Animal Models of COVID-19 II. Comparative Immunology

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Abstract

Developing strong animal models is essential for furthering our understanding of how the immune system functions in response to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection. The alarming speed at which SARS-CoV-2 has spread, and the high mortality rate of severe Coronavirus Disease 2019 (COVID-19), has required both basic science and clinical research to move at an unprecedented pace. Models previously developed to study the immune response against SARS-CoV have been rapidly deployed to now study SARS-CoV-2. To date, both small and large animal models are remarkably consistent when infected with SARS-CoV-2; however, certain models have proven more useful when answering specific immunological questions than others. Small animal models, such as Syrian hamsters, ferrets, and mice carrying the hACE2 transgene, appear to reliably recapitulate the initial cytokine surge seen in COVID-19 as well as show significant innate and adaptive cell infiltration into the lung early in infection. Additionally, these models develop strong antibody responses to the virus, are protected from reinfection, and genetically modified versions exist that can be used to ask specific immunological questions. Large animal models such as rhesus and cynomologus macaques and African green monkeys are critical to understanding how the immune system responds to SARS-CoV-2 infection because they are considered to be the most similar to humans. These models are considered the gold standard for assessing vaccine efficacy and protection, and recapitulate the initial cytokine surge, immune cell infiltration into the lung, certain aspects of thrombosis, and the antibody and T-cell response to the virus. In this review, we discuss both small and large animal model studies previously used in SARS-CoV-2 research that may be useful in elucidating the immunological contributions to hallmark syndromes observed with COVID-19.

Key words: animal models, immunology, SARS-CoV-2, COVID-19

INTRODUCTION

This is the second installment of a 2-part review that focuses on which animal models best recapitulate Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection and Coronavirus Disease 2019 (COVID-19) disease. The first installment focuses on the virology and pathogenesis of SARS-CoV-2, and this installment focuses on the immune response to SARS-CoV-2. Since the beginning of the COVID-19 pandemic, there have been many reports linking the virus to various hallmark disorders such as acute respiratory distress syndrome, cytokine release syndrome, lymphopenia, thrombocytopenia, and central nervous system (CNS) manifestations. It is likely that these symptoms are driven by dysregulation of the immune system, and to understand what is occurring in patients we need animal models that can carefully recapitulate the effects of the virus and the immune response. In this review, we will discuss the most prominent immunological characteristics of COVID-19 in humans and how animal models have been used thus far to address immunological questions regarding SARS-CoV-2 infection and COVID-19 disease as well as how animal models...
have been used to help develop successful vaccine strategies and therapeutic treatments. In an effort to facilitate a clear understanding of the complexities of SARS-CoV-2 animal research, we have included Table 1 with a summary of the immunological characteristics for each animal model and Table 2 with the definitions and functions of all immunological proteins mentioned in this review. It should be noted that due to the need to facilitate rapid sharing of scientific studies throughout the COVID-19 pandemic, a large proportion of the studies cited in this review have not been qualified though normal peer review methods. Therefore, it is essential post publication of this review to revisit the citations and confirm that the information presented here has been further validated through the proper channels.

**INNATE IMMUNE SYSTEM**

The innate arm of the immune system serves as the first line of defense against invading pathogens. Today, our understanding of the specific innate immune responses to SARS-CoV-2 is extremely limited. However, because SARS-CoV-2 is an enveloped RNA virus, we must assume that the response of the immune system would be similar to how it responds to other RNA viruses. Presumably the innate system would respond after recognition of pathogen-associated molecular patterns by pattern recognition receptors. Recognition of the pathogen would lead to type-I and type-III interferon (IFN) responses, upregulation of interferon stimulated genes (ISGs), and the release of cytokines and chemokines to combat the infection. Previous studies on SARS-CoV, Middle East Respiratory Syndrome (MERS), and other coronaviruses have elucidated how coronaviruses can evade the innate immune system by inhibiting MAVs, TBK1, and NfkB signaling. In vitro or in vivo work has yet to confirm if SARS-CoV-2 evades the innate system in a similar fashion; however, proteomic studies suggest that some SARS-CoV-2 proteins, which are homologous to SARS proteins, are able to evade the IFN response in a similar manner. The details of how coronaviruses evade downstream innate immune pathways are discussed in detail in other published reviews, and will not be a focus of this review. In this review, we will focus on clinical outcomes that involve the dysregulation of the immune system and animal models that best recapitulate those outcomes.

**Cytokine Storm**

Most individuals infected with SARS-CoV-2 clear the infection and the immune response recedes, allowing recovery. However, in some patients, a dysfunctional immune response occurs, which leads to a massive release of cytokines and widespread lung inflammation. Patients with severe COVID-19 demonstrate remarkably impaired IFN-I signatures compared with mild or moderate cases. Severe cases present signatures that show low IFN production and activity, with consequent downregulation of ISGs, suggesting that type I IFN deficiency in the blood may be a hallmark of severe COVID-19 and define a high-risk population. However, it has not been determined if the lack of an IFN response is due to the virus interfering with IFN downstream signaling or host-specific effects. Perhaps more importantly, the timing of IFN responses may be key, because IFN is known to be protective early in disease and later becomes pathologic. It is clear that the dysregulation of IFN signaling leads to an imbalance of proinflammatory cytokines. Patients with severe COVID-19 exhibit higher blood plasma levels of Interleukin(IL)-2, IL-7, IL-10, granulocyte colony stimulating factor (G-CSF), Interferon gamma-induced protein 10 (IP-10), Monocyte Chemoattractant Protein-1 (MCP-1), Macrophage inflammatory protein-1α (MIP1α), and Tumor necrosis factor. Significantly elevated systemic levels of the pro-inflammatory cytokine IL-6 have been reported in several COVID-19 patient cohorts and are shown to correlate with disease severity. Increased IL-6 has also been associated with higher levels of IL-2, IL-7, IFN-γ, and GM-CSF as seen in severe COVID-19 cases with secondary hemophagocytic lymphohistiocytosis. SARS studies have shown that non-structural and structural proteins can enhance inflammasome activation, leading to secretion of IL-1β and IL-18, which likely contribute to pathological inflammation in the absence of IFN. Although direct activation of the inflammasome by SARS-CoV-2 has not been reported, IL-18 and IL-1β are elevated during SARS-CoV-2 infection, suggesting inflammasome involvement. Similarly, some SARS-CoV-2 proteins may lead to the induction of IL-6 and IL-8 production by blocking the inhibition of NFκB. Collectively, these pro-inflammasome processes likely contribute to the “cytokine storm” observed in COVID-19 patients and suggest a role for targeted immunosuppressive treatment regimens that modulate these inflammatory responses.

Moving forward, a clear understanding of the delicate balance between antiviral and inflammatory innate immune programs will be essential to developing effective biomarkers and therapeutics for COVID-19. Animal data in general have not matched human data regarding IFN responses, suggesting that there is more to learn about the timing and degree of the IFN response required to prevent viral spread and avoid enhanced pathology. A recent study showed that pangolins, thought to be a possible intermediate host for SARS-CoV-2, lack IFIH1/MDA5 signaling entirely, leading to no disease when infected with coronaviruses. MDA5 binds to double-stranded RNA in the cytosol signaling through MAVS to activate expression of IFNs and induce inflammation. The authors hypothesized that this antiviral defense was harmful to the species and the loss of this signaling mechanism provided an evolutionary advantage by increasing tolerance to infections by certain RNA viruses. However, the hypothesis in this study conflicts with other human cohort studies that suggest a diminished IFN response leads to more inflammatory disease. It is likely that the timing and control of the IFN response, not necessarily the magnitude, is most important.

To date, no animal model has perfectly recapitulated the cytokine storm associated with severe COVID-19 disease. This is likely because the current animal models for COVID-19 are best suited for studying mild disease outcomes. However, studies with both small and large animal models have observed the initial cytokine surge associated with acute viral infections. Studies in Syrian golden hamsters, K18-ACE2 transgenic mice, African green monkeys (AGM), and rhesus macaques have all reported elevated levels of proinflammatory cytokines in serum within the first week of infection. Additionally, studies utilizing genetic knockouts to elucidate the role IFN signaling programs play in viral control of SARS-CoV-2 have been reported. However, it should be noted that IFN signaling overlaps substantially. Type I IFNs (IFN-α and β) signal through the IFN-α/β receptor (IFNAR), whereas type III IFNs (IFN-λ) signal through the IL28RA/IL10Rβ receptor leading to the formation of both a heterodimer of STAT1-STAT2 and a STAT1 homodimer, which then enter the nucleus and induce transcription of ISGs. Type II IFNs (IFN-γ) signal through the IFN-γ receptor leading to the formation of...
Table 1. Immunological Features of SARS-CoV-2 Infection in Animal Models of COVID-19

| Animal Model       | Innate Immunity                                                                 | Adaptive Immunity                                                                 | Vaccine Design/Therapeutic mAbs                                                                 |
|--------------------|---------------------------------------------------------------------------------|-----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|
| Rhesus macaque     | Observed initial cytokine surge within wk 1; infiltration of myeloid cells into lungs; mild neutropenia | Exposure results in protective B and T-cell responses by 21 DPI, protective against reinfection. The higher the inoculum, the stronger the adaptive immune response. T cell infiltrates into lungs; mild lymphopenia | DNA vaccine induced strong B- and T-cell responses that were protective against a high inoculum challenge, nAbs were on the order of what is observed in convalescent human serum; Adeno (ChAd)-vectored vaccine elicited a robust humoral and T-cell–mediated response and provided protection from subsequent challenge; purified inactivated virus vaccine provided protection from challenge. |
| Cynomolgus macaque | No studies to date                                                               | Seroconversion by 14 DPI, no studies to date on T-cell responses                   | Recombinant protein vaccine induced high nAbs titers, no challenge work to date; RNA vaccine induced high nAbs titers, no challenge work to date. |
| Pigtailed macaque  | No studies to date                                                               | No studies to date                                                                | No studies to date. |
| African green monkeys (AGM) | Observed initial cytokine surge within wk 1, mild thrombocytopenia, myeloid cell infiltration in lungs | Seroconverted by 5 DPI peaked 15 DPI with high titers of nAbs; no T-cell studies to date | No studies to date. |
| Ferret             | Observed innate cells infiltration in lung, associated with pathology; no virus detected in brain | Seroconversion of all animals by terminal time point, had varying titers of nAbs based on dose and inoculation route; no T-cell studies to date; protected from rechallenge | No studies to date. |
| Syrian hamster     | Observed initial cytokine surge within wk 1, attributed lung pathology to type I IFN responses, mononuclear cell infiltration in lungs of infected animals | Seroconversion of all animals, nAb detected by 14 DPI; no T-cell studies to date; no rechallenge studies to date | No vaccine studies to date; mAb and convalescent serum prophylactic studies showed protection. |
| Cat                | No studies to date                                                               | Seroconversion of all animals, nAb detected by 20 DPI; Abs/nAbs are found in domestic and stray cats without known exposure; no T-cell studies to date | No studies to date. |
| Dog                | No studies to date                                                               | <50% of animals inoculated seroconverted, no exposed animals seroconverted; no T-cell studies to date | No studies to date. |
| Pig                | No studies to date                                                               | No animals seroconverted; no T-cell studies to date | No studies to date. |
| Mouse adapted SARS-CoV-2 | Observed initial cytokine surge in aged mice; inflammatory cell infiltration into lungs of young and old mice; Type III IFN may play a protective and therapeutic role | Not assessed except in context of vaccination | Recombinant protein or virus replicon particle vaccination lead to protection on challenge; strong nAbs developed in response to vaccine; A mAb prophylactic study showed protection; T cells not assessed. |
| Knockin-CRISPR/Cas9—hACE2 mouse | Observed initial cytokine surge in aged mice; inflammatory cell infiltration into lungs of young and old mice, neutrophils and macrophages; SARS-CoV-2+ macrophages in lung; viral RNA detected in brain | No studies to date | No studies to date. |
| Animal Model | Innate Immunity | Adaptive Immunity | Vaccine Design/Therapeutic mAbs |
|--------------|----------------|------------------|-------------------------------|
| hACE2 transgenic mouse (driven by mouse promoter) | Inflammatory cell infiltration into alveolar interstitium and alveolar spaces | All inoculated animals seroconverted, 50% of exposed cage mates seroconverted; no T-cell studies to date | No vaccine studies to date; A mAb prophylactic study showed protection |
| HFH4-hACE2 transgenic mouse | Observed monocyte and lymphocyte infiltration into lung; viral RNA detected in brain; cytokines not assessed | Animals that survived long term seroconverted and were protected from reinfection | No studies to date |
| K18-hACE2 transgenic mouse | Observe initial cytokine surge in blood and tissues 2–4 DPI; early recruitment of alveolar macrophages, monocytes, and neutrophils in BAL, viral RNA detected in brain, possibly due to aggressiveness of model | No studies to date | No studies to date |
| Adeno-associated virus 9 (AdV9)—hACE2 induced mouse | Increase in activated monocytes, macrophages, neutrophils, and NK cells in lung; have similar IFN signatures to COVID patients; Type I IFN drive pathogenic response | Seroconversion of all animals, nAbs detected by 7 DPI; increase in activated T cells in lung | No studies to date |
| Adeno-associated virus 9 (AdV9)—hACE2 induced + IFNAR blockade mouse | Observed immune cell infiltrates into lung; increase in proinflammatory cytokines in lung; Type 1 IFN signaling shown to be protective early in infection | No studies to date | No vaccine studies to date; A mAb prophylactic study showed protection |

wk = week; DPI = days post infection; IFN = interferon; nAb = neutralizing antibodies; mAb = monoclonal antibodies; NK = natural killer; BAL = Bronchoalveolar lavage.

