[141] It is evident that the immune system may contribute to immunopathology through a variety of mechanisms. However, the details and timeline of these maladaptive interactions still remain to be unraveled.

4.2 | Lung pathology

The lung tissue is the main site of immune and virus-mediated pathology induced by SARS-CoV-2 infection. In the case of all three recent coronavirus epidemics/pandemics the primary lung pathology is diffuse alveolar damage which accounts for the ground glass opacity on chest x-rays.[142] During the early/mild phase of disease, SARS-CoV-2 does not seem to result in the formation of hyaline membranes in the lung. However, by the later/more severe phase the extensive hyaline membrane formation and desquamation of pneumocytes occurs. Significant alveolar damage and progressive respiratory failure lead to the onset of severe disease and subsequent death.[142,143] Furthermore, there seems to also be persistent damage in the lungs of COVID-19 recovered patients. A study noted that 6 weeks following hospital discharge 88% of the participants had some visible damage on their lung CT and by 12 weeks the incidence of visible damage reduced to 56%.[144] A comprehensive long-term study looking at the long-term effects of SARS-CoV infection published earlier this year, found that 15 years after infection 4.6% of the participants still had visible lesions of the lung and 38% displayed reduced diffusion capacity.[145] Furthermore, a prospective observational 3-month follow-up study found that although lung high-resolution computed tomography (HRCT) returned to normal in most of the patients, in 42% of them mild pulmonary abnormalities persisted and 50% suffered from symptoms such as dyspnea, cough, chest tightness, and palpitations on exertion 3 months after discharge.[146] Another study has reported that 35 out of 55 recovered participants exhibited different degrees of radiological abnormalities 3 months after discharge.[147] In some follow-up patients, lung interstitial thickening and pure ground-glass opacity were among the most common features on HRCT scans.[147] A study carried out in Austria evaluated long-term lung damage in recovered patients at 1.5, 3, and 6 months following discharge from the hospital.[148] At the first visit, 56% of recovered patients experienced at least one persistent symptom, mainly breathlessness and coughing, and 88% of patients displayed lung damage on CT scans.[148] At 3-month visit, while breathlessness had improved with lung damage rates reduced to 56%, coughing persisted in 13 patients. Results from 6-month visit is still pending from this study.[148] Clearly, such persistent lung injuries in some of recovered patients may underpin some of the clinical symptoms experienced by the "long haulers" and call for increased scientific attention and continuing medical care even after the pandemic is behind us.

4.3 | Cardiac pathology

It has become clear that SARS-CoV-2 or COVID-19 has a detrimental effect on tissue organs including the cardiovascular system other than the respiratory system. COVID-19-associated cardiac complications include, but are not limited to, heart failure, myocarditis, pericarditis, vasculitis, and cardiac arrhythmias.[149] Around 8%-28% of COVID-19 patients exhibit a marked troponin release early in the onset of

disease, reflecting cardiac stress, or injury.[149] Whether this injury is caused directly by the SARS-CoV-2 virus or a result of heightened immune responses to danger signals from the cardiac system remains to be understood. Since ACE2 and TMPRSS2 are widely expressed throughout the cardiac tissue and if the virus makes its way to the cardiac tissue, the virus could potentially infect and cause injury in the tissue. The initial release of troponin for many patients can foreshadow a poor prognosis, conferring a five times higher risk of requiring ventilation.[149] A few autopsy reports have found viral RNA and mild inflammation in cardiac tissue of deceased patients.[150,151] Further investigation is required to understand whether there are long-term consequences of cardiac damage particularly in those who recovered from severe disease.

4.4 | Pathology in renal, hepatic, reproductive, and neural tissues

ACE2 is expressed throughout the renal tissue, namely in the apical brush border and podocytes and SARS-CoV-2 is capable of infecting renal tubular epithelium and podocytes. Infection of these cells has been associated with acute kidney injury (AKI) and proteinuria.[152] The incidence of AKI in different COVID-19 clusters ranges from 0.9% to 29%. The CD147 receptor is also highly expressed in renal tissue, likely playing a role in other renal diseases.[152] Although incidence levels of AKI are lower than other pathologies, renal injury in diabetic patients or others with pre-existing conditions can result in worsened health outcomes associated with COVID-19.

