How to Survive COVID-19 Even If the Vaccine Fails

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Coronavirus disease 2019 (COVID-19) has created an emergency of epic proportions. While a vaccine may be forthcoming, this is not guaranteed, as discussed herein. The potential problems and ominous signs include (1) lung injury that developed in animals given an experimental vaccine for the severe acute respiratory syndrome coronavirus (SARS-CoV)-1; (2) a perversion of adaptive immune responses called antibody-dependent enhancement of infection that occurs in SARS-CoV-1 and that may occur in people vaccinated for COVID-19; (3) the frequent and recurrent infections that are caused by respiratory coronaviruses; and (4) the appearance of mutations in SARS-CoV-2 proteins, which raise the specter of vaccine escape mutants. Because success is uncertain, alternatives to vaccines need to be vigorously pursued during this critical moment in the pandemic. Alternatives include (1) engineered monoclonal antibodies that do not cause antibody-dependent enhancement; (2) cocktails of antiviral drugs and inhibitors of the cellular proteins required for SARS-CoV-2 replication; (3) interferons; and (4) anticoagulants, antioxidants, and immune modulators. To organize and coordinate the systematic investigation of existing therapies and new therapies (as they emerge), a Covid-19 clinical trials network is needed to provide (1) robust funding (on a par with vaccine funding) and administration; (2) an adaptive trial design committee to prioritize interventions and review results in real time; (3) a computer interface to facilitate patient enrollment, make data available to investigators, and present findings; (4) a practice guidelines study group; and (5) a mobile corps of COVID-19 experts available for rapid deployment, to assist local health care providers and enroll patients in trials as outbreaks occur. To combat the COVID-19 pandemic and future mass contagions, the network would be a cornerstone of a comprehensive infectious diseases research program. (Hepatology Communications 2020;4:1864-1879).

For decades, zoonotic disease experts warned of a looming apocalypse. Their warning came to life in 2019 when severe acute respiratory syndrome coronavirus (SARS-CoV)-2 jumped into humans and set off a global pandemic of coronavirus disease 2019 (COVID-19). Within a matter of months everything changed. By the middle of May 2020, SARS-CoV-2 had infected over 1.5 million people in the United States and killed over 90,000 of them, up-ending personal life, commerce, and health care. In New York City, the U.S. epicenter, liver fellows and faculty were redeployed to care for patients with COVID-19, sometimes practicing in makeshift wards set up in hospital lobbies and in tents pitched in Central Park: It was, “All hands on deck.” Overnight, office visits were converted to video televisits—medicine’s version of social distancing. Noon conferences and pizza were replaced by connectivity problems and mute buttons.

The discovery of live SARS-CoV-2 in feces and evidence that it infects intestinal cells raised concerns...
about the safety of endoscopies.\(^1-3\) A case report of acute hepatitis in a patient with COVID-19 and autopsy data showing that SARS-CoV-2 infects the liver alerted hepatologists to the possibility that this new pathogenic virus might directly cause liver damage.\(^4-6\) This added to worries about collateral damage (i.e., increased alcohol consumption due to cabin fever\(^7\) and drug-induced liver injury caused by home remedies and experimental COVID-19 treatments) and to concerns that SARS-CoV-2 poses special risks for obese patients with fatty liver disease\(^8\) and for immunosuppressed transplant patients. Many patients sensed the threat and took action to protect themselves, avoiding the hospital at all cost, even if this meant delaying necessary treatments and liver cancer screening.

Questions abound: What makes SARS-CoV-2 so virulent? Where is the pandemic headed? What is the timeframe for herd immunity and a vaccine? How soon will a better understanding of COVID-19 pathogenesis lead to more effective clinical management? This article focuses on the SARS-CoV-2 entry process, evolution, immune responses, and prospects for vaccine development and improved clinical management. Studies that have not yet undergone peer review are marked by an asterisk.

Coronavirology

Coronaviruses are plus-strand RNA viruses, meaning that the infectious virus particle contains a single-stranded RNA that is capable of functioning as a messenger RNA (mRNA) and directing the synthesis of viral proteins.\(^9,10\) Coronavirus RNA genomes are about 30,000 bases long, giving them about 3 times the coding capacity of hepatitis C. To maintain the integrity of the exceptionally long RNA genome, coronaviruses have a proof-reading exonuclease that removes many of the copying errors introduced by the viral RNA–dependent RNA polymerase. Genetic changes still occur, however, through a combination of point mutations and recombination. Recombination allows sets of mutations in two parental viruses to combine into a single progeny, as occurs during sexual reproduction in plants and animals.