Table 1. Continued
| Abbreviation | Full Name | Function in Relation to Viral Immunity* |
|--------------|-----------|----------------------------------------|
| MAVS | Mitochondrial Antiviral Signaling Protein | Required for innate immune defense against viruses, acts downstream of innate immune proteins that detect intracellular dsRNA produced during viral replication, leads to activation of NFκB, IRF3, and IRF7 and to subsequent induction of antiviral cytokines. |
| TBK1 | TANK Binding Kinase 1 | Serine/threonine kinase that plays an essential role in regulating inflammatory responses to viruses. Following activation of toll-like receptors by viral components, TBK1 associates with TRAF3 and TANK and phosphorylates IRF3 and IRF7. |
| NFκB | Nuclear Factor Kappa B | NFκB is a major transcription factor that regulates genes responsible for both the innate and adaptive immune response to viral infections. Activation of NFκB results in systemic inflammation. |
| IFIH1/MDA5 | Interferon Induced With Helicase C Domain 1/Melanoma Differentiation-Associated Protein 5 | Innate immune receptor that acts as a cytoplasmic sensor of viral nucleic acids and plays a major role in sensing viral infection and in the activation of a cascade of antiviral responses, including the induction of type I interferons and pro-inflammatory cytokines. |
| IRF3 and IRF7 | Interferon Regulatory Factor 3 and 7 | Key transcriptional regulators of type I IFN-dependent immune responses and plays a critical role in the innate immune response against DNA and RNA viruses. Regulates the transcription of type I IFN genes (IFN-alpha and IFN-beta) and ISGs by binding to an ISRE in their promoters. IRF3 acts as a more potent activator of the IFN-beta gene than the IFN-alpha gene, IRF7 can efficiently activate both the IFN-beta and the IFN-alpha genes. |
| STAT1 | Signal Transducer And Activator Of Transcription 1 | Signal transducer and activator of transcription that mediates signaling by type I, II, and III IFNs |
| STAT2 | Signal Transducer And Activator Of Transcription 2 | Signal transducer and activator of transcription that mediates signaling by type I IFNs and type III IFNs |
| IFNAR | Interferon Alpha/Beta Receptor Subunit 1 | Component of the receptor for type I interferons, a heterodimer with IFNAR2. Activation leads to downstream STAT proteins, ISGs, as well as type I IFN themselves. |
| IL28RA | IL-28 Receptor Subunit Alpha | The IL28RA/IL10RB dimer is a receptor for type III IFNs, IFN-lambda 2 and IFN-lambda 3 and mediates their antiviral activity. |
| IFNγR | Interferon gamma receptor | Heterodimer of IFNγR1 and IFNγR2, the receptor for type II IFN (IFNγ) |
| Type I IFNs | Type I Interferons | IFN-alpha (IFNα), IFN-beta (IFNβ) |
| Type III IFNs | Type III Interferons | IFN-lambda (IFNλ) |
| IL-2 | Interleukin 2 | Produced by T-cells in response to antigenic or mitogenic stimulation, required for T-cell proliferation and other activities crucial to regulation of the immune response. |
| IL-6 | Interleukin 6 | Produced by macrophages and endothelia cells in response to tissues damage and acts as a potent inducer of the acute immune response. Plays an essential role in the final differentiation of B-cells into Ab-secreting cells and involved in lymphocyte and monocyte differentiation. |
| IL-7 | Interleukin 7 | An important growth factor for T- and B-cell development and maturation. |
| IL-8 | Interleukin 8 | A chemotactic factor that attracts neutrophils, basophils, and T cells, but not monocytes. |
| IL-10 | Interleukin 10 | Major immune regulatory cytokine that has anti-inflammatory functions, limiting excessive tissue disruption caused by inflammation. Mainly produced by monocytes and to a lesser extent by lymphocytes. |
| IL-18 | Interleukin 18 | A proinflammatory cytokine primarily produced by macrophages as a result of inflammasome activation. |
| IL-1β | Interleukin 1 beta | A proinflammatory cytokine primarily produced by macrophages as a result of inflammasome activation. |
| IFNγ | Interferon gamma | Produced by lymphocytes activated by specific antigens or mitogens, has antiviral activity, and is a potent activator of macrophages. |
| G-CSF/CSF3 | Granulocyte Colony-Stimulating Factor | Granulocyte/macrophage colony-stimulating factors are cytokines that act in hematopoiesis by controlling the production, differentiation, and function of 2 related white cell populations of the blood, the granulocytes and the monocytes-macrophages. |

(Continued)
a STAT1 homodimer and transcription of ISGs.23 These signaling cascades clearly share common signaling proteins; therefore, it can be difficult to differentiate which pathway is the most prominent in response to viral infections. Boudewijns et al attempted to determine the importance of type I vs type III IFNs in genetically altered hamsters.24 The authors infected mice using an adenovirus (AdV5) encoding the core domain (RBD) of SARS-CoV or SARS-CoV-2. The authors then proceeded to block IFNAR signaling before and throughout infection and observed enhanced disease progression and a marked increase in immune cell infiltration into the lung when IFN signaling was absent.25 An additional study using AdV9-hACE2 WT mice infected with SARS-CoV-2 found gene signatures in the lung that focused on acute ISG responses, which clustered with type I IFNs. Additionally, when the animals introduced hACE2 into IFNAR-deficient and IRF 3/7-deficient genetic backgrounds, they observed that type I IFNs did not control viral replication but did drive pathologic responses. IFNAR-deficient animals result in a blockade of type I IFN signaling, but the IFN proteins would still be produced, whereas IRF 3/7-deficient animals would result in a blockade in the production of type I IFNs in response to viral infection. The authors observed mild increases in viral load measures in IFNAR-deficient animals but no differences when the animals were deficient in IRF 3/7 compared with WT. Interestingly, when IFN signaling was absent (IFNAR-deficient), there was a significant decrease in the number of activated myeloid cells and lymphocytes recruited to the lung. These data suggest that IFN is likely a major driver of both recruitment and activation of proinflammatory immune cells in SARS-CoV-2 infection, which may lead to the cytokine imbalance and pathological damage.27 Finally, very recent studies have linked the expression of ACE2 directly with IFN signaling.28,29 The first study showed that IFNAR-deficient mice have lower expression levels of ACE2 in epithelial cells compared with WT mice.28 The second showed that the ACE2 gene is an ISG, with exogenous IFN able to upregulate the gene in human epithelial cells.29 The data from these studies suggest that SARS-CoV-2 could exploit IFN-driven upregulation of ACE2 to enhance infection and disease severity. Collectively, these studies give evidence of the importance of IFN regulation in clearance of SARS-CoV-2 infection and pathology. However, more work is needed to decipher why some individuals experience severe disease and others do not.