Likewise, hepatic endothelial cells also express ACE2 and cell culture work with SARS-CoV demonstrated that SARS-CoV-specific protein 7a was capable of inducing apoptosis in liver cell lines through caspase-dependent pathways.[153] Whether SARS-CoV-2 can induce apoptosis in vivo and via a similar mechanism is unclear at the moment. The incidence of liver injury ranged from 14.8% to 53% in COVID-19 patients with higher incidence in more severe cases. However, it remains to be understood if this is due to virus/immune-mediated pathology or due to drugs utilized in treatment against the virus.[153] Although specifics of this interaction are largely unreported at the time of writing, it is worth mentioning that ACE2 is also highly expressed in the bile duct tissue, higher levels than in hepatic tissue, and the bile duct plays a vital role in the immune response and liver regeneration.[153] More studies are needed in these areas.

Similar to renal and hepatic tissues, the reproductive tissue in both males and females has been shown to exhibit ACE2 expression. The adult reproductive tissues, spermatogonia, Leydig, and Sertoli cells, enriched for ACE2 receptor expression, are potential targets for SARS-CoV-2 infection.[154] While a few studies have reported on testes-related clinical manifestations of COVID-19, the data on gonadal tissue-associated pathophysiology remains scarce.[155-157] Sex hormone imbalance in the serum has been reported in severe COVID-19 patients.[158] Notably, SARS convalescent male patients exhibited orchitis as a complication of infection along with decreased spermatogenesis.[159] Another growing concern is about the possibility of sexual transmission of COVID-19. While the data regarding whether SARS-CoV-2 is present in semen of infected or recovering men remains conflicting, most of the studies suggest that it is highly unlikely that the virus is sexually transmitted.[160] Indeed, three studies that examined the sexual transmission of COVID-19 in women by examining lower genital tract via vaginal swab for the presence of SARS-CoV-2 at the time of admission or during the period of hospitalization have reported negative results.[161-163] Similar results were reported with vaginal swabs and breast milk from pregnant women.[164] However, like the male reproductive tissue, ACE2 receptor is expressed in the placenta, ovaries, uterus and vagina, raising a concern about vertical transmission of the virus.[165,166] A systematic review of 38 cohort studies found an infection rate of 3.25% neonates from COVID-19-infected pregnant women.[167]

In comparison, there have been many reports of COVID-19 patients presenting with neurological abnormalities. ACE2 is primarily expressed in neurons and glial cells of the brain.[168] Similar to SARS-CoV and MERS-CoV, SARS-CoV-2 may also take a direct transsynaptic route via the olfactory bulb following inhalation without the use of ACE2, which may be one of the reasons for loss of smell. Following invasion into neural tissue, the virus can cause reactive astrogliosis and activation of microglia and concurrently, systemic inflammation can compromise the blood-brain barrier leading to a disturbance in homeostasis and neuronal cell death.[168] Moreover, some case studies suggest the possibility of direct infection of the CNS tissue based on the presence of SARS-CoV-2 RNA in the cerebrospinal fluid.[169] Furthermore, systemic hyperinflammatory responses in severely ill patients could also contribute to some of the neurological manifestations.[170] These events can ultimately lead to the development of acute encephalitis, infectious toxic encephalopathy, or acute cerebrovascular attacks. Thus, as seen in the lung it is likely that if some of the neurological pathologies persist in COVID-19-recovered patients, they could be the basis of lingering neurological symptoms such as the "brain fog" experienced by some of these patients. Continuing investigation will be critical to developing clinical management strategies for treating COVID-19 patients with neurological symptoms in stages of disease and recovery.

5 | VACCINE DEVELOPMENT

5.1 | Lessons learned from SARS and MERS vaccines

Vaccine candidates attempted for SARS and MERS included RNA-, DNA-, recombinant protein-, viral vector-, live attenuated virus-, and inactivated virus-based platforms which are also currently being used for developing COVID-19 vaccines.[171,172] The development of vaccines for SARS and MERS did not go beyond phase 1 clinical trials due to quickly diminished demand.[171,173,174] Mice vaccinated with a SARS inactivated viral vaccine produced high amounts of neutralizing antibodies (NAb) against S, N, and M proteins.[175,176] In a phase 1 clinical trial, subjects given two doses of an inactivated SARS vaccine developed SARS-CoV-specific NAb and the vaccine was well-tolerated.[173] Mass production of inactivated viral vaccines requires large amounts of virus to be properly inactivated to ascertain safety.[171,177]