Taxonomically, SARS-CoV-2 is in the family Coronaviridae in the genus beta-coronavirus. Across the entire genome, SARS-CoV-2 is over 95% identical to Yunnan 2013 RatG13,\(^11-13\) a virus isolated from a horseshoe bat in China. It is possible, indeed likely, that bats harbor other viruses that are even more closely related to SARS-CoV-2 than RaTG13. Only a tiny fraction of the world’s coronaviruses has been sampled. The millions of contacts that occur every year between humans and virus-infected animals provide recurring opportunities for zoonotic transmission. It is sobering to realize that SARS-CoV-2 is the third highly pathogenic coronavirus known to have infected humans during the past 20 years. It was preceded in 2002 by SARS-CoV-1, and in 2012 by the Middle East respiratory syndrome coronavirus (MERS-CoV). SARS-CoV-2 is about 80% identical to SARS-CoV-1, which was eradicated by quarantine. This was possible in large part because patients with SARS were not infectious until they had obvious symptoms, allowing timely isolation. MERS-CoV continues to infect camels in the Middle East. Person-to-person transmission is rare. Most infections are acquired from dromedary camels, the intermediate host. As revealed by analysis of banked camel specimens, MERS-CoV circulated in camels for at least 20 years before the first human case of MERS was identified.\(^14\) In addition to the highly pathogenic viruses, four respiratory coronaviruses infect humans and cause “common colds” seasonally.

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SARS-CoV-2 Entry Mediated by Angiotensin Cleaving Enzyme 2 and the Viral Spike Protein

Viruses need to get into cells to replicate. Entry is not easy. In general, viruses are restricted to cells that have a surface protein the virus can attach to with high affinity. The viral entry receptor of SARS-CoV-1 and SARS-CoV-2 is angiotensin cleaving enzyme 2 (ACE2), a protein involved in blood pressure regulation (Fig. 1). ACE2 is expressed on cells in many organs, including the eye, throat, lung, kidney, liver, heart, and intestine. Its wide distribution helps SARS-CoV-2 infect multiple organs. The viral spike (S) protein binds ACE2. It has two subunits, S1 and S2.\(^{(9,10,15)}\) S1 is shaped like a club. It has the entry receptor binding domain (RBD) at its tip (Fig 1, insert, left side). S2 harbors a domain capable of fusing with the cell's surface membrane. After the RBD binds ACE2, the fusigenic peptide in S2 needs to be unmasked and activated. For this to occur, the linker between S1 and S2 must be severed (Fig. 1, insert, right side). Importantly, in SARS-CoV-2, the linker can be cut by several widely distributed cellular proteases, including furin.\(^{(11,12)}\) This cut site is not present in SARS-CoV-1, and it may enhance transmissibility of SARS-CoV-2.

An Alternative Entry Process Mediated by Antiviral Antibodies and Fc Receptors

Several viruses, including SARS-CoV-1, human immunodeficiency virus (HIV), and dengue virus, can infect cells by an alternative process called antibody-dependent enhancement (ADE) of infection.\(^{(16-18)}\) SARS-CoV-2 might also use ADE to infect cells. ADE is important because it increases pathogenesis during natural infections and can be a barrier to vaccine development, as illustrated by the fatal disease that occurred in cats given an experimental vaccine for feline infectious peritonitis virus, a coronavirus.\(^{(14,19,20)}\) In ADE, antiviral immunoglobulin G (IgG) antibodies attach to the surface of a virus particle and form an antibody-virus complex that binds to Fc\(\gamma\) receptors on target cells, allowing the complex to be internalized (Fig. 2A). Innate immune cells of the myeloid lineage (monocytes and macrophages) are frequent ADE targets. Paradoxically, the antibodies that mediate ADE are often capable of viral neutralization. Neutralizing antibodies bind viral surface proteins and prevent infection through the classical entry pathway (Fig. 2B). While many neutralizing antibodies are
protective, others mediate ADE and exacerbate disease.

Data from studies in Chinese rhesus macaques and in cultured cells can be used to construct a model of how ADE might increase lung injury in patients with pathogenic coronaviruses.\textsuperscript{(17,18)} These studies reveal that SARS-CoV-1-infected animals given anti-S antibodies had worse lung damage than infected animals given control antibodies.\textsuperscript{(17,18)} Vaccination with a platform that induced high levels of anti-S neutralizing antibodies increased lung injury in animals that were later challenged with the virus, even though viral replication was significantly reduced by vaccination. Interestingly, compared with patients who recovered from SARS, patients who died had higher titers of neutralizing anti-S antibodies. Their sera provoked greater proinflammatory responses in virus-infected macrophages than the sera of patients who recovered. Convalescent patients had relatively high levels of nonneutralizing antibodies directed against other viral proteins. The inflammatory responses could be reduced by blocking Fcγ receptors on the cells.\textsuperscript{(17)} Of interest, the elevated pro-inflammatory responses only occurred in the subset of macrophages polarized toward a wound-healing response. Studies carried out in HL-CZ cells, a human promonocyte cell line, confirm that anti-S antibodies promote SARS-CoV-1 infection.\textsuperscript{(16)} Several monoclonal antibodies against the S protein were tested. Only one performed as desired, preventing infection. All others had mild-to-moderate ADE effects, enhancing infection.

In addition to ADE, antibodies can also enhance viral infectivity through pathways that do not involve Fc receptors, as demonstrated by Wang et al., who investigated monoclonal antibodies directed against the SARS-CoV-1 S protein.\textsuperscript{(21)} They propose that the antibodies enhance virion attachment and/or membrane fusion by subtly altering the conformation of the S protein. At high doses, one of the enhancing antibodies increased lung damage in SARS-CoV-1-infected macaques, demonstrating its pathogenic effects.

Taken together, these studies lead to the following testable model and tentative conclusions: (1) Anti-S antibodies, including neutralizing antibodies, may contribute to the precipitous “crash” that many patients with SARS/COVID-19 experience 7-14 days after the onset of symptoms when virus-specific antibodies appear; (2) macrophages infected via ADE and/or activated by engagement of their Fc receptors produce excessive amounts IL-6 and other pro-inflammatory cytokines, damaging lungs and other organs; (3) drugs capable of down-modulating pro-inflammatory macrophages may reduce organ damage; (4) the biological effects of anti-S antibodies—whether they protect or increase damage—depends on their concentration and structure (i.e., exactly where they bind the S protein).
protein and their isotype, which governs interactions with Fc receptors); and (5) vaccines need to be scrutinized to make sure their benefits exceed their risks in people of all ages and with a range of underlying conditions. Decreased viral replication is not the only important endpoint. Organ damage needs to be assessed as well.

**Mutation in the S Protein and the Global Spread of a New Clade**

Mutations in the SARS-CoV-2 S protein are important because of their potential to impact both viral transmission and vaccine development. A recent report describes a mutation located near the RBD in the center of an antibody binding site previously identified in the SARS-CoV-1 S protein. The mutation changes aspartic acid (D) to glycine (G) at amino acid 614. Most of the G-clade viruses (i.e., viruses with the G614 mutation) have two additional mutations: a synonymous substitution in the nonstructural protein 3 gene and a P323L mutation in the RNA dependent RNA polymerase. In early March 2020, the G-clade was about 4% (7 of 183) of all recorded sequences globally. By 6 weeks later, it had exploded to 56%. Wherever G614 appeared, it rapidly increased in frequency, in many cases becoming predominant in only a few weeks.

This expansion could indicate that the G-clade is more fit than the (parental) D-clade or it could be caused by a founder effect, meaning the G-clade is spreading for reasons unrelated to its inherent fitness, such as by infecting people who are more likely to transmit the infection to others because they travel to densely populated cities, as discussed. However, data from clinical specimens suggest the G-clade has an inherent replication advantage: G-clade viruses replicated to higher levels in specimens of patients hospitalized in Sheffield than D-clade viruses. For further testing of their potential fitness advantage, head-to-head comparisons of G-clade and D-clade viruses need to be carried out, with each of the three G-clade mutations tested individually and in combination.

SARS CoV-2 is a very recent émigré to the human population and is in the early stages of adapting to this new environment. Some mutations may be part of this adjustment and allow the virus to exploit its new host more efficiently. Others may result from immune pressure. Regardless of their origins, the mutations are important because they are likely to slow the development of herd immunity and could confound vaccine development. It is too early to know whether SARS-CoV-2 stimulates a durable and protective immune response, or whether vaccines will cause immunopathologies. Defining the immunological outcomes of SARS-CoV-2 infection is a priority for research. While awaiting the results, it is useful to examine data on other coronaviruses.

**Impermanence of Adaptive Immune Responses to the Human Respiratory Coronaviruses**

Clearly, the human respiratory coronaviruses do not induce durable immunity. Reinfections are common and recur at frequent intervals. A longitudinal study in New York City used weekly nasal swabs to detect episodes of infection by two respiratory beta-coronaviruses, HKU1 and OC43. Many of the participants had recurrent infections of the same virus within 18 months. The median time between episodes of OC43 infection was 9 months. At one extreme, sequential infections occurred within 4 weeks of each other. Children were more prone to recurrent infections, which could be an encouraging sign that adults build up resistance over time, or it could just reflect more frequent exposures in children. A previous study of 10 families in Seattle, Washington, had similar results. Reinfections were frequent and more common in children. One 37-year-old woman had three episodes of OC43 infection in 12 months.

In both studies, the infections were caused by community-acquired virus; thus, intrastrain variation could have contributed to the absence of protective immunity and reinfection. Similar results, however,
were obtained in an experimental trial that used
a single isolate of 229E, an alpha-coronavirus.\(^{26}\)
After initial infection, antibody titers rose quickly,
but declined over the next 12 months. Several volun-
teers challenged with the same virus isolate became
re-infected, although the infections were asymptom-
atic and the duration of viral shedding was reduced,
hinting that perhaps the initial infection produced a
degree of protection.\(^{26}\)
Taken together, these studies establish that both
antibody responses and protective immunity are fleet-
ing and only modestly effective, at best. They show
that the respiratory coronaviruses vex the human
adaptive immune system and can return to the same
person time after time. Their easy comings and goings
are reminiscent of the way Obi-Wan Kenobi bypassed
the Imperial storm troops in *Star Wars* by waving at
them and saying, “These aren’t the droids you are
looking for.”

**Interlude: How Viruses Avoid Extinction**

This next section expands on the Obi-Wan analogy
and provides information about the countermeasures
that viruses use to undermine host defenses and avoid
extinction. The battle begins during the first hours of
an infection. A network of internal sensors in cells
constantly monitors for pathogen-associated molecu-
lar patterns (PAMPS)—molecules that are emblem-
atic of microbial infection. Double-stranded RNA,
which is produced during the replication of every
RNA virus, is an example of a viral PAMP. Viruses,
including coronaviruses, use many mechanisms to
counter innate defenses, hiding their double-stranded
RNAs in vesicles and deploying viral proteases to
obliterate the sensors.

Despite active viral countermeasures, the cellular
sensors are exquisitely sensitive and nearly impossi-
bile to evade completely. Once activated, they trigger
the production and secretion of interferons. Type I
and type III interferons activate antiviral defenses in
neighboring cells, inducing local herd immunity at
the cellular level. Over 300 genes participate in the
response. The array of interferon-stimulated genes
(ISGs) includes molecules that establish an “anti-
viral state” that is hostile to viral replication, and other
molecules, including pro-inflammatory cytokines,
chemokines, and human leukocyte antigens (which
help the immune system recognize infected cells).
Importantly, infected, dying, and dead cells express
disease-associated molecular patterns (DAMPs)
that amplify the pro-inflammatory distress signal.
Immune cells receive this information and rush to
the site of infection.

Innate immune cells are the first responders. They
attempt to wall off and contain the infection and
immediately begin messaging to cells of the adap-
tive immune system. While innate defenses are
broad-spectrum and in some cases ham-fisted—killing
both infected cells and their neighbors—the T and B
lymphocytes of the adaptive immune system are more
selective. T cells home in on virus-infected cells. B cells
differentiate into antibody-producing cells. Ideally, at
the conclusion of an infection, the body is left with
memory T and B cells that are ready to react quickly,
preventing infection if re-exposure occurs. The goal
of vaccines is to mimic this process and stimulate the
production of memory T and B cells.

Durable protective immune responses and their
manifestation at the population level—acquired herd
immunity—are existential threats to viruses. Viruses
use an array of weapons to undermine adaptive
immune responses, just as they strive to undermine
innate defenses. Their weapons evolved over millen-
nia. They are highly sophisticated and incompletely
understood. Research is needed to uncover exactly how
the respiratory coronaviruses manage to repeatedly re-
infect the same person. Evidently, they somehow abort
the development of durable immunity. Each infection
is cleared, but the person is left susceptible to future
infections by the same virus. If SARS-CoV-2 has a
similar ability, herd immunity may be slow in coming.

**Dwindling Immunity to the SARS Viruses and
Cross-reactivity With the Respiratory Viruses**

The durability of adaptive immune responses
has been studied in patients who recovered from
SARS.\(^{27,28}\) The results are not encouraging.
Antibody titers plunged during the first 2 years after SARS-CoV-1 infection and were undetectable in most patients by 5 years following infection (Fig. 3A). At 5 years, no detectable virus-specific memory B cells, the progenitors of antibody-producing cells, remained. Virus-specific T cells did remain and interestingly they were higher in patients who had experienced more severe disease (Fig. 3B), suggesting that the size of the memory T-cell population is a marker of disease severity and greater exposure to viral antigens, rather than an indicator of the effectiveness of the immune response. Unless SARS-CoV-2 is stopped by a vaccine or differs from its relatives and induces strong long-lasting protective immunity, it is likely to join the ranks of the viruses circulating in human populations, causing endless cycles of disease.

Data about adaptive immune responses to SARS-CoV-2 are emerging. Lymphopenia is often profound, at least in patients with severe disease, suggesting that infection suppresses and dysregulates T cells. Antibodies are present in about 50% of patients by 1 week after symptoms appear, and develop in nearly all patients by day 19. There is a nonsignificant trend toward higher antibody titers in patients with higher serum levels of C-reactive protein (CRP) \( (P = 0.064) \), indicating that antibody levels are higher in patients with greater inflammation and more severe COVID-19. A study of neutralizing antibodies in 175 people who recovered from COVID-19 showed that the titer was higher in elderly and middle-aged patients than in patients 15 to 39 years of age. Only 14% of the patients developed high-titer neutralizing antibodies. Titers of neutralizing antibodies correlated positively with blood levels of CRP, a marker of inflammation, and negatively with lymphocyte counts. Ten patients who recovered completely did not develop detectable neutralizing antibodies, suggesting that neutralizing antibodies are not required to clear mild infections, although they might protect against future re-infection.

A small, but rigorous, study of 9 patients identified by contact tracing showed that neutralizing antibodies appear at about the same time as other virus-specific antibodies. The neutralizing antibodies did not affect the trajectory of viremia, which was already in decline by the time of seroconversion when antibodies became detectable (Fig 4, red-brown arrows). In most patients, the virus was
cleared from swabs of the oropharynx (Fig 4, yellow lines) before it was cleared from sputum and stool (Fig. 4, orange and gray lines, respectively), indicating that infection continues at internal sites after the oropharynx tests negative. (Note to self: Do not discontinue antiviral drugs based on declining viral load in nasal swabs.)

Of potential importance for both vaccine design and for understanding natural resistance/susceptibility to COVID-19, Long et al. found that SARS-CoV-2 infection increases the titer of antibodies that recognize the respiratory coronaviruses. Among other possibilities, this finding could indicate that the respiratory viruses and SARS-CoV-2 share cross-reactive antibody epitopes, and it raises the possibility that antibodies stimulated by respiratory viral infections alter the outcome of SARS-CoV-2 infection. On the one hand, cross-reactive antibodies could contribute to the mild course of COVID-19 disease that occurs in most children. Children have more frequent colds than adults, as noted previously, and thus may have higher titers of protective cross-reactive antibodies. On the other hand, cross-reactive antibodies might have a role in the pediatric multisystem inflammatory syndrome and/or in ADE in adults.

To terminate a viral infection and prevent a recurrence, antibodies work with T cells. A recent study showed that most convalescent patients with COVID-19 had T cells that recognized both the N-terminal and C-terminal domains of the SARS-CoV-2 S protein, and revealed that 34% of healthy donors (who had not had COVID-19) had T cells that reacted to peptides representing the C-terminal domain of the SARS-CoV-2 S protein, presumably reflecting prior exposure to respiratory coronaviruses. Research is needed to determine whether the cross-reactive T cells and cross-reactive antibodies affect the course of COVID-19 disease.