### Dysregulation of Myeloid Cells

SARS-CoV-2 infection of lung cells triggers a local immune response that then recruits macrophages and monocytes to the site of injury. These cells respond to the infection by releasing cytokines that act on virally infected cells, surrounding cells,
as well as priming and recruiting the adaptive immune system. In most cases, this coordinated effort leads the resolution of the infection, but as we have seen with COVID-19, this is not always the case. Patients with severe disease show a more aggressive response by the innate system, which is likely due to monocytes and macrophages recruited to the lung. These patients also have a significantly higher percentage of pro-inflammatory monocytes (CD14+CD16+) in the peripheral blood compared with patients with mild disease.32–35 These cells are responsible for secreting inflammatory cytokines, such as MCP-1, IP-10, and MIP-1α, which may contribute to the cytokine imbalance observed in COVID-19. Unrestrained inflammatory cell infiltration results in damage to the lung through excessive secretion of proteases and reactive oxygen species in addition to the damage caused by the virus itself. Additionally, viral infection of monocytes and macrophages can result in abnormal cytokine production even if the viral infection is not productive.32–35 The ability of SARS-CoV-2 to infect other cell types, aside from epithelial cells in the lung, is not yet known. SARS is known to infect T cells,36 macrophages, and monocyte-derived dendritic cells,33–34 which results in cell death and excessive activation. SARS-CoV-2 has not been shown to productively infect macrophages, and given the lack of ACE2 expression on the cell surface it is likely that they are not targets of the virus.37 However, that does not mean the virus is unable to enter macrophages via other mechanisms and cause cellular distress. One study reported the presence of SARS-CoV-2 nucleocapsid protein in lymph node and spleen-associated macrophages in COVID-19 patients,38 suggesting that the virus may enter macrophages via another mechanism. A growing body of evidence points to dysregulation of myeloid responses as potential drivers of the COVID-19 hallmark syndromes such as acute respiratory distress syndrome and cytokine release syndrome.1

Understanding the precise drivers of myeloid immune dysfunction is critical to help guide the development and application of appropriate immunotherapies against SARS-CoV-2. Animal models will prove to be a valuable tool to decipher the involvement of myeloid cells because these models give access to tissues that are typically difficult to obtain in human studies. Though no animal model has fully mimicked human disease, there are several promising candidates that have observed significant myeloid cell dysregulation during SARS-CoV-2 infection. One study using hACE2 transgenic mice, driven by the mouse promoter, observed typical histopathology in the lung and significant infiltration of lymphocytes and monocytes into the alveolar interstitium along with an accumulation of macrophages within the alveolar spaces.39 Additionally, this study found viral antigens were present in lung epithelial cells and alveolar macrophages, suggesting that though these cells may not express ACE2, the virus is able to enter and may be effecting macrophage function. An additional study with AdV9-hACE2 mice, where hACE2 was specifically introduced into the lungs of WT mice, observed an acute inflammatory immune response in the lung characterized by infiltrating monocytes, macrophages, and neutrophils as well as activated T and NK cells.37 An additional study with K18-hACE2 transgenic mice showed a significant increase in recruitment of alveolar macrophages, monocytes, and neutrophils in the lung bronchial lavage fluid (BAL) early in infection.40 Two additional studies, one using the HFH4-hACE2 transgenic mouse and the other CRISPR/Cas9-hACE2 knock-in mice, both showed marked increases in monocyte and lymphocyte infiltration into the lung and the development of pneumonia in SARS-CoV-2-infected mice41,42 as well as detected virus positive macrophages within the lung.1 Other small animal models such as hamsters and ferrets have also shown pneumonia in the lungs, and the pathology has also been linked to infiltrating myeloid cells, neutrophils, and lymphocytes.39–43

Large animals models of COVID-19 have also observed myeloid cell infiltration into the lung during SARS-CoV-2 infection. Rhesus macaques infected with SARS-CoV-2 show signs of leukocytosis, neutrophilia, monocytosis, and lymphopenia between 1 and 3 days post infection (DPI).21 There is obvious macrophage infiltration into the lung, and uninfected F4/80+CD68+/CD206+ macrophages were frequently found next to infected epithelial cells, suggesting the macrophages may be engulfing infected cells. Two additional studies, 1 with aged and the other with young rhesus macaques, detected viral antigen within both alveolar epithelial cells and alveolar macrophages in the lung.44,45 Additionally, AGM studies have shown recruitment of monocytes to the lungs of infected animals within 5 DPI,46,47 and reported the detection of SARS-CoV-2 N protein in the cytoplasm of macrophages as well as lung epithelial cells.39 Genomic SARS-CoV-2 RNA has been detected by in situ hybridization in lung epithelial cells and associated with the alveolar macrophages within acute inflammation centered on terminal bronchioles.39 Increases of MCP-1 and IL-6 in blood have also been reported and correspond to subsequent recruitment of monocytes and neutrophils to the lung. An additional study in both rhesus and AGM specifically focused on the acute phase of infection and cellular trafficking to the lung.46 This study reported that the acute phase of infection was characterized by a rapid migration of CD16+ monocytes from the blood and simultaneous increase in CD16+ macrophages in the lungs. The CD16+ cell populations observed consisted of interstitial macrophages (HLADR+CD206−), a transitional population (CD11c+CD16+), that was directly associated with IL-6 levels in plasma, and 1 long-lasting population (CD11b+CD16+). Additionally, blood monocytes were a correlate of viral replication in bronchial brushes and levels of TARC (CCL17), a chemokine produced by myeloid cells that drives the recruitment of T cells. Finally, this study also reported that worse disease outcomes in both species were associated with high levels of cell infiltration and CD11b+CD16+ macrophage accumulation in the lungs. Most importantly, this study reported that the accumulation of myeloid cells in the lungs was long lasting and detectable in animals with mild or no signs of disease. These studies give evidence that myeloid cell function may be an important driver of COVID-19 disease, and further studies are needed to determine if this cellular response can be modulated.

