VIEWPOINT: COVID-19

How does SARS-CoV-2 cause COVID-19?

The viral receptor on human cells plays a critical role in disease progression

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Viruses enter cells and initiate infection by binding to their cognate cell surface receptors. The expression and distribution of viral entry receptors therefore regulates their tropism, determining the tissues that are infected and thus disease pathogenesis. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the third human coronavirus known to co-opt the peptidase angiotensin-converting enzyme 2 (ACE2) for cell entry (*1*). The interaction between SARS-CoV-2 and ACE2 is critical to determining both tissue tropism and progression from early SARS-CoV-2 infection to severe coronavirus disease 2019 (COVID-19). Understanding the cellular basis of SARS-CoV-2 infection could reveal treatments that prevent the development of severe disease, and thus reduce mortality.

As with all coronaviruses, SARS-CoV-2 cell entry is dependent on its 180-kDa spike (S) protein, which mediates two essential events: binding to ACE2 by the amino-terminal region, and fusion of viral and cellular membranes through the carboxyl-terminal region (*2*). Infection of lung cells requires host proteolytic activation of spike at a polybasic furin cleavage site (*3*). To date, this cleavage site is found in all spike proteins from clinical SARS-CoV-2 isolates, as well as some other highly pathogenic viruses (e.g., avian influenza A), but it is absent from SARS-CoV and is likely to have been acquired by recombination between coronaviruses in bats. Cleavage by the furin protease therefore expands SARS-CoV-2 cell tropism and may have facilitated transmission from bats to humans (*1*). Membrane fusion also requires cleavage by additional proteases, particularly transmembrane protease serine 2 (TMPRSS2), a host cell surface protease that cleaves spike shortly after binding ACE2 (*3*). SARS-CoV-2 tropism is therefore dependent on expression of cellular proteases, as well as ACE2.

Other proteins that enable SARS-CoV-2 cell entry are also emerging, including neuropilin 1 (NRP1), a receptor that binds the carboxyl-terminal RXXR motif in spike that is exposed after furin cleavage. How NRP1 promotes cell entry is unclear, but it may further increase the cell types infected (*4*).

Of the seven known human coronaviruses, three are highly pathogenic [SARS-CoV, SARS-CoV-2, and Middle East respiratory syndrome (MERS)-CoV], and the remaining four (HCoV-NL63, HCoV-229E. HCoV-OC43, and HCOV-HKU1) are less virulent, causing "common colds." SARS-CoV, SARS-CoV-2, and HCoV-NL63 use ACE2 as their cell entry receptor. MERS-CoV binds DPP4 (dipeptidyl peptidase 4) and HCoV-229E uses CD13 (aminopeptidase N) (*2*). No host protein receptors have been identified for the other two viruses. It seems a notable coincidence that all known human coronavirus receptors are cell surface peptidases, particularly because the interactions do not involve the endopeptidase active site (*2*). The presence of a specific region within ACE2, targeted by three coronaviruses, is particularly noteworthy (*1*). Conversely, the receptor-binding domain of spike is encoded by the most variable part of the coronavirus genome (*1*). This implies that diversification of these viruses generated different sequences that converged on the same region of the same protein using alternative structural solutions. What, then, is so special about ACE2?

ACE2 is a transmembrane protein best characterized for its homeostatic role in counterbalancing the effects of ACE on the cardiovascular system (*5*). ACE converts angiotensin I to angiotensin II, a highly active octapeptide that exhibits both vasopressor (vascular contraction to increase blood pressure) and proinflammatory activities. The carboxypeptidase activity of ACE2 converts angiotensin II to the heptapeptide angiotensin-(1-7), a functional antagonist of angiotensin II. Because ACE is highly expressed in vascular endothelial cells of the lungs, angiotensin II is also likely to be high in the pulmonary vasculature. Indeed, *Ace2* deletion in mouse models of acute lung injury results in more severe disease, suggesting a protective role for ACE2 in lung tissue (*5*). In many host-virus interactions, expression of the viral receptor is down-regulated in infected cells, and expression of ACE2 in the lungs of mice was reduced by SARS-CoV infection. Depletion of ACE2 may thus play a causative role in the lung injury caused by SARS-CoV and SARS-CoV-2, and high plasma angiotensin II is reported in patients with COVID-19. However, MERS-CoV causes a similar lung disease without targeting ACE2, so other factors must also be important.

As a respiratory virus, SARS-CoV-2 must initially enter cells lining the respiratory tract. Single-cell sequencing and RNA in situ mapping of the human respiratory tract show ACE2 and TMPRSS2 expression to be highest in ciliated nasal epithelial cells, with lesser amounts in ciliated bronchial epithelial cells and type II alveolar epithelial cells (*6*). This translates to greater permissivity of upper versus lower respiratory tract epithelial cells for SARS-CoV-2 infection in vitro and fits disease pathology: Upper respiratory tract symptoms are common early in disease, with nasopharyngeal and throat swabs positive for SARS-CoV-2 at clinical presentation (*7*). In contrast to SARS-CoV, infectivity of patients with SARS-CoV-2 peaks before symptom onset (*8*). Indeed, presymptomatic transmission makes SARS-CoV-2 impossible to contain through case isolation alone and is a key driver of the pandemic (*8*). This alteration in the pattern of disease may relate to the acquisition of the furin cleavage site in spike or increased binding affinity for ACE2 in SARS-CoV-2, compared with SARS-CoV (*9*).

If the main role of ACE2 is to cleave angiotensin II, it is unclear why expression in lung tissue is more prominent in epithelial than in endothelial cells. Furthermore, the Human Cell Atlas highlights ACE2 expression in intestinal enterocytes, rather than in the lungs. This distribution may reflect non-enzymatic roles of ACE2, such as chaperoning amino acid transporters. Indeed, SARS-CoV-2 infection of the gastrointestinal (GI) tract is common, with viral RNA detectable in stool in up to 30% of COVID-19 patients. This likely contributes to the frequency of GI symptoms. Conversely, whereas fecal-oral transmission of coronaviruses is thought to be prominent among bats, it appears to be a minor transmission route for SARS-CoV-2 in humans, perhaps because colonic fluid inactivates the virus. Whether extrapulmonary ACE2 expression and concomitant viral infection account for other clinical manifestations of SARS-CoV-2 is unclear. The association between SARS-CoV-2 infection and anosmia (loss of smell) may reflect ACE2 and TMPRSS2 expression in sustentacular cells, which maintain the integrity of olfactory sensory neurons. Olfactory epithelial cells also express NRP1 and could provide a direct route to the brain (*4*).

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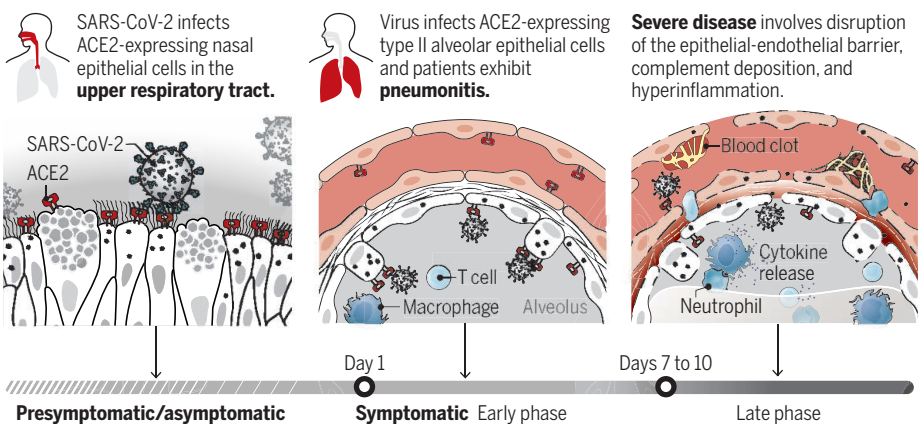
Although a substantial proportion of SARS-CoV-2–infected individuals report few, if any, symptoms and recover completely, chest computed tomography (CT) evidence of viral pneumonitis is present in >90% of symptomatic cases within 3 to 5 days of onset (*10*). This presumably reflects viral replication in the lower respiratory tract, with infection of type II pneumocytes and accompanying inflammation (see the figure). Lung pathology in this early phase is poorly reported because most patients recover. Histopathology from cynomolgus monkeys, 4 days after inoculation with SARS-CoV-2, shows a viral pneumonitis with alveolar edema, capillary leakage, inflammatory cell infiltration, interstitial thickening, and cell fusion (a feature of coronavirus infection), with viral spike expression on alveolar epithelial cells (*11*).

Key phases of disease progression

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) binds to angiotensin-converting enzyme 2 (ACE2). Initial infection of cells in the upper respiratory tract may be asymptomatic, but these patients can still transmit the virus. For those who develop symptoms, up to 90% will have pneumonitis, caused by infection of cells in the lower respiratory tract. Some of these patients will progress to severe disease, with disruption of the epithelial-endothelial barrier, and multi-organ involvement.



Around 80% of patients with COVID-19 pneumonitis recover without specific treatment (*12*). However, ~20% of patients deteriorate, often rapidly, ~7 to 10 days after symptom onset. This is when patients are most frequently admitted to hospital, with worsening fever, hypoxia, lymphopenia, rising inflammatory markers [C reactive protein (CRP), interleukin-1 (IL-1), and IL-6], coagulopathy, and cardiovascular involvement. About 25% of these patients will require mechanical ventilation, which is associated with high mortality (50 to 80%). The demographic of this "at risk" group is reproduced across many countries: older men with hypertension, diabetes, and obesity, as well as a less well defined contribution of ethnicity (*12*). Similar factors regulate ACE2 expression, which may therefore contribute to disease severity. Nonetheless, the amount and distribution of ACE2 expression cannot be the only factor affecting disease progression, because the three human coronaviruses that use ACE2 for cell entry exhibit markedly different pathogenicity.

What causes the sharp deterioration that leads to severe systemic COVID-19? The lung pathology in severe disease is different from the earlier pneumonitis, with progressive loss of epithelial-endothelial integrity, septal capillary injury, and a marked neutrophil infiltration, with complement deposition, intravascular viral antigen deposition, and localized intravascular coagulation (*13*). If the earlier viral pneumonitis reflects direct ACE2-mediated infection of type II pneumocytes, what drives this next, potentially deadly phase of acute lung injury, with the concomitant breakdown of the respiratory epithelial barrier, endothelial damage, and patient deterioration (*14*)? Most importantly—how can it be stopped?

The timing of deterioration suggests a role for the adaptive immune system, either antibodies or T cells, and has many hallmarks of immune-driven inflammation. Endothelial injury may result from immune-mediated damage, through complement activation, antibody-dependent enhancement, and/or cytokine release. Attention has therefore focused on the use of immunomodulatory therapies in patients with severe disease. Nonetheless, the most obvious culprit for severe disease is the virus itself, either alone or with immune pathology. Breakdown of the lung epithelial-endothelial barrier might trigger endothelial damage and viral dissemination, with more widespread infection (*14*). Studies documenting the time course of SARS-CoV-2 RNA shedding are mainly limited to mild disease (*7*) and typically show a progressive decline after a peak around symptom onset. However, viral load from lung swabs may correlate with disease severity (*15*), and patients with severe lung disease remain RNA-positive for longer. It is critical to determine how long active viral replication really persists in the lungs of patients with severe disease, and how frequently viral replication occurs at extrapulmonary sites where ACE2 (or other receptors) is expressed, such as vascular endothelium.

Although there are huge efforts to understand and treat severe COVID-19, it would be preferable to prevent the development and progression of clinical disease. How might this be achieved? Vaccine candidates are mainly aimed at eliciting neutralizing antibodies, to prevent the binding of spike to ACE2. The same rationale underpins the use of passive immunization, with convalescent plasma or monoclonal antibodies, or the administration of recombinant, soluble ACE2. Alternatively, antiviral drugs may be used to target essential viral enzymes such as the RNA-dependent RNA polymerase. Experience from other infections, such as influenza, emphasizes that treatment with antiviral agents is most effective when administered as early as possible in infection. Therefore, it is essential to identify individuals with early SARS-CoV-2 infection who are at high risk of progression to severe disease, and test antiviral therapies to prevent viral entry and replication. It should not be too difficult to identify these "at risk" patients who are in danger of progressing to severe disease through contact tracing and testing, even prior to symptom onset. Conversely, delaying candidate antiviral treatment until patients are hospitalized with severe lung injury may be too late, and combination with immune modulation is likely to be required. ■

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