Prospects for a safe COVID-19 vaccine

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Rapid development of an efficacious vaccine against the viral pathogen severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the cause of the coronavirus disease 2019 (COVID-19) pandemic, is essential, but rigorous studies are required to determine the safety of candidate vaccines. Here, on behalf of the Accelerating COVID-19 Therapeutic Interventions and Vaccines (ACTIV) Working Group, we evaluate research on the potential risk of immune enhancement of disease by vaccines and viral infections, including coronavirus infections, together with emerging data about COVID-19 disease. Vaccine-associated enhanced disease has been rarely encountered with existing vaccines or viral infections. Although animal models of SARS-CoV-2 infection may elucidate mechanisms of immune protection, we need observations of enhanced disease in people receiving candidate COVID-19 vaccines to understand the risk of immune enhancement of disease. Neither principles of immunity nor preclinical studies provide a basis for prioritizing among the COVID-19 vaccine candidates with respect to safety at this time. Rigorous clinical trial design and postlicensure surveillance should provide a reliable strategy to identify adverse events, including the potential for enhanced severity of COVID-19 disease, after vaccination.

INTRODUCTION

The new human viral pathogen, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the cause of the coronavirus disease 2019 (COVID-19) pandemic, emerged in Wuhan, China in late 2019. The global COVID-19 pandemic continues to expand in many countries, including the United States. A protective vaccine will be required to achieve sufficient herd immunity to SARS-CoV-2 infection to ultimately control the COVID-19 pandemic (1). The World Health Organization (WHO) has listed more than 200 COVID-19 vaccines as under development (2), and expectations for effective prophylactic COVID-19 vaccines are high. The hope that preventive vaccines will control COVID-19 is justified by the impact of vaccines on preventing disability and death from other infectious diseases (3). Vaccines against infectious diseases are estimated to have saved at least 23 million lives between 2011 and 2020 (4).

An essential part of developing any vaccine is to ensure that known and theoretical safety risks are identified, quantified, and weighed against potential benefits. Among the potential risks raised in the context of COVID-19 vaccine development is whether the immune responses elicited by a vaccine could enhance SARS-CoV-2 acquisition or make the disease worse when infection occurs after vaccination. Recent commentaries have provided background and assessments of aspects of this question as it relates to COVID-19 vaccines (1, 5–9). Here, we review the relevant literature and evaluate the possibility of enhanced disease caused by COVID-19 vaccines.

For this Review, we define immune-associated enhanced disease as an infection that is made worse because the person has a preexisting immune response against the pathogen. Vaccine-associated enhanced disease (VAED) is defined as an immune response to a vaccine that is causally linked to a higher risk of adverse outcomes upon infection compared with infection without prior vaccination. Pathogen-specific antibodies have been associated with disease enhancement, called antibody-dependent enhancement (ADE), in rare cases of secondary dengue infection (6–8). VAED was observed in children given formalin-inactivated whole-virus vaccines against respiratory syncytial virus (RSV) and measles virus in the 1960s. Here, we assess in vitro data, animal model data, and human data relevant to forms of VAED to provide background for vaccine scientists and developers, health care providers, policymakers, and public health advocates.

Immune enhancement of viral infections after vaccination or natural infection

RSV is the leading cause of bronchiolitis and pneumonia in the first 1 to 2 years of life and is also a cause of severe respiratory illness in older persons. VAED was observed when a formalin-inactivated vaccine for RSV (FI-RSV) was given to infants and young children in clinical trials in the 1960s (Fig. 1) (1–3, 5, 10–12). In these studies, the overall incidence of RSV infection was not increased when compared with either an unimmunized or a formalin-inactivated parainfluenza-vaccinated group (FI-PV) (11, 12). However, hospitalization rates for severe RSV were higher in children vaccinated with FI-RSV from 6 to 11 months of age, with two fatal cases in this age group, and, to a lesser extent, in those immunized at 12 to 23 months of age, but not in children vaccinated at >2 years. These age-associated differences indicated that the risk was highest in infants with an immature immune system or when FI-RSV was administered before the child’s first encounter with RSV. Notably, parainfluenza virus hospitalizations were not increased among children given FI-PV, despite formalin inactivation of the vaccine (11).