Central Nervous System

In addition to issues observed in the periphery, there is growing evidence that SARS-CoV-2 also affects the CNS, which is heavily modulated by the innate immune system.48 Patients with COVID-19 experience a range of neurological symptoms that include loss of smell, delirium, cognitive impairment, and neuroinflammation.49 Other human coronaviruses are known to enter the CNS shortly after infection, leading to neuroinflammation, and it has been reported that SARS-CoV-2 is present in cerebrospinal fluid and brain parenchyma of some COVID-19 patients post mortem.10–52 To date, there have been several COVID-19 magnetic resonance imaging studies that suggest wide range of neurologic manifestations are observed in patients with severe COVID-19.53,54 Additionally, a subset of
Thrombosis

Though thrombosis is not specifically an immunological phenomenon, platelets, the cellular mediator of thrombosis, are innate immune cells and have been shown to impact inflammatory processes.\(^71\) There is growing evidence that platelets are involved in the acute phase of infection and contribute to the production of acute phase proteins. These proteins have been shown to inhibit further spread of infection by producing procoagulants and trapping pathogens within blood clots.\(^72\)

A hypercoagulable state has been described in a large number of COVID-19 cases.\(^73\)\(^-\)\(^75\) It has become standard practice to administer blood thinners on entry into the ICU in an effort to combat thrombotic complications that are related to increased mortality in COVID-19.\(^73\)\(^-\)\(^75\)\(^,\)\(^76\) Thrombocytopenia and increased levels of D-dimers have been proved as early predictors of outcome in critically ill COVID-19 patients.\(^73\)\(^,\)\(^77\) However, the mechanisms underlying COVID-19–associated hypercoagulability are still to be determined. It has been suggested that increased platelet activation and platelet-monocyte aggregates may play a critical role in severe COVID-19 patients,\(^73\) because these cells may initially localize to the lung,\(^73\) leading to endothelitis\(^60\) and other complications. Platelets are not only considered innate and inflammatory immune cells but have also been shown to assist in the adaptive immune response,\(^81\) and it is possible that increased platelet activation and aggregation will alter the immune response to SARS-CoV-2 infection.

Animal models will likely provide a helpful tool to study platelet activation and function during SARS-CoV-2 infection. Though few studies have been published thus far, there have been reports of unusual clotting activity in both small and large animal models of SARS-CoV-2. In K18-hACE2 transgenic mice, increased levels of hematocrit and plasma hemoglobin were observed as well as a modest increase in clotting time at 7 DPI.\(^73\) Additionally, an increase in D-dimer concentrations at 2 and 4 DPI was reported; however, blood clots were not observed in any of the extra-pulmonary organs examined.\(^66\) In an AGM study, following intratracheal and intranasal inoculation, fibrinogen levels surged in a subset of animals examined.\(^66\) In an AGM study, following intratracheal and intranasal inoculation, fibrinogen levels surged in a subset of animals examined.\(^66\)

To date, there are no large animal studies that have focused on SARS-CoV-2 effects on the CNS; however, there have been a few studies that have assessed whether viral RNA can be detected in the brain. One study with rhesus macaques was unable to detect viral RNA in the brain,\(^61\) whereas an additional study with AGM detected SARS-CoV-2 RNA in the frontal cortex and olfactory bulbs.\(^23\) The lack of consistent results in large animal models are rather consistent with the current conversation in the literature that either suggests there is no obvious CNS manifestation of SARS-CoV-2 humans\(^69\) or that the neurotropic ability of SARS-CoV-2 remains very controversial.\(^70\) More studies are needed in large animal models, where the brain and nerves are easily accessible to determine the effect of SARS-CoV-2 in the CNS and the role myeloid cells and cytokines may play in facilitating the clinical manifestations observed in the clinic.
ADAPTIVE IMMUNE SYSTEM

Unlike SARS and MERS, SARS-CoV-2 is likely to persist in society long term due to the ability of the virus to spread before symptoms emerge, and as we are observing in real time, it is unlikely simple public health measures alone will be able to contain the pandemic. Therefore, it is imperative to understand the development of the adaptive immune response and the development of immunological memory against SARS-CoV-2 infection. The adaptive response is composed of a virus-specific B-cell and T-cell response. The humoral immune response, composed of B cells that produce virus-specific antibodies, and T-cell response, composed of CD8+ and CD4+ T cells, are critical for clearance of viral infections and prevention of reinfection. CD8+ T cells are important for directly killing virally infected cells, whereas CD4+ T cells are needed to prime both CD8+ T cells and the B-cell response. CD4+ T cells also produce the cytokines needed to drive immune cell recruitment and differentiation at the site of infection. The adaptive immune response also develops immunologic memory. Immunologic memory, comprised of memory B cells, plasma B cells, and memory T cells, allows for a streamlined response by the immune system when presented with a pathogen a second time and rapid clearance of the infection.82 Understanding the development of the initial and memory adaptive immune response against SARS-CoV-2 infection is essential to defeat this pandemic.

B-Cell Immunity

SARS-CoV-2 has been shown to elicit a robust B-cell response, as evident by the rapid development of virus-specific IgM, IgG, and IgA neutralizing antibodies (nAbs) following infection. The kinetics of the antibody response to SARS-CoV-2 has been well described, seroconversion occurs in most COVID-19 patients between 7 and 14 days after the onset of symptoms, and antibody (Ab) titers remain elevated in the weeks following viral clearance.83–86 Abs binding to the internal N protein and external S glycoproteins are the most common SARS-CoV-2–specific Abs detected.81–86 Abs against the RBD, found within the S1 subunit of the virus spike protein, are thought to be the most important because they are able to neutralize viral entry preventing infection.81–83 The kinetics and binding domains reported for SARS-CoV-2 resemble those observed with SARS and MERS infections, and some cross-reactivity has been reported between SARS-CoV-2–specific Abs and SARS and MERS N and S proteins.83 Cross-reactivity of Abs against the RBD of SARS-CoV-2 and SARS has also been reported; however, Abs that are cross-reactive are thought to be very rare.83,84 A few monoclonal Abs derived from SARS infection have been shown to be able to neutralize SARS-CoV-2 infection using a pseudovirus system and large quantities of SARS-specific Abs.85 These data suggest that some SARS-specific Abs may work as therapeutics to neutralize the current pandemic virus; however, using SARS-CoV-2–specific Abs as a therapeutic would be preferable.

The B-cell response that develops during an infection serves not only to clear the initial infection but also to provide long-term immunity that protects against future exposure. Currently, there is a growing interest in determining the life span of memory B cells that recognize SARS-CoV-2. Protection from reinfection has direct medical and social consequences, because immunological protection will be essential in allowing the world to resume normal activities and likely the development of a successful vaccine. COVID-19 patients have shown evidence of near-universal seroconversion and few if any reinfections, which suggests a robust and effective antibody response. Case studies of patients who have recovered from COVID-19 have shown the presence of SARS-CoV-2–specific plasma cells and memory B cells circulating in blood post infection,92,96,97 which may wane as early as 8 weeks post infection.98 These data suggest that a B-cell response does exist and persists post clearance of the infection, but may not be long lasting. Additionally, convalescent plasma has been deployed as a potential therapeutic, emphasizing the effectiveness of the B-cell responses against SARS-CoV-2.99 However, 1 study has suggested that most convalescent plasmas obtained from individuals who recover from COVID-19 do not contain high levels of neutralizing activity and may not be protective.100 Protection may be linked to the level of SARS-CoV-2–specific IgG in the donor serum as shown in 1 study that found improved mortality rates based on time of administration and level of IgG in administered serum.101 Due to how recent this outbreak is, it is not possible to know how long these memory B-cell responses will remain in circulation and be protective. Work with other human coronaviruses can help to estimate the potency of these responses against reinfection over time. Infection with human CoV-229E induces specific IgG and nAbs rapidly, but responses wane approximately 1 year post infection and result in minimal protection against reinfection.102,103 The Abs response to SARS is also thought to be short lived, with specific IgG and nAb responses diminishing 2–3 years after infection and being nearly undetectable in 25% of individuals by 3 years.104,105 An additional study that followed SARS-infected healthcare workers over 13 years also found that virus-specific IgG declined after several years but could still be detected up to 12 years later. However, because SARS has not re-emerged, it is difficult to assess if these detectable B-cell responses would be protective against reinfection.106 In the case of MERS, antibodies were detected in 6 out of 7 volunteers 3 years after infection; while similar to SARS, it is unknown if these responses would be fully protective.107 Overall, these data demonstrate that virus-specific Ab responses against coronaviruses wane over time and may result in only partial protection against reinfection. More studies are needed to determine the degree of protection Ab immunity against SARS-CoV-2 will provide long term.

To better understand the development of protective B-cell responses against SARS-CoV-2 infection, we need reliable animal models to elucidate how immunologic memory develops and can be sustained. Studies using small animals such as hamsters, mice, ferrets, and cats have all observed the development of virus-specific IgG and nAbs after SARS-CoV-2 infection.108,109–110 Hamsters develop high titers of nAbs and virus-specific IgG by 14 DPI.109 The mouse promoter hACE2 transgenic and the AdV9-hACE2 mouse models develop virus-specific Abs and nAbs within 7 DPI, and in the case of hACE2 transgenic mice the titers of Abs that develop appear to be associated with the amount of inoculum.109,110 Animals directly inoculated via the intranasal route developed strong Ab titers compared with those that were exposed to infected cage-mates, respiratory droplets, and even aerosol inoculation; however, regardless of route of exposure, virus-specific Abs were developed, only differing in the strength of response.108 These data add insight into whether level of exposure can determine the degree of immunity developed and, if studied further, may shed light on whether...
asymptomatic cases offer protective immunity. Additionally, hamster and mouse studies have begun to assess if the administration of mAbs or convalescent serum known to neutralize SARS-CoV-2 will offer protection against infection. Two different studies, one that used the hACE2 transgenic mouse model and the other the hamster model, showed that the administration of 2 different exogenous mAbs, known to neutralize SARS-CoV-2, offered some protection against infection, with all animals showing better outcomes compared with controls. A third study showed that passive transfer of serum from previously infected and recovered hamsters inhibited viral replication in the lungs of naive hamsters; this same study also reported that hamsters were protected against subsequent challenge. These data suggest that the antibody response may be protective against reinfection and that the use of convalescent serum and or mAbs against SARS-CoV-2 as therapeutic treatments may help resolve infection.

Studies with large animal models have reported the development of antibody responses that are similar to those observed in COVID-19 patients. Two studies with AGM reported that infected animals seroconverted against SARS-CoV-2 as early as 5 DPI, and titers of SARS-CoV-2–specific Abs peaked between 15 and 20 DPI. One of these studies also reported that IgG and IgM responses were developed simultaneously. This contradicts the classic immunology paradigm in which antigen-specific IgM develops prior to IgG in response exposure but mirrors what has been reported in COVID-19 patients. Additionally, this study reported that mucosal inoculation led to the development of higher Ab titers compared with those that received the virus via the aerosol route, as was also described in the hACE2 transgenic mouse model. One study with cynomolgus macaques observed the development of anti-SARS-CoV-2 IgG Abs and nAb by 10 DPI. A study with rhesus macaques reported that nAbs developed in all infected animals by 5 weeks post infection regardless of inoculum size; however, the higher the inoculum, the more animals within a group that developed a response and the higher the Ab titer. Additionally, several studies using rhesus macaques suggest that antibody responses do protect against reinfection. These studies do not address whether protection will exist long term. However, they present potential models that can be used to determine the length of time these responses are protective and how many exposures or what type of exposure may be necessary to develop long-term protective B-cell immunity.

T-Cell Immunity

T-cell responses to SARS-CoV-2 are detected in the blood of infected patients within 2 weeks after the onset of COVID-19 symptoms. Initial reports from COVID-19 patients have shown an accumulation of mononuclear cells, likely T cells and monocytes, in the lungs and lower levels of T cells in the periphery. It has been suggested that multiple mechanisms may work together to cause lymphopenia, SARS-CoV-2 may directly attract lymphocytes or destroy lymphoid organs, cytokines may play a role, or reported increases in blood lactic acid levels may drive lymphopenia. Regardless, it is clear that T cells are attracted away from the blood and to the site of infection, likely to help control it. The extent of lymphopenia, more extreme for CD8+ vs CD4+ T cells, correlates with COVID-19–associated disease severity and mortality. Patients with mild symptoms present with normal or slightly higher T-cell counts in the periphery, SARS-CoV-2–specific CD4+ T cells in blood display a predominately central memory phenotype, whereas CD8+ T cells predominately display an effector phenotype. CD4+ T-cell SARS-CoV-2–specific responses are primarily against the M, N, and S proteins of the virus, whereas CD8+ responses are against the M and S proteins. Several COVID-19 cases have increased levels of T-cell exhaustion and reduced functional diversity, suggesting that the T-cell response may be affected by the chronic nature of COVID-19. To date, we do not have a good understanding of how SARS-CoV-2 infection is controlled, though T cells likely play an important role, and dysregulation of the T-cell response, much like the innate immune system, can result in immunopathology.