A study examined four candidate SARS vaccines including a virus-like particle-, two whole virus-, and a recombinant DNA S protein-based vaccines, with or without alum, in animal models.[178] While all four vaccines-induced NAb and provided protection against SARS-CoV infection, they also elicited Th2 immunopathology.[178] Recombinant adenovirus (Ad) vector is popular for its safety, well-characterized genome, and suitability for mucosal administration.[177] Ad-vectored SARS vaccine delivered via the intramuscular route-induced T cell responses against the N protein and NAb against the S1 in rhesus macaques and mice.[179,180] Although the intramuscular route produced higher levels of NAb in sera, the intranasal route-induced SARS-specific IgA antibodies and significantly reduced SARS-CoV replication in the lungs, suggesting the benefit of mucosal immunity in protection against SARS-CoV infection.[181] Although recombinant viral-vectored vaccines can induce humoral and cellular immunity, their potency following the first or repeated immunization can be affected by pre-existing immunity against the viral backbone[125] and their global production capacity may be limited.[171]

Both recombinant DNA- and RNA-based vaccines do not involve infectious viruses; their safety and simplicity makes them an attractive alternative to live vaccines.[171,177] A DNA vaccine expressing the S protein was shown to induce T cells and NAb and protective immunity in mice.[182] Similar results were seen in a phase 1 clinical trial where three doses of a plasmid DNA vaccine encoding S protein-induced NAb and $CD4^+$ T cell responses.[174] However, while most DNA vaccines showed promising results in preclinical models, few made their way into clinical trials due to their weak immunogenicity and requirement for more than two repeated injections.[177]

A few studies assessed immunogenicity and protective efficacy of a live attenuated recombinant SARS-CoV lacking the E gene in mice and hamsters.[183-185] This live attenuated SARS viral vaccine was shown to have reduced ability to replicate in experimental animals.[184,185] Hamsters immunized with this vaccine produced high levels of NAb and were protected from SARS-CoV infection in the upper and lower respiratory tracts.[183] These studies suggest that certain viral structural proteins such as E may be depleted to develop safe and live attenuated SARS-CoV-2 vaccines.

5.2 | Current vaccine candidates and strategies for COVID-19

Since COVID-19 is new to mankind, it is imperative to develop different vaccine platforms and strategies in parallel. Currently, there have been more than 200 vaccine candidates in pre-clinical and clinical development around the world.[3,125] Recently, there have been very encouraging results from ongoing phase III COVID-19 vaccine trials, indicating high protective efficacies by two mRNA vaccines (Moderna & Pfizer) and one chimpanzee adenoviral-vectored vaccine (AstraZeneca) and leading to their emergency use authorizations in a number of countries (Figure 1). At the time of writing, these front-runner vaccines are rolling out and administered to prioritized human populations in these countries while their long-term safety and efficacy data still remain to be obtained. However, as predicted,[125] due to limited supplies, and different economic status and geopolitical policies in various regions and countries, the type of vaccines, vaccine distribution, and vaccination roll-out time have been highly uneven and heterogenous. This situation may significant delay the effective global control of the pandemic. Furthermore, with recent emergence of highly transmissible virus variants (Figure 1), the question remains whether the currently approved first-generation vaccines will remain as efficacious.

The six main COVID-19 vaccine platforms include live attenuated virus, recombinant viral-vectored vaccines, inactivated or killed virus, protein subunit vaccines, virus-like particles, and nucleic-based (DNA or mRNA) vaccines. The main immunological property of each vaccine platform is thoroughly discussed in a separate review and will not be discussed in detail.[125] Vaccine development against most acute viral infections focuses on mimicking the immune response elicited during natural infection.[186] Due to a high degree of similarities in the genome, structural proteins and surface receptors between SARS-CoV and SARS-CoV-2 the immune responses should be comparable and can, therefore, aid in finding immunogenic determinants for vaccine development.[172]

Studies have demonstrated that during SARS-CoV-2 infection, $CD4^{++}$ and $CD8^{++}$ T cell responses were not only directed to the S protein but also to M and N proteins.[186] This

indicates that $CD4^{++}$ and $CD8^{++}$ T cells have many potential targets and the next generation of COVID-19 vaccine candidates should include additional structural antigens to provide a broader immune protective response.[125] This consideration is of particular relevance to the possibility of diminished efficacy of current vaccines against new SARS-CoV-2 variants. Considerable apprehension also exists regarding potential candidate vaccines causing ADE or Th2 immunopathology.[187] In previous SARS vaccine studies, young and aged mice exhibited Th2 immunopathology, with predominant eosinophil infiltration, and were not protected against rechallenge.[178,187] Animal studies and clinical trials of candidate vaccines for COVID-19 must be properly assessed for potential immune complications.

Several companies have been developing vaccines that express the SARS-CoV-2 S protein and aim to induce both antibody-mediated humoral and T cell responses.[188] All platforms have unique advantages and disadvantages making it tough to predict which strategy will ultimately be most successful in terms of long-term safety and protective efficacy although the current information favors the mRNA vaccine platform. Immunogenicity and safety of a DNA vaccine targeting the SARS-CoV-2 S protein was tested in different animal models and produced robust T cell responses and neutralizing antibodies blocking the binding of S to ACE2.[189,190] The DNA vaccine provided optimal protection with expression of the full-length S immunogen and significantly reduced viral load in upper and lower respiratory tracts of non-human primates.[190] Inovio Pharmaceuticals recently started a phase 1 clinical trial to evaluate the DNA vaccine, INO-4800 via two intradermal injections. Inovio announced positive interim data regarding the INO-4800 vaccine and was deemed safe and well-tolerated in participants, a phase 2/3 efficacy study is underway.[191] A purified inactivated virus vaccine candidate, PiCoVacc, protected non-human primates against SARS-CoV-2 infection and no ADE was observed. The safety of this vaccine was evaluated; no clinical signs of illness were observed; no notable changes in pro-inflammatory cytokines and no pathology.[192] Sinovac Research and Development Co. started phase 1/2 clinical trials testing the candidate vaccine CoronaVac, an inactivated vaccine, in April 2020 and preliminary results of the COVID-19 vaccine showed good safety and immunogenicity.[193] The phase 3 trial has recently commenced to test the efficacy and safety of CoronaVac.

A few dozen vaccine candidates have moved or will soon progress into clinical development to evaluate safety, immunogenicity, and efficacy in humans.[125] Meissa Vaccines has started phase 1 clinical trials to evaluate safety and immunogenicity of intranasal delivery of a live attenuated respiratory syncytial virus (RSV) vaccine in healthy adults.[194] The MV-014-210 vaccine expresses the SARS-CoV-2 S protein and is adjuvant free.[194] Many countries in Europe, Australia, and the United States have also begun clinical trials to evaluate the efficacy of intradermal BCG vaccination in health care workers and the elderly.[195] CanSino Biologics developed a recombinant adenovirus type-5 (Ad5) vectored coronavirus vaccine, expressing the SARS-CoV-2 S protein, via intramuscular injection. The Ad5-vectored COVID-19 vaccine is tolerable and immunogenic and induces humoral responses (28 days post-vaccination) as well as T cell responses (14 days post-vaccination) in healthy adults.[196] In the phase 2 trial, Ad5-vectored COVID-19 vaccine was safe and induced significant immune responses following a single immunization.[197] Now that a vaccine dose has been determined, ongoing phase 3 trials will evaluate the efficacy of the vaccine.[197] It is important to note that pre-existing Ad5 immunity may slow down rapid immune responses and negatively affect the quality of protective immunity.

AstraZeneca/University of Oxford developed a chimpanzee adenovirus (ChAdOx1)-vectored vaccine, encoding the full-length S protein, referred to as AZD1222. The ChAdOx1 nCoV-19 vaccine-induced humoral and cellular immune responses and reduced viral load in the respiratory tract of vaccinated animals.[198] The immune response was not Th2 dominated and no signs of disease enhancement were observed in lungs of non-human primates.[198] Preliminary data from a phase 1/2 trial revealed that ChAdOx1 nCoV-19 is safe and homologous boosting increased antibody responses and induced cellular immune responses.[199] Following two repeated intramuscular injections, the vaccine is recently announced to be 70%-90% effective based on its ongoing phase III trial (Figure 1). Moderna started clinical testing of intramuscular injection of mRNA-1273, which is a novel lipid nanoparticle encapsulated mRNA-based vaccine encoding full-length S.[200] The study evaluated safety and reactogenicity of mRNA-1273 and immunogenicity measured by IgG levels to the SARS-CoV-2 S protein. Moderna reported positive interim clinical data of the mRNA-1273 vaccine from the phase 1 trial.[200] The vaccine-induced seroconversion by day 15 and elicited neutralizing antibodies in all eight participants. In addition, the vaccine was safe, well-tolerated, and elicited an immune response of the same magnitude of natural infection. The encouraging results supported its phase 3 trial beginning in July 2020. In November 2020, Pfizer/BioNTech announced their mRNA-based COVID-19 vaccine candidate, BNT162b2, met all of the study's primary efficacy endpoints, with a efficacy rate of 95%.[201] Shortly after, Moderna announced their mRNA-1273 vaccine has met statistical criteria with a 94.5% vaccine efficacy (Figure 1).[202] At the time of writing this review, while the Pfizer/BioNTech vaccine has been authorized for emergency use in the United Kingdom, Bahrain, Canada, United States, Mexico, and European Union,[203-205] United States and Canada have also approved the Moderna vaccine for emergency use.[206,207] Furthermore, as of December 30th, 2020 the United Kingdom became the first country to provide emergency authorization for the

use of the AstraZeneca ChAdOx1 vaccine[208] (Figure 1). Novavax has developed a recombinant SARS-CoV-2 S subunit vaccine (NVX-CoV2373) that is constructed from full-length S. In August 2020, the company announced positive data for the phase 1 portion of the phase 1/2 trial indicating that the NVX-CoV2373 vaccine was well-tolerated and safe.[209] It also induced strong neutralizing titers in all participants and the Matrix-M adjuvant-induced robust $CD4^{++}$ T cell responses.[209] In September 2020, the company announced it has initiated its first phase 3 trial in the United Kingdom to evaluate the efficacy, safety, and immunogenicity of NVX-CoV2373. In addition to Pfizer/BioNTech and Moderna mRNA vaccines, an inactivated vaccine developed by Chinese state-owned Sinopharm has been approved by United Arab Emirates (UAE) and Bahrain.[210] The approval was based on Sinopharm announcement stating that the two-dose regimen was 86% effective which included trials in 31 000 people in the UAE and 7700 in Bahrain. However, neither of these involved entities released detailed phase III data used to calculate the effectiveness. Sinopharm's vaccine is also undergoing phase III trials in other countries including Egypt, Jordan, and Argentina. Notably, a double-blind, randomized, placebo-controlled phase 1/2 trial designed by the Wuhan Institute of Biological Products Co Ltd and Henan Provincial Center for Disease Control and Prevention has reported that vaccine was well-tolerated at varying doses with the most common adverse reaction being pain at the injection site in only 15% of subjects.[211] The vaccine effectively induced antibody responses but the longer interval between first and second injections produced stronger antibody responses. Of importance, according to Chinese state media more than 100 countries have pre-ordered Sinopharm vaccine despite the data gap, as this vaccine formulation is appealing over mRNA vaccines in their multi-antigenicity, storage conditions, and availability.

5.3 | Other considerations for COVID-19 vaccine development

5.3.1 | Animal models

Preclinical evaluation of vaccine candidates for COVID-19 requires the use of relevant animal models. The safety, immunogenicity and protective efficacy of the vaccine is first evaluated and established in animal models before moving to clinical trials. However, due to the pandemic, the preclinical and clinical stages of COVID-19 vaccine development are condensed and move forwards in parallel.[125] An animal model should mimic infection seen in humans and requires the pathogen to infect the animal using the same receptor as human host cells and to be able to effectively replicate and cause similar disease.[171] Testing the disease in certain animal models can be challenging. For instance, wild-type mice are not infected by SARS-CoV-2 since its ACE2 does not effectively bind the S1 subunit of the S protein and transgenic mice that express human ACE2 only display mild infection.[171] Other animal models have been utilized to study pathogenicity of the virus including hamsters, ferrets, and non-human primates.[171] Syrian hamsters are a good model to study pathogenesis, vaccination strategies, and antiviral treatment for COVID-19 since they are readily available, small and resemble the clinical and pathological characteristics seen in human SARS-CoV-2 infection.[212] SARS-CoV-2 effectively replicates in the lungs of Syrian hamsters, causing pathological lesions in the lungs, making this model attractive for understanding pathogenesis of lung injury.[213] Syrian hamsters are also able to transmit the disease to naïve hamsters through close contact following SARS-CoV-2 infection.[212] This animal model would allow for the rapid evaluation of vaccines at a low cost in comparison to other animal models including ferrets and non-human primates.[213] While ferrets are highly susceptible to SARS-CoV-2 infection, viral RNA, and infectious virus has only been found within the upper respiratory tract and is undetectable in other organs.[214,215] SARS-CoV-2 can efficiently replicate in the upper respiratory tract and effectively transmit to naïve ferrets through direct contact, suggesting they are a good model for evaluating the efficacy of COVID-19 vaccines in hindering transmission.[214,215] Macaques are permissive to SARS-CoV-2 infection, shed virus, and display COVID-19 like symptoms.[216] SARS-CoV-2 was able to efficiently replicate in the upper respiratory tract including the nasal cavity, bronchi, bronchioles, and alveoli and lower respiratory tract of macaques.[137,216] In addition, SARS-CoV-2 infection provided protection against reinfection in macaques, thus making them a promising animal model to test preventative and therapeutic strategies for COVID-19.[171]

5.3.2 | Route of vaccination

Although the selection of vaccine antigens and appropriate animal models is important, the route of vaccination is another key consideration of vaccine design. This is especially important for mucosal pathogens including SARS-CoV-2 as optimal protection requires the induction of both humoral and cellular immunity.[125] Since SARS-CoV-2 is a respiratory disease, inducing memory responses in the respiratory tract through the intranasal route or inhaled aerosol would be beneficial[217] (Figure 3). The two main vaccination routes include parenteral and mucosal and induce different immune responses. Parenteral vaccination induces IgG antibodies within the respiratory mucosa but is unable to induce mucosal IgA antibodies or T_{RM} cells in the lung.[125] In contrast, respiratory mucosal vaccination is proficient at inducing mucosal antibodies, T_{RM} cells and macrophage-mediated

trained innate immunity[125] (Figure 3). During SARS-CoV and SARS-CoV-2 infection, memory $CD4^{++}$ T cells in the respiratory tract play a vital role in providing protection.[218] Inducing antibody and cellular responses in the respiratory tract through intranasal vaccination is essential for protecting against SARS-CoV and MERS-CoV.[181,218] However, there are some challenges with mucosal vaccination including the need for safe/effective mucosal adjuvant and vaccine platform and the resources associated with the delivery method.[125,219]

Based on available information, there are at least half dozen COVID-19 vaccine candidates designed for mucosal route of administration including a DNA-based vaccine developed at the University of Waterloo that can be given via the nasal route, a vaccine by Intravacc that contains a Newcastle disease virus (NDV) vector expressing the SARS-CoV-2 S protein, an intranasal vaccine AdCOVID developed by Altimmune, and the CoroFlu nasal vaccine developed by FluGen, University of Wisconsin-Madison and Bharat Biotech.[219] More recently, MediciNova has begun to develop a recombinant parainfluenza viral-vectored intranasal COVID-19 vaccine.[220] BlueWillow Biolgics has been developing an oil-in-water nanoemulsion-adjuvanted spike protein subunit vaccine for intranasal delivery.[221]