The FI-RSV studies were terminated because of VAED, but specific characteristics of FI-RSV–induced immunity were not established as causative. RSV neutralizing antibodies are primarily directed...
against the fusion protein that exists in a metastable prefusion conformation before virus entry into host cells, which changes to a postfusion form upon host cell receptor engagement. The postfusion state is the predominant conformation after formalin inactivation (13). In the FI-RSV clinical trials, neutralizing antibodies were induced by the vaccine in fewer children (43%; 10 of 23) than after natural infection (75%; 12 of 16) (12). Although 14 of 15 vaccinees who developed RSV disease had neutralizing antibodies at the onset of illness (12), later studies indicated that vaccinees had a higher ratio of fusion protein binding antibodies than RSV neutralizing antibodies compared with controls with natural RSV infection (13). Later animal studies also showed that the FI-RSV lot used in the clinical studies failed to elicit neutralizing antibodies in cotton rats and that the animals developed more severe lung pathology upon RSV challenge than did mock vaccinated animals (15). How-ever, understanding immunological correlates of protection in the vaccinated children was limited because the only assay to measure T-cell immunity was lymphocyte transformation, which did not allow the assessment of antigen specificity, cytokine profiles, or cytotoxic functions of T cells induced by FI-RSV. Pathologically, the two infants with fatal infections had severe alveolitis with neutrophilic and lymphocytic infiltrates and peribronchial inflammation (16) as well as evidence of immune complex formation in lung tissues (17). Potential mechanisms of VAED suggested by these studies of children given the FI-RSV vaccine include antibodies directed against nonprotective fusion protein epitopes, a failure to elicit high-avidity neutralizing antibodies to RSV fusion protein, aberrant antibody responses to other RSV proteins, activation of the complement pathway by immune complex deposition, and abnormal T-cell responses (Fig. 1). Animal model studies support other potential factors including a bias toward T-helper type 2 (Th2) cell cytokine responses, a lack of antibody affinity maturation that may occur in young children because of several putative mechanisms, including poor Toll-like receptor stimulation (18), insufficient regulatory T-cell activity, and poor priming of cytotoxic T cells (19). Without a defined mechanism for VAED due to FI-RSV, the recent Vaccines and Related Biological Products Advisory Committee report concluded that “In the absence of a reliable method for differentiating between enhanced respiratory disease and severe RSV infection, identification of possible vaccine-associated enhanced respiratory disease will likely rest on detecting a significant difference in rates of severe RSV disease between vaccine and control groups” (19).

A formalin-inactivated measles virus vaccine licensed in the 1960s was withdrawn because some immunized children developed atypical measles with high fever, an unusual petechial/papular rash, and atypical pneumonia (20). Measles neutralizing antibodies persisted in only 25% of immunized children at 1 year of age, and 8 of 125 vaccinees developed atypical measles after a known exposure two or more years later (20). When live attenuated measles virus was given after formalin-inactivated measles virus, papular lesions that showed immune complex deposition appeared at the inoculation site. In contrast, the live attenuated measles virus vaccine has high protective efficacy with no enhanced disease (21).

Dengue infections are caused by one of the four related dengue virus serotypes. Rarely, these viruses cause dengue hemorrhagic

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**Fig. 1. Immune enhancement of human viral disease.** Immune enhancement of human viral disease through viral reinfection or vaccination has been documented in (top) natural dengue virus infection and (bottom) vaccination with a formalin-inactivated vaccine for RSV. (Top) During natural dengue virus infection, IgG antibodies protect against dengue virus of one serotype by causing uptake of virus particles and their degradation when the Fab fragment of IgG binds to a surface viral protein and the Fc portion of IgG binds to Fcγ receptors expressed by macrophages and other immune cells. A second infection with a different dengue virus serotype creates a risk of ADE of disease because cross-reactive antibodies against the first serotype that have limited neutralizing capacity can mediate internalization of the virus by Fcγ receptor-bearing cells. Viral immune evasion mechanisms then allow the production and release of new virions. (Bottom) Vaccine-associated enhancement of disease (VAED) occurred in some children given a formalin-inactivated RSV vaccine in the 1960s. Although the immunological mechanisms of VAED remain undefined, fatal RSV infection occurred in two children after vaccination and was associated with complement activation. This was attributed to the formation of immune complexes and their deposition in the lungs, and peribronchial infiltrates and alveolitis associated with pulmonary infiltration by neutrophils and eosinophils, which is consistent with a Th2-biased CD4+ T-cell response. To date, none of these mechanisms are known to apply to SARS-CoV-2 infection.
Experience with other viral infections and viral vaccines

Despite the high antigenic diversity and prevalence of influenza viruses, extensive annual surveillance has not revealed correlations between more severe illnesses and preexisting immunity. When an antigenic shift caused the 2009 H1N1 pandemic, a cohort of middle-aged patients was reported to have low-avidity antibodies against the H1N1-2009 virus, and six people in this age group with fatal pneumonia had evidence of pulmonary immune complex formation (26). Thus, decades of surveillance suggest that immune enhancement of natural influenza virus infection is rare despite the prevalence of cross-reactive antibodies with limited neutralizing activity. In addition, influenza immunization programs demonstrate that inactivated vaccines per se do not potentiate the risk of VAED, even though vaccine antigens used to induce immunity may not be matched to the influenza viruses that emerge (27). Whereas some epidemiological studies of the 2009 H1N1 pandemic reported more medically attended illnesses among vaccinated people (28), others supported vaccine efficacy (26, 29), partial protection, or infection but without evidence of VAED (30).

Although cross-reactive antibodies to parainfluenza viruses 1, 2, and 3 are elicited and the same individual is typically infected with the other virus serotypes over time, preexisting immunity is not known to result in severe disease due to a different parainfluenza virus serotype.

Infection by different rotavirus serotypes is another example of a circumstance where cross-reactive immunity typically provides some protection and does not potentiate disease. Inactivated vaccines, such as the polio vaccine, may induce less potent neutralizing antibodies against one or more viral serotypes, but VAED has not been reported. Thus, vaccines made from inactivated viruses do not have an intrinsic potential to elicit deleterious immune responses.

Immune enhancement of disease in animal models of human coronaviruses

The outbreak of SARS caused by the SARS-CoV-1 coronavirus emerged in Southern China in 2002, and the Middle Eastern respiratory syndrome (MERS) outbreak caused by MERS-CoV was first reported in Saudi Arabia in 2012. Although multiple animal models of SARS-CoV-1 and MERS-CoV infection and of the related coronavirus SARS-CoV-2 have been developed, they do not fully recapitulate the pathology or clinical symptoms of severe coronavirus infections in humans. Some elements similar to human pulmonary disease can be observed in mice, hamsters and Syrian hamsters, ferrets, and nonhuman primates. Animal models of SARS-CoV-2 infection have not shown evidence of VAED after immunization, whereas cellular immunopathology has been demonstrated after viral challenge in some animal models administered SARS-CoV-1 or MERS-CoV vaccines (Table 1). Whether cellular immunopathology is directly linked to VAED remains unclear as, in many cases, cellular pulmonary infiltrates are not associated with clear respiratory signs or illness. Whereas some in vitro experiments suggest the potential for ADE, their relationship to VAED in animal models has not been established.

SARS-CoV-2 studies in rhesus macaques. African green macaques, or cynomolgus macaques (31–36) have demonstrated acute, transient, and resolving interstitial pneumonia after virus inoculation, but infection elicits mild to moderate pulmonary disease with no progression to respiratory failure or death, unlike COVID-19 in humans with severe illness (32). COVID-19 exhibits greater severity in older humans; two studies in small numbers of aged macaques have suggested greater pulmonary disease due to either SARS-CoV-1 (37) or SARS-CoV-2 infection (38) compared with young macaques. Similarly, modified SARS-CoV-1 induces more severe disease in aged versus young mice (39). However, whereas expression of angiotensin-converting enzyme 2 (ACE2), the host cell receptor for SARS-CoV-2, has been reported to be higher in the endothelium of aged compared with young cynomolgus macaques (40), humans exhibit an age-associated decline in ACE2 expression (41), indicating that factors beyond ACE2 are likely to be critical for disease severity.

In animal models of SARS-CoV-2 infection, rhesus macaques were found to be resistant to SARS-CoV-2 reinfection in two studies, and there was no evidence of enhanced disease from prior infection (31, 32). In one study, neutralizing antibody titers correlated with protection from reinfection with SARS-CoV-2 (32). Several COVID-19 vaccines expressing the SARS-CoV-2 spike protein have now been tested in rhesus macaque SARS-CoV-2 challenge models. Vaccines tested include DNA vaccines (35), an inactivated virus vaccine with an alum adjuvant, an adenovirus vector vaccine (33), and a vaccine comprising mRNA encapsulated in lipid nanoparticles (42). Protective
efficacy has correlated with the titers of neutralizing antibodies against the spike protein (35), although analyses of T cell immunity are needed. SARS-CoV-2–infected macaques do develop some lung pathology, but they do not show clinical manifestations of COVID-19 or death; VAED or other evidence for immunopathology has not been observed after vaccination followed by SARS-CoV-2 challenge. Observations with SARS-CoV-1 and MERS-CoV vaccines also confirm that high titers of neutralizing antibodies against the spike protein correlate with protection from infection in ferrets and macaques (43–45).

In addition to evidence for protection, cellular infiltrates and immunopathology have been documented in some animal models of SARS-CoV-1 and MERS-CoV infection including mice, hamsters, rats, ferrets, and nonhuman primates (Table 1). Ferrets immunized with recombinant modified virus vaccinia Ankara (MVA) expressing SARS-CoV-1 spike protein followed by SARS-CoV-1 virus challenge developed cellular infiltrates in the liver and hepatitis (46). Cellular immunopathology was noted in BALB/c mice immunized with recombinant vaccinia virus expressing the SARS-CoV-2 spike protein or the nucleocapsid antigen, which was linked to increased production of proinflammatory cytokines, especially interleukin-6 (IL-6) (47). Cellular immunopathology was also observed in BALB/c mice immunized with Venezuelan equine encephalitis virus replicon particles expressing the nucleocapsid protein of SARS-CoV-1 (48).

In a SARS-CoV-1 infection and reinfection model in African green macaques, alveolitis and interstitial pneumonitis associated with dysregulated cellular inflammatory and cytokine responses were observed, