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COVID-19: Coronavirus replication, pathogenesis, and therapeutic strategies

HUMAN CORONAVIRUSES, along with influenza virus, human metapneumovirus, respiratory syncytial virus, and rhinovirus, are endemic and cause approximately 15% to 30% of annual respiratory tract infections. Coronavirus infections are generally mild in healthy adults, obviating any urgent need to develop treatments or vaccines. However, outbreaks of acute respiratory distress syndrome (ARDS) due to novel, highly pathogenic strains—severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and now, SARS-CoV-2—have revealed the potency and danger of this expanding family of pathogens that have the capacity to kill many thousands of people around the world if not geographically contained.¹

As in severe SARS and MERS disease, the mortality rate is disproportionately high in the elderly and patients with preexisting comorbidities such as heart disease, diabetes, hypertension, and renal disease.² Higher morbidity in the elderly may partly be attributed to muted interferon antiviral responses (although the suggestive study has not yet been peer-reviewed)³ as well as overall lower adaptive immunity,² resulting, paradoxically, in longer courses of hyperactivity of the innate immune system (“cytokine storm”).

■ A ZONOTIC INFECTION

Bats have been implicated as the likely source of SARS-CoV-2, as both SARS-CoV and MERS-CoV are genetically similar to viruses recovered from bats, and bat coronaviruses can use human receptors for cell entry.⁴ How-

ever, phylogenetic studies, looking at sequence-based virus evolution, suggest that the virus is not transmitted directly from bats to humans but rather first infects intermediate animal hosts in close contact with humans. In the case of SARS-CoV, these can be civets or raccoon dogs sold at crowded markets; for MERS-CoV, they can be domesticated dromedary camels.⁴

Transmission from bats to intermediate hosts and then to humans, as well as from human to human, all involve viral adaptation, slight changes in viral sequence to improve fitness in the new host. This is not unique to coronaviruses, as endemics and pandemics also occur when novel influenza A virus strains emerge in the human population from an animal host.⁵ Similar to introduction of Ebolavirus and human immunodeficiency virus 1 by mammals, many other viruses circulating in wild animals have the potential for zoonotic transmission.⁶

SARS-CoV-2, the causative agent for the pandemic corona virus disease of 2019 (COVID-19) outbreak, was first found in Wuhan, China, and initial analysis of viral RNA obtained from patients hospitalized in late 2019 revealed it was 96% identical at the whole-genome level to a bat SARS-like coronavirus.⁷

Uniquely, SARS-CoV-2 can be transmitted by people who are infected but have no symptoms, not just by symptomatic patients. Concern about potential spread of SARS-CoV-2 to household cats has emerged from a news report of infection in a tiger in the Bronx Zoo. Ferrets can be infected, with intraspecies transmission,⁸ and cats can also be infected and transmit the virus to other cats, while dogs have low susceptibility. However, it is unknown if any of these animals can transmit the virus to humans.⁹

Understanding of the virus and how it causes disease points the way to potential treatments

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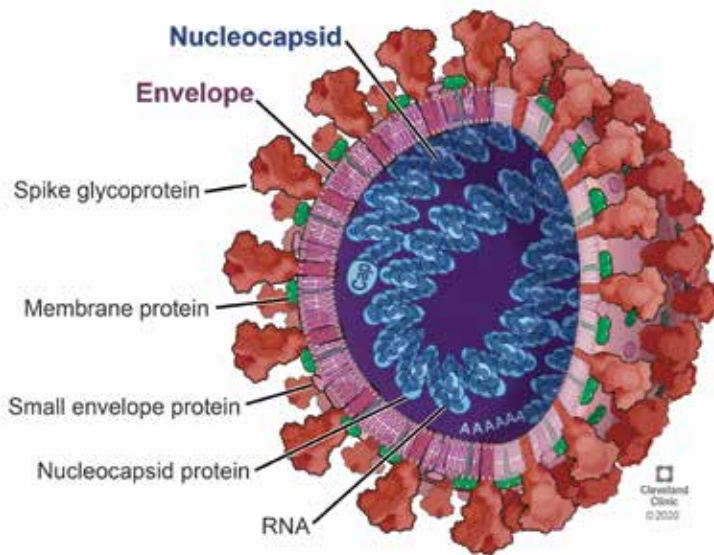


Figure 1. Structure of coronaviruses.

■ STRUCTURE AND GENOME OF CORONAVIRUSES

Coronaviruses are spherical enveloped viruses containing a single strand of positive-sense RNA (similar to host mRNA) of approximately 26 to 32 kb.¹⁰ Their defining morphologic features are club-shaped projections from the viral envelope resembling a crown or a solar corona, made of a highly glycosylated protein, named spike protein. Their other 3 structural proteins are the envelope, membrane, and nucleocapsid proteins (Figure 1).

The first two-thirds of the genome consists of 2 large overlapping open reading frames, which encode 16 nonstructural proteins, including proteases, RNA-dependent RNA polymerase (prRdRp), RNA helicase, primase, and others, that form the viral replicase complex, a platform to propagate viral mRNAs. These nonstructural proteins are all potential targets for therapies, which would in theory work against all coronaviruses (Figure 2).

The remaining portion of the genome includes interspersed open reading frames for the structural proteins, as well as a number of accessory proteins generally nonessential for replication in tissue culture but capable of suppressing immune responses and enhancing pathogenesis.^{10,11}

■ HOW THE VIRUS GETS IN

Features of coronavirus transmission, replication, and pathogenesis are determined by both the viral genome and the human host (Figure 2).

Coronavirus spike proteins are key determinants for virus attachment and entry into target cells. The receptor for both SARS-CoV and SARS-CoV-2 is angiotensin-converting enzyme 2 (ACE2),^{12,13} a cell-surface enzyme contributing to control of blood pressure. SARS-CoV cell entry is independent of ACE2 catalytic activity.

Entry involves 2 spike protein subunits, which mediate distinct functions. The S1 subunit mediates ACE2 attachment through the receptor-binding domain. The S2 subunit, containing the fusion peptide and transmembrane domains, drives fusion of viral and host cell membranes. To be activated for fusion, the spike protein must be cleaved at 2 sites directly at the cell membrane, through endosomes, or both. The sequence of the cleavage sites, one located at the border of S1 and S2 subunits, the other (S2') within S2 just upstream of the fusion peptide, provide substrates for a variety of cellular proteases and determine cleavage efficiency.

The route or routes of infection thus depend on the proteases available in different cell types and the protease cleavage sites.¹⁴ This is also demonstrated by involvement of the cellular serine protease TMPRSS2 (transmembrane protein serine protease 2) and activities of furin and endosomal cathepsins B and L in SARS-CoV-2 entry.¹² TMPRSS2 activity is also involved in viral spread and pathogenesis in SARS-CoV-infected and MERS-CoV-infected mouse models.¹⁵

Host proteases that cleave the S protein are also potential targets for antiviral drugs (Figure 2). A higher rate of SARS-CoV-2 infections compared with SARS-CoV infections may be at least partially explained by a higher affinity of spike protein for ACE2.¹³ The sequence divergence in both the receptor-binding domain and cleavage domains in the spike protein between SARS-CoV-2 and the bat virus highlight how only a few changes are needed to adapt an animal virus to humans.^{7,12,13,16}