**Prospects for a COVID-19 Vaccine: Accelerate Alternatives Just as Vigorously!**

News reports make it seem that a COVID-19 vaccine is just around the corner, but success is not
guaranteed. Decades of work have not yielded vaccines for HIV or hepatitis C virus (HCV), and, as noted previously, an experimental SARS vaccine caused lung injury in nonhuman primates.\(^{17}\) Based on the many unsuccessful human vaccine programs that increased disease, Huisman et al. cautioned, “There may well be a delicate balance between the induction of protective immunity on the one hand and the induction of enhanced susceptibility on the other.”\(^{19}\) Several barriers may stand in the way of developing a COVID-19 vaccine: (1) ADE and antibody-mediated enhancement of SARS indicate that SARS-CoV-1 repurposed adaptive immune responses and turned them to its own advantage (therefore, a COVID-19 vaccine may be subject to similar exploitation); (2) memory B cells were undetectable in patients who had recovered from SARS-CoV-1 infection several years earlier, and antibody levels declined rapidly over time; (3) respiratory beta-coronaviruses cause repeat infections, suggesting that adaptive immune responses to this group of viruses are subpar; and (4) the D614G mutation proves that SARS-CoV-2 is evolving in real time and therefore may be capable of escaping from any potent responses that the adaptive immune system is able to mount.

Despite the challenges, modern vaccine technologies are powerful and varied. They may yield an effective vaccine. Vaccines can succeed where natural immunity fails, as illustrated by the ability of the hepatitis B virus vaccine to induce protective immunity in newborns who would otherwise become chronically infected as a consequence of vertical transmission. Vaccines for SARS-CoV-2 must be given adequate funding and time for testing in large phase 3 trials. A very large study group will be needed to ensure that both the impact of the vaccine on the infection rate and its impact on disease severity and ADE can be evaluated in a wide spectrum of people, including older patients and patients with cirrhosis and other conditions that weaken vaccine responses. Efforts to develop a vaccine may fail if political resolve wanes and the number of candidate vaccines narrows prematurely, and/or if a few vaccine recipients experience severe adverse effects that spark an antivaccine movement. Because success is uncertain for both scientific and sociological reasons, alternatives to vaccines need to be vigorously pursued during this critical moment in the evolution of the pandemic.

Currently, several groups are testing the utility of convalescent plasma. A recent randomized trial showed some promising trends that were generally not statistically significant\(^{34}\); the trial was stopped early due to the inability to complete enrollment. A second trial of convalescent plasma was also stopped early when the investigators discovered that although the patients had been symptomatic for only 10 days, 44 of 66 (67%) had neutralizing antibodies, raising questions about the likelihood that donor plasma would not improve outcomes.\(^{35}\) To reduce the risk of ADE and other immunopathologies that might be caused by convalescent plasma, a cocktail of engineered monoclonal neutralizing antibodies lacking the Fc region could be developed. This type of passive immunization would require repeated administration, raising costs; however, it could buy time for other interventions and vaccines to be developed. Costs could be contained by reserving the monoclonal cocktail for people in assisted living facilities (nursing homes) and other high-risk populations.

Antiviral drug development is also an essential area for research. SARS-CoV-2 provides many attractive drug targets, including the RNA-dependent RNA polymerase, viral proteases, and the viral RNA itself. Cellular proteins required for viral replication are also potential drug targets. In today’s laboratories, thousands of compounds can be evaluated for activity and toxicity through high-throughput screens in cell culture, with the most promising molecules selected for chemical modification and accelerated medicinal development. Although antiviral drugs are typically used to treat infections, they can also be used prophylactically to prevent them, as illustrated by pre-exposure prophylaxis for HIV. Efforts to develop oral drugs that prevent SARS-CoV-2 infection should be given a high priority. Prophylactic drugs could be coupled with enhanced surveillance to recognize and contain nascent outbreaks. Clinical trials of existing drugs offer the best immediate hope for reducing the case/fatality rate and must be part of the containment plan, as discussed in detail subsequently. A double-blinded, randomized, placebo-controlled trial of the antiviral drug remdesivir showed a shortened time to recovery among hospitalized adults who had evidence of lower respiratory tract involvement.\(^{36}\) Among the large subgroup of patients receiving oxygen but no
ventilation, the Kaplan-Meier estimate of deaths by day 14 were 2.4% in the remdesivir group versus 10.9% in the control group, a 4.5-fold difference. The next generation of antiviral drugs is expected to yield even better results.

In addition to vaccines, passive immunotherapies (monoclonal antibodies, convalescent serum, and hyperimmune globulin) and antiviral drugs, there is interest in combating SARS-CoV-2 by activating innate defenses. As predominant drivers of the antiviral state, the alpha, beta, and lambda interferons are attractive therapeutic candidates.