Mucosal vaccinations for SARS-CoV produced significantly higher levels of secretory IgA in the lung and neutralizing antibodies.[222,223] When comparing mucosal to systemic routes of administration of a recombinant adeno-associated virus encoding the RBD of SARS-CoV S protein, intranasal vaccination induced a systemic humoral response of similar strength and shorter duration than intramuscular vaccination but a much stronger local humoral response.[224] The intranasal vaccination induced stronger cytotoxic T cell responses but provided comparable protection against SARS-CoV infection compared to intramuscular vaccination.[224] The near-sterilizing protective immunity was seen in a murine model of SARS-CoV-2 only by single intranasal, not by repeated intramuscular, route of immunization with a ChAd-vectored COVID-19 vaccine expressing the S protein.[225] Furthermore, in a hamster model, a single intranasal or intramuscular immunization with the ChAd-vectored COVID-19 vaccine protected Golden Syrian hamsters from developing pneumonia.[226] Intranasal immunization provided higher antibody titers in respiratory passageways and provided a greater degree of protection compared to intramuscular immunization, again speaks to the capacity of local mucosal immunization to provide better protection than traditional systemic routes.[125,226] Thus, based on the great potential of ChAd platform, the Imperial College London is conducting clinical trials to evaluate the safety and effectiveness of two COVID-19 vaccines, Oxford's ChAdox1 nCoV-19 and Imperial's ssRNA vaccine platform.[227] The two vaccine candidates will be delivered directly to the respiratory tract by inhalation through the mouth via a nebulizer and three doses will be assessed.[227] Furthermore, Beijing Wantai Biological Pharmacy Enterprise with Xiamen University and Hong Kong University will soon begin a phase 1 clinical trial to evaluate a duo-flu & COVID-19 vaccine via intranasal spray.[228] It is thus anticipated that the next couple of years may see further increased knowledge in the feasibility, safety and immune potency of mucosal COVID-19 vaccine strategies from both preclinical and clinical studies.

5.3.3 | Trained innate immunity in vaccine design

It has been long thought that only the adaptive immune system exhibits memory. However extensive evidence suggests that innate immune cells (monocytes, macrophages etc) also exhibit memory-like characteristics.[229] Various microbial components including *C albicans, β-glucan,* and live attenuated vaccines such as BCG, measles and smallpox provide non-specific protection against unrelated pathogens.[109,229,230] This process of heterologous protection is mediated by innate immune memory cells and has now been termed trained innate immunity (TII). Innate immune memory represents a reset state following exposure to an antigen or microbe, leading to an altered responsiveness to the same or unrelated antigen or microbe.[109] Innate immune cells of TII exhibit key hallmarks of a pro-defense signature, increased cytokine responses, epigenetic and metabolic reprograming, and enhanced protection to secondary infections.[109,231,232]

Bacillus Calmette-Guerin (BCG) has been and is currently being studied for its non-specific protective effects against unrelated pathogens and induction of TII.[233,234] In a randomized controlled trial BCG vaccination at birth in low-birthweight infants lowered mortality caused by neonatal sepsis, respiratory infection, and fever.[235] Circulating monocytes isolated from healthy volunteers vaccinated with BCG showed an increase in IFN-γ, TNF-α, and IL-1β in response to unrelated bacterial infection, persisting for up to 3 months.[236,237] Furthermore, BCG vaccination protected SCID mice, which lack functional T and B cells, against lethal *C albicans* infection further supporting the importance of innate immune cells in mediating protection.[237] Various research groups are postulating that BCG vaccination may help to prevent SARS-CoV-2 infection and/or reduce disease severity.[234] Phase 1 clinical trials have begun in numerous countries to evaluate the efficacy of BCG vaccination in preventing COVID-19 by strengthening the innate immune system. Another clinical trial is evaluating the effectiveness of the measles vaccine in decreasing the incidence of SARS-CoV-2 infection.

With this new knowledge on innate immune memory and TII, vaccine strategies for COVID-19 should aim to target both innate and adaptive immune systems in the

respiratory tract to best control COVID-19 in early stages of SARS-CoV-2 infection[109,125] (Figure 3). In doing so, besides protection from COVID-19, vaccine-induced TII may also offer non-specific protection against other respiratory pathogens.[109]

6 | CONCLUSIONS

The world is in urgent need of a safe and effective COVID-19 vaccine. To date globally SARS-CoV-2 has infected more than 100 million people and has caused over 2.15 million deaths and counting. Many of the specifics about this virus and COVID-19 still remain incompletely understood including its high transmissibility, asymptomatic state, disparate susceptibility between the young and elderly, and protective immune correlates. Therefore, it is imperative for us to continue to expand our knowledge on all of these fronts. Such knowledge is critical to developing further improved COVID-19 vaccine strategies beyond the first-generation vaccines developed according to a compressed pandemic vaccine scheme.[125]

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

R. Singh, A. Kang, and X. Luo with Z. Xing developed the manuscript. R. Singh, A. Kang, and X. Luo wrote and finalized the manuscript and with S. Afkhami and M. Jeyanathan, constructed the figures. M. Jeyanathan, A. Gillgrass, S. Afkhami, and Z. Xing edited the entire manuscript.

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