Only a few changes are needed to adapt an animal virus to humans

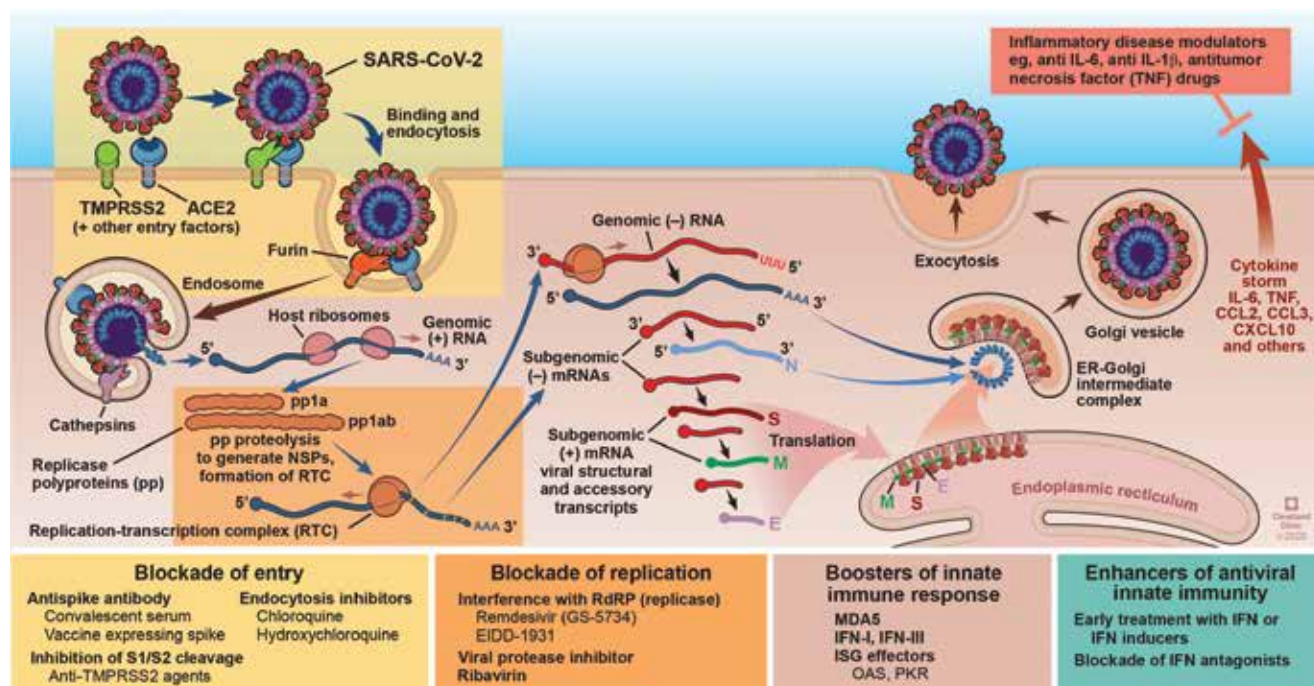


Figure 2. Overview of COVID-19, SARS-CoV-2 replication, and therapeutic targets.

Upper left. Virus entry entails binding the angiotensin-converting enzyme 2 (ACE2) receptor and cleavage by the serine protease TMPRSS2 (in green) to allow fusion with the host membrane. Other cellular proteases, eg, furin (in orange), facilitate pH-dependent entry through the endocytic pathway. The predominant entry routes are cell type-specific and dependent on availability of select proteases.

Middle. Following uncoating and release of viral RNA into the cytoplasm, translation of open reading frame 1a (ORF1a) and ORF1ab produces the polyproteins pp1a and pp1ab. These in turn are processed by viral proteases (encoded by ORF1a) to yield 16 nonstructural proteins. Formation of the RNA replicase–transcriptase complex (RTC) uses rough endoplasmic reticulum (ER)-derived membranes. The RTC drives synthesis of (–)RNAs. Full-length (–)RNA copies of the genome provide templates for full-length (+)RNA genomes. Transcription further produces a subset of subgenomic RNAs, including those encoding all structural and accessory proteins.

Right. The translated structural proteins and genomic RNA are assembled into the viral nucleocapsid and envelope in the ER–Golgi intermediate compartment, and are subsequently released by exocytosis.

Bottom. Potential strategies for treatment. Anti-TMPRSS2 or chloroquine treatment in experimental animals will reveal efficacy of targeting select proteases or entry pathways in limiting infection, while simultaneously monitoring effects on innate and adaptive immunity. The replication cycle can be blocked at several stages using single or combined treatment paradigms: virus entry can be inhibited by antispike antibodies elicited by vaccines to block attachment or by preventing fusion using relevant protease inhibitors.¹² RTC formation and transcription-replication events can be targeted using viral protease inhibitors or nucleoside analogues (GS-5734 or EIDD-1931).⁴⁸ Interferon (IFN) responsiveness can be increased by early exogenous IFN treatment,²⁴ IFN inducer treatment, repression of viral IFN antagonists, and enhancement of host antiviral IFN pathways. The “cytokine storm” induced as a host response to rampant virus replication may be targeted by administration of select anti-inflammatory immune modulators, which are already given to patients with inflammatory disorders. Drugs targeting viral replication may also be combined with treatments that control detrimental immune responses. The ferret model will provide a useful tool to test multiple therapeutic and preventive treatments.⁸

Based on information in references 1, 10, 13, and 47

THE BODY MOUNTS AN INNATE IMMUNE RESPONSE

Interferons I and III are cytokines with critical roles in the innate immune response against viral infections.¹⁷ Virus-infected cells induce and secrete interferon I molecules that bind to the cell surface receptor IFNAR (interferon III uses a different receptor), thereby triggering the Jak-Stat (Janus kinase/signal transducer and activator of transcription) signaling pathway that switches on many antiviral genes. The interferon-stimulated genes are then transcribed into RNA and translated into proteins that suppress viral replication and spread.

HOW THE VIRUS EVADES THE HOST RESPONSE

During coevolution with their hosts, viruses have learned to counteract the interferon antiviral response. Like other human coronaviruses, SARS-CoV-2 can at least partly evade innate immunity to gain a foothold in humans, a critically important step in the infection cycle. Although mechanistic insights are as yet unavailable, we do have a good understanding of how other coronaviruses evade interferon's antiviral activity,¹⁸ and also how we could engage antiviral factors to promote interferon activity.¹⁹

In general, coronaviruses can potentially antagonize antiviral innate immunity by interfering with both interferon production and the cellular antiviral response.²⁰ For instance, mouse coronaviruses and MERS-CoV have accessory proteins that block an interferon response pathway that degrades the viral RNA (by oligoadenylate synthetase and ribonuclease L).^{21,22}

The large number of host antiviral mechanisms and distinct viral antagonism at different steps in the virus replication cycle have made it difficult to identify the most relevant ones. Not only does each type of coronavirus encode different accessory proteins responsible for allowing the virus to escape cellular innate immune mechanisms, but distinct cell types may respond differently.

While we are only just beginning to understand the functions of the SARS-CoV-2 accessory proteins, it is clear that there are similarities and differences between the acces-

sory proteins of SARS-CoV-2 and those of its closest human pathogen relative, SARS-CoV. A better understanding of the precise functions of the SARS-CoV-2 accessory proteins, especially their interaction with innate immune pathways could lead to novel antiviral drugs that promote the innate immune response. The finding (not yet peer-reviewed)²³ that SARS-CoV-2 was more sensitive to interferon than SARS-CoV raises hope that giving interferon or interferon inducers very early in the infection could be beneficial, and, perhaps, less likely to cause harm than using interferon later in COVID-19.²⁴

WHY DO SOME PEOPLE GET SO SICK, BUT OTHERS ARE FINE?

One of the most problematic features of SARS-CoV-2 infection is the broad spectrum of disease, ranging from no symptoms to mild flu-like symptoms, anosmia, fever, nonproductive cough, dyspnea, and fatigue to acute respiratory distress syndrome, the main cause of death. While multiple organs, including the heart, kidneys, liver, and gastrointestinal tract, are injured, it remains to be resolved to what extent tissues are damaged by infection, hypoxia, or the immune response. Complications may also involve the central nervous system, either by direct infection or secondary damage.^{25,26}

Different ACE2 expression? ACE2 is expressed in various cell types of the lung, including alveolar epithelial cells, pneumocytes, and bronchial transient secretory cells, as well as enterocytes of the small intestine, heart (pericytes), and kidney. These are the same tissues that the virus affects, but studies with SARS-CoV indicate that ACE2 expression is not the only determinant of susceptibility.^{27–30} More research is needed to assess to what extent ACE2 surface expression or polymorphisms, or other coreceptors and proteoglycan moieties, are markers of tissue susceptibility.

Renin-angiotensin system dysregulation? The finding that ACE2 is a primary SARS-CoV-2 receptor has further led to extensive discussion of dysregulation of the renin-angiotensin system, which regulates blood pressure and electrolyte balance.^{31–33} Conversion of angiotensin I to angiotensin II by angiotensin-converting enzyme (ACE) activates pathways

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that lead to inflammation, vasoconstriction, oxidation, and fibrosis. ACE2 activity counterbalances this pathway by cleaving both angiotensin I and angiotensin II to shorter peptides, which use distinct receptors to promote vasodilation, as well as anti-inflammatory, antioxidant, and antifibrosis activity.

ACE inhibitors and angiotensin II receptor blockers are thus commonly used in patients with cardiovascular diseases, hypertension, and diabetes, promoting the protective effects of ACE2. However, increased expression of ACE2 by use of antihypertensive drugs in animal models raised concerns of higher virus infection risk for patients receiving these drugs. Nevertheless, current recommendations are to continue treatment.^{32,33} The complexity of the renin-angiotensin system will require more extensive retrospective analysis of larger and ethnically diverse patient groups.

Protective host features? The apparently large percentage of infections that are asymptomatic is unique to SARS-CoV-2, but many of the pathogenic features resemble those observed in SARS and MERS-CoV infections. The protective host features underlying the asymptomatic infections are currently unknown, as testing within many countries is limited to people presenting with symptoms such as severe shortness of breath, coughing, and fever. Retrospective studies incorporating serum antibody testing and health status will provide much-needed insights.