To better understand the role of T-cell responses in controlling SARS-CoV-2 infection, we will need to develop animals models that can successfully answer the following questions: (1) what is the contribution of T cells during the acute and chronic response to the infection? (2) how effective are the T-cell responses? Do they lead to successful viral clearance, tissue damage, or both? and (3) how long do SARS-CoV-2–specific memory T cells persist, and will they provide long-term protective immunity upon reinfection? Several promising animal models have begun to emerge that may help to answer these questions. First, it is worth noting that to study these complex immune responses, it is essential to use models with intact immune systems to draw final conclusions; however, using knockout models can provide information on the immune programs that are important to focus on. The leading small animal models for COVID-19, hamsters, hACE2 transgenic mice, and ferrets, have yet to show T-cell responses against SARS-CoV-2 infection in depth. However, studies on SARS have suggested that Th1 CD4+ T cells may control the infection, as depletion of these cells in mice results in slower clearance of the virus and exacerbated lung inflammation. Studies using a mouse-adapted strain of SARS have also shown that higher numbers of virus-specific CD4+ and CD8+ T cells that accumulate in the lungs lead to increased survival. Additionally, adoptive transfer of these virus-specific cells into immunodeficient mice result in protection against infection. One SARS-CoV-2 study infected WT hamsters that were treated with cyclophosphamide, known to deplete lymphocytes from the blood producing a transient immunosuppressed phenotype, resulting in a more aggressive model of SARS-CoV-2 in hamsters, with a 50% survival rate compared with the 100% seen with immunocompetent hamsters. This study also infected RAG2-deficient hamsters, animals without B or T cells, resulting in 100% mortality and more rapid onset of disease as measured by weight loss. These data suggest the importance of T-cell responses, even very early on in infection, and although a pro-inflammatory T-cell profile may be an aggravating factor for immunopathogenesis, a balance of these cells will be important for control of SARS-CoV-2 infection.

Studies using large animal models, similar to small animal models, have yet to spend significant time on understanding the development of the T-cell responses to SARS-CoV-2. One study on rhesus macaques assessed the development of T-cell responses to natural infection and if these responses could prevent subsequent reinfection. The authors reported that all animals infected with a high dose of virus developed ELISpot responses to N and S peptide pools by 5 weeks post infection and resolved the infection in BAL 10–14 DPI; however, animals continued to shed virus up to 28 DPI as measured by detectable viral RNA in nasal swabs. On reinfection, the animals had a fivefold log decrease in median viral loads in BAL and nasal mucosa compared with primary infection, and all animals showed an
increase in T- and B-cell responses, suggesting that protection was mediated by immunologic control.\textsuperscript{118} These data give evidence that SARS-CoV-2 infection induces protective immunity against re-exposure in nonhuman primates; however, they also indicate that the size of the inoculum matters, as the animals infected with the lower doses of virus developed weaker responses. An additional study in rhesus macaques showed that CD4+ T-cell responses in the blood, lung, and lymph nodes were predominately Th1 skewed, reflective of the cytokine environment following infection.\textsuperscript{137} This same study also reported the generation of germinal center T follicular helper cells specific for the SARS-CoV-2 S and N proteins, which corresponded to early appearance of SARS-CoV-2 serum IgG. More studies that investigate the T-cell response are needed to further determine how long T-cell responses may last and protect from reinfection and what type of response a vaccine would need to induce to successfully protect against infection.

**VACCINES, IMMUNOGENICITY, AND PROTECTION**

Coronavirus-specific T cells and B cells will inevitably be important in controlling SARS-CoV-2 infection and disease. However, the type and strength of adaptive responses needed to prevent infection need further study. Elucidating these aspects of the immune response will be essential for the development of a successful vaccine. Multiple studies have been published thus far on the safety and effectiveness of a SARS-CoV-2 vaccine, all showing relatively promising results.\textsuperscript{138–142} However, there are numerous candidates currently in trial that have yet to be reported on (ClinicalTrials.gov). We know first-hand from SARS and MERS vaccine studies that the S protein on the surface of the virus is an ideal target, as it is very immunogenic. The structure of the S protein in SARS-CoV-2 was solved in record time at high resolution, which in addition to the development of novel and advanced vaccine platforms will allow us to develop a SARS-CoV-2 vaccine more rapidly than ever before.\textsuperscript{143,144} However, there are many considerations to be taken into account prior to developing and implementing a vaccine against a novel pathogen. As discussed above, the possibility of waning antibody and T-cell responses should be taken into consideration in vaccine design and dose scheduling. Infection with other human coronaviruses does not result in the induction of long-lived antibody responses; in fact, antibody titers against SARS and MERS have been shown to wane 2–3 years post infection.\textsuperscript{145–147} Additionally, SARS-CoV-2 infection causes greater pathology in individuals over the age of 50 years for reasons unknown. Therefore, it will be essential to develop a vaccine platform that will protect older populations as well as younger ones. Unfortunately, older individuals have less robust immune responses to vaccination due to immune senescence, which leads to less protection.\textsuperscript{146} Therefore, different formulations of a vaccine, as has been done with influenza, may be necessary to induce protection in this population.\textsuperscript{147,148} If protective vaccination is not possible in older individuals, they will still benefit from the vaccination of younger individuals, which presumably will stop a large proportion of transmissions. An effective SARS-CoV-2 vaccine will need to overcome these issues to protect in a scenario where the virus becomes endemic and causes seasonal epidemics similar to influenza.

Animal models have been an excellent tool to determine immunogenicity of vaccines and will help facilitate the development of the best SARS-CoV-2 vaccine possible. Though there are already ongoing vaccine clinical trial studies, it is likely that we will need to improve on first-generation vaccines, and having animal studies underway will help to facilitate the development of multiple vaccines that can be implemented throughout the world. Animal models will allow for testing vaccine strategies in both young and aged animals to see if certain strategies are more appropriate in different age groups. Similar to COVID-19 in humans, studies in hamsters and macaques have shown that older animals have worse disease outcomes compared with younger animals.\textsuperscript{149,150} The importance of using animals first is highlighted by some of the vaccines that were tested for SARS. Of the vaccines developed for SARS, including a wide variety of platforms,\textsuperscript{150} most protected animals from subsequent challenges with the virus. However, they did not induce sterilizing immunity and in some cases resulted in complications, including liver damage and infiltration.\textsuperscript{151,152} One vaccine resulted in the enhancement of disease and determined that responses to certain epitopes on the S protein were protective, whereas immunity to others seemed to enhance disease.\textsuperscript{153} Similar findings have been reported for MERS vaccines.\textsuperscript{154,155} Formulation of a vaccine will also be important, because vaccination with inactivated whole virus is more likely to lead to the development non-neutralizing Abs that may result in antibody-dependent enhancement. In SARS and other viruses, antibody-dependent enhancement results in the internalization of virus–antibody immune complexes that promote inflammation and tissue injury by activating myeloid cells via Fc receptors.\textsuperscript{156} As discussed above, inflammation is an important component of the innate response but when left unregulated can result in negative side effects. Overall, vaccination studies with SARS and MERS have been associated with greater survival, reduced virus titers, and less morbidity compared with no vaccination. Therefore, it is reasonable to use these same platforms and animal models to determine if the vaccines developed against SARS-CoV-2 are efficacious and safe.

Mice, guinea pigs, rats, and rabbits have been used in several SARS-CoV-2 studies specifically to test the immunogenicity of vaccine candidates.\textsuperscript{157–159} The benefits to using WT small animal models that are otherwise not susceptible to the virus are that they are readily available, easier to house, and have robust humoral and adaptive immunological responses similar to humans and can therefore provide rapid results as to whether a candidate is worth pursuing further. One study assessing the immunogenicity of the INO-4800 vaccine, a DNA-based vaccine candidate, found that strong antigen-specific T-cell responses, functional nAbs, and a biodistribution of other SARS-CoV-2-targeting Abs were developed shortly following immunization in WT mice and guinea pigs.\textsuperscript{157} This is only 1 of many studies that have used this method to test the immunogenicity of RNA,\textsuperscript{155} recombinant protein,\textsuperscript{156} adenovirus-vectored,\textsuperscript{157} and inactivated virus\textsuperscript{160} vaccines and highlight the importance of using small animal models to determine which candidates are worth advancing. However, the drawback of using small animal models that are not susceptible to SARS-CoV-2 infection is that subsequent challenge studies cannot be performed in the same animals. There are small animal models that are susceptible to SARS-CoV-2; however, the most promising models such as Syrian golden hamsters and hACE2 transgenic mice have not yet been used in published challenge studies. Two studies using mouse-adapted strains of SARS-CoV-2 have been published and include vaccine challenge components. Both studies show that vaccination with an RBD vaccine or a virus-like-particle vaccine provided a certain degree of protection and resulted in less severe disease as measurable by viral titers in the lung and nasal turbinate.\textsuperscript{25,161}
Large animal models have also been shown to be valuable tools in assessing vaccine efficacy and protection. The negatives to using large animal models are mostly the expense and availability; however, these model systems, short of human studies, often give the best insight as to whether a vaccine candidate can provide protection using both short- and long-term studies. Rhesus macaques and pigtailed macaques have been the current front-runners in vaccine studies. One study showing that an adenovirus-vector vaccine, ChAdOx1 nCoV-19, encoding the spike protein of SARS-CoV-2 was very immunogenic in both mice and rhesus macaques and elicited a robust humoral and T-cell-mediated response. This same vaccine was then moved into human clinical trials and has since been published with promising results. An additional study that assessed an inactivated virus, PiccoVac, found a similar outcome; this vaccine protected rhesus macaques from infection due to development of robust nAb and T-cell response. A third study in rhesus macaques found that a DNA-based vaccine, shown to be immunogenetic in mice, led to protection in subsequently challenged animals. The authors reported that vaccinated animals developed humoral and cellular immune responses, including nAb titers comparable with those found in convalescent human serum, and led to a 3.5-log reduction in median viral loads in BAL and nasal mucosa in subsequently challenged animals. These data present in these studies suggest an immune correlate of protection generated by effective vaccines. Though no long-term studies have been completed to date, the use of large animal models offers the option to assess whether certain vaccines result in sterilizing immunity or how long protective immune responses last, something that cannot be accomplished using small animal models. For the current SARS-CoV-2 pandemic, vaccines will not be available to help with the first or even second wave of infections. However, as additional waves occur or in a post-pandemic scenario where SARS-CoV-2 continues to circulate as a seasonal virus, having a large toolbox of efficacious vaccines will be essential.

SUMMARY

Developing strong animal models is essential for furthering our understanding of how the immune system functions in response to SARS-CoV-2 infection. Animal models provide access to tissues and cells that would otherwise be inaccessible as well as allow us to probe immune function using many laboratory techniques that would not be feasible in human studies. The alarming speed in which SARS-CoV-2 has spread and the high mortality rate of severe COVID-19 has required both basic science and clinical research to move at an unprecedented pace. Within months, thousands of scientific studies have been published online in reference to this subject. Therefore, it is imperative to carefully assess the value of the studies and determine which reports are worth pursuing further. Here, we have aimed to review published animal studies to provide a resource that could be used to determine what animal model is right for the question at hand. We have focused on small and large animal models that were previously shown to be useful in coronavirus research. As with all models, there are of course limitations. Appropriate small animal models are essential for research and antiviral therapeutic development. Mouse models are popular because of their affordability, availability, and clear genetic backgrounds and have been widely used for studying pathogenesis of human coronaviruses. However, mice are not naturally susceptible to SARS-CoV-2 infection and require engineering or virus modifications to allow productive viral infection. Hamsters and ferrets do not require genetic manipulation to be susceptible to infection, but reagents and genetic knockouts are currently limited. Large animal models, such as nonhuman primates, are instrumental for the preclinical evaluation. However, they are restricted by high costs, availability, and the complexity of husbandry facilities required. Choosing the appropriate model will depend on the question and ability to house and conduct the research in question because all COVID-19 animal models require access to biosafety level 3 facilities. In this review, we have discussed both small and large animal model studies that have been previously used in SARS-CoV-2 research and may be useful in elucidating the immunological contributions to hallmark syndromes observed with COVID-19, because it is likely that these hallmark syndromes are driven by dysregulation of the immune system.

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Potential conflicts of interest

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