Interferons: Pharmaceutical Activation of Innate Immune Responses

Interferon alpha is well-known to all but the youngest of hepatologists, because it played a central role in HCV treatment before 2014, when direct acting antiviral drugs became widely available. Interferon alfacon-1 was examined in an open-label study of patients with SARS in combination with corticosteroids. Patients on interferon alpha resolved their radiologic lung abnormalities and dependence on supplemental oxygen more quickly than patients receiving corticosteroids alone. Promising results were also obtained in patients with COVID-19 who were treated with aerosolized interferon alpha and in patients treated with interferon beta that was part of a cocktail. Interferon lambda also has activity against human coronaviruses, including SARS-CoV-1, and MERS-CoV, a case can be made for further testing. Interferon lambda also has activity against human coronavirus, including SARS-CoV-1, and MERS-CoV, and it causes fewer side effects than interferon alpha, most likely because its receptor is preferentially expressed on epithelial cells, whereas the interferon alpha/beta receptor is expressed on virtually all cells, including immune cells.

SARS-CoV-2-infected intestinal organoids up-regulate interferon response pathways, providing a valuable model system for investigating the ability of interferons to reduce viral replication. It will be interesting to see whether there is an inverse relationship between the intensity of the interferon response and the duration of local viral shedding. A pilot study showed that treatment with aerosolized interferon alpha accelerated clearance of SARS-CoV-2 from nasal swabs and decreased serum level of IL-6 and CRP.

Pathogenesis of the COVID-19 Cytokine Storm

There has been some concern about treating patients with COVID-19 with interferons out of fear that interferons might worsen the cytokine storm in patients with severe disease. However, a recent study demonstrated that SARS-CoV-2 stimulates only a very modest pro-inflammatory cytokine response from infected primary human alveolar epithelial cells, blood-derived macrophages, and Caco2 cells (Fig. 5 D-F, green bars). The response was far lower than the one provoked by two influenza viruses (Fig. 5 D-F, rust and lavender bars).

The very low amplitude raises the possibility that the initial pro-inflammatory response stimulated by SARS-CoV-2 is too low during the early stages of clinical infection, rather than too high, and contributes to worse outcomes by failing to alert the immune system to the gravity of the threat at a time when fast action could contain the infection locally, like what happened in cities that delayed lockdown because leaders did not see the warning signs soon enough. The attenuated pro-inflammatory response might be part of the strategy that coronaviruses use to keep durable immune responses from developing. SARS-CoV-2 evolved in bats, and its survival strategy may be a bridge too far in people—attenuating T-cell responses to the point that local infection in the oropharynx gets out of control and catastrophic dissemination into the alveoli, heart, and kidney occurs. Failure of early containment would prolong the period of active viral replication; therefore, increasing the chances that viral replication would still be going on when anti-S antibodies appear, providing the conditions necessary for ADE and other immunopathologies.

It’s the Virus

A recent autopsy study showed that SARS-CoV-2 replicates in lungs, pharynx, heart, kidney, liver, and brain, and confirmed that it is often detectable in
FIG. 5. Viral gene and TNF-α mRNA levels of control cells compared to cells infected with SARS-CoV-2 and other viruses. (A-C) Expression of viral mRNAs (SARS-CoV-2 and SARS-CoV-1 open reading frame 1b gene; MERS-CoV UpE gene; influenza matrix gene). (D-F) Quantitation of TNF-α mRNA. Analysis was performed on alveolar epithelial cells, human macrophages, and Caco-2 cells. Graphs show mRNA copies normalized to β-actin. Abbreviations: AEC, alveolar epithelial cell; H1N1pdm, 2009 pandemic influenza H1N1; and H5N1, highly pathogenic avian influenza H5N1 virus. Reproduced from Mahlakõiv et al. (40)
blood during the late stages of disease. These data suggest that active viral replication occurs in multiple organs right up until death, implying that uncontrolled viral replication and an insufficient antiviral response contributes to the cytokine storm during late-stage progressive disease. A cross-sectional study of patients from various points in the disease spectrum, ranging from mild to critical, supports this interpretation.
It revealed that patients with mild/moderate disease had high blood levels of interferon alpha, and their white blood cells expressed high levels of interferon-stimulated genes (ISGs). In contrast, patients in critical condition had low levels of interferon alpha, a “striking” down-regulation of ISGs, and a marked decrease in plasmacytoid dendritic cells, the premier interferon alpha producing cells. These patients also had a marked decrease in T cells and natural killer cells in blood and elevated levels of IL-6, tumor necrosis factor α (TNF-α), and lactate dehydrogenase, a marker of cell injury and necrosis. Despite down-regulation of ISGs, their white blood cells retained the ability to respond to interferon, as demonstrated experimentally, a positive sign that interferon treatment might be effective. The investigators concluded that IL-6 and TNF-α likely contribute to pathogenesis and propose that type I interferon deficiency is a hallmark of severe COVID-19. They suggest that type I interferon treatment could mitigate disease, potentially when used in combination with anti-inflammatory therapies targeting IL-6 and/or TNF-α. A direct acting antiviral drug (remdesivir) might be a useful addition to the cocktail.\(^{(36)}\)

**Individualized Treatment Across the Evolving Course of Disease**

As additional data about the clinical management of patients with COVID-19 emerge, it will become

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**FIG. 8.** Boom-bust funding for coronavirus research (A) and chronic underfunding of hepatitis C research (B). (A) Before the COVID-19 pandemic, public and philanthropic funding for coronavirus research was only 0.5% of global funding on infectious disease, according to the Research Investments in Global Health Study, University of Southampton. Previously, funding spiked briefly after outbreaks of SARS and MERS. (B) National Institutes of Health funding for HCV research is 30-fold lower than for HIV research, whereas HCV-related mortality is over 2-fold higher. Reproduced from Head et al.\(^{(46)}\) and Saab et al.\(^{(47)}\)
feasible to individualize treatment to match a patient’s evolving pathophysiology (Fig 6). Antiviral drugs and interferons may be helpful for patients with mild/moderate symptoms. Management of the cytokine storm will need to be based on its causes in an individual patient. ADE-mediated infection and excessive inflammation might require a combination of antivirals, antioxidants, IL-6/TNF-α blockers, and immunosuppressants. A randomized open-label trial of dexamethasone versus standard of care showed that dexamethasone reduced 28-day mortality by 3%, from 24.6% in the control arm to 21.6% in the active treatment arm; among patients receiving invasive mechanical ventilation, mortality was reduced from 40.7% to 29.0%.34 Adjustment of ventilator settings and repositioning of patients into the prone position (to reduce labored breathing) may lessen mechanical lung damage and allow some patients to avoid mechanical ventilation. The judicious use of anticoagulants may mitigate coagulopathy. After viral replication has ceased, interventions that promote tissue repair, shorten convalescence, and reduce long-term pulmonary, kidney, and cardiac dysfunction will be extremely important.

Re-imagine

The COVID-19 pandemic is an emergency. Unprecedented measures, including a national/international COVID-19 clinical trials network, are needed to optimize existing therapies while awaiting new therapies and vaccines. The network could provide an infrastructure for testing both existing interventions and new interventions when they become available. As illustrated by the increases in survival achieved through a coherent research program in childhood leukemia35 (Fig. 7), a systematic series of clinical trials can greatly improve the effectiveness of treatment by allowing optimization of patient selection, dosage, timing, and synergism between the components of drug cocktails and treatment bundles (combinations of drugs and procedures, such as the use of ventilators). It is likely that the COVID-19 case/fatality rate can be cut in half (or more) just by learning how to use existing therapies most effectively.

An established network will be especially important for combating COVID-19, because the number of cases in a geographic location changes quickly. By the time researchers can put the necessary infrastructure in place, their city may no longer have cases. The network would address this problem by providing (1) funding; (2) an administrative core to enroll sites, oversee compliance, and distribute funds; (3) an adaptive trial design committee to prioritize interventions for testing and to review results in real time; (4) a computer interface allowing clinicians and investigators to find open trials, enroll patients, and review results; and (5) a mobile corps of clinical investigators with experience treating patients with COVID-19 who would rapidly deploy to local hospitals, assisting the health care professionals and enrolling patients in clinical trials. The network would regularly compile and update evidence-based practice guidelines.

The COVID-19 network could be part of a larger initiative to prevent future pandemics and combat existing infectious diseases. The world is lucky that a small, but unstoppable, group of coronavirus researchers persisted despite the crisis-management approach that has governed funding: Support increased briefly after outbreaks of SARS and MERS, but soon disappeared (Fig. 8A).36 Boom/bust cycles are not ideal for combating microbes, nor is the related pattern of chronic underfunding of research. As discussed by Saab et al.,37 inadequate funding contributes to the persistence and spread of hepatitis C, which remains widespread (and rapidly expanding in many communities), despite the availability of curative treatments (Fig. 8B). For the United States to manage the current pandemic and move forward, stronger infrastructure and more stable and adequate funding for basic, translational, clinical, and public health research must be provided. This will save lives. Now is the time, before the next pathogenic virus takes the big leap into humans.

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