Cytokine storm? Severe disease is associated with lymphopenia and an uncontrolled systemic inflammatory response called a cytokine storm, which ultimately leads to multiple organ failure and death.^{34,35} Autopsy results reveal severe damage to endothelial tissue, vasculitis-like manifestations, and atrophy of secondary lymphoid tissues.³⁵ Early studies in COVID-19 patients showed higher plasma levels of interleukin 2 (IL-2), IL-7, granulocyte colony-stimulating factor, C-X-C motif chemokine 10, monocyte chemoattractant protein 1, macrophage inflammatory protein 1a (chemokine [C-C motif] ligand 2), and tumor necrosis factor (TNF), but also anti-inflammatory IL-10, in intensive care patients than in nonintensive care patients.³⁶ Several reports also confirm high levels of IL-6 in severely ill patients.^{2,37} Retrospective clinical

investigation of more patient cohorts without or with preexisting conditions and of those being treated with distinct anti-inflammatory immune modulators, eg, anti-TNF, anti-IL-6, anti-IL12/IL23 or anti-IL-1 beta for immune-mediated inflammatory conditions will provide much-needed guidance on treatment to stem severe COVID-19.

■ ONCE YOU GET IT, ARE YOU IMMUNE FOR LIFE?

Another critical unresolved aspect of COVID-19 is the establishment of adaptive immunity. Lessons from the SARS-CoV epidemic indicate that CD4 and CD8 T-cell memory lasts for up to 11 years in recovered individuals.^{38–40} A study of a limited number of patients hospitalized with mild or severe COVID-19 revealed humoral immunoglobulin M (IgM) and immunoglobulin G (IgG) serum responses to the viral nucleocapsid and spike proteins emerging at 10 days after symptom onset, with seroconversion in a sizable majority of patients by 3 weeks.^{2,41,42} Moreover, the IgG levels correlated with virus neutralization titers.

Transfusion of convalescent plasma from recovered patients had beneficial outcomes in a small number of SARS and COVID-19 cases.⁴³ Based on preliminary results of convalescent serum as well as in vitro and in vivo neutralization studies, clinical trials will be launched to evaluate the efficacy of spike protein-based vaccines.

A concern is the mutation rate of the virus as it spreads through the population. Viral genomes are being analyzed throughout the world and compiled in large, publicly available databases, which collate sequenced isolates and look at relationships (<https://nextstrain.org/ncov/global>). Although such databases currently reflect a population naïve to the virus, similar studies can be conducted once vaccines become available to test the effects of immune pressure on the virus.

■ VIRAL AND HOST TARGETS FOR THERAPIES AND VACCINES

There are at least 4 potential therapeutic strategies against COVID-19, apart from supportive and oxygenation therapies such as use of ventilators (Figure 2):

There are at least 4 potential therapeutic strategies against COVID-19

- Direct antiviral drugs against SARS-CoV-2 (eg, remdesivir)
- Indirect antiviral agents (eg, interferon I, interferon inducers, and drugs that target host proteins required for infections)
- Convalescent plasma that contains antibodies against SARS-CoV-2
- Drugs that tamp down the pathogenic hyperactive inflammatory response and cytokine storm later in disease progression.

However, we would like to emphasize that at present, these strategies are investigational only, including the off-label use of existing drugs, and may prove to show no efficacy and could be harmful in controlled clinical trials.

The emergence of 3 highly pathogenic human coronaviruses within the past 20 years predicts that more of them will continue to come along. As the timing is unpredictable, monitoring and transparent reporting of local outbreaks is imperative for early intervention.

With respect to currently circulating SARS-CoV-2 and limited overall testing, it is also unknown whether the virus is affected by seasonal changes. While physical distancing is an effective control measure to limit acute infection rates, asymptomatic carriers will likely

continue to spread the virus, leading to ongoing hotspots of symptomatic infection.

A major factor influencing the future of COVID-19 is the ability of recovered people to develop protective immunity. However, the ongoing yearly infection rates by historically circulating coronaviruses,⁴⁴ as well as evidence for already distinct SARS-CoV-2 variants⁴⁵ suggest that established immunity may be insufficient to avoid recurring infections.

Clinical trials with drugs targeting viral proteins will reveal tolerance of the SARS-CoV-2 to selective pressure and guide in development of strategies that target host proteins required for replication.⁴⁶ Efficacy of vaccination strategies to elicit protective antibodies may further uncover the potential need for seasonal vaccines like those for circulating influenza viruses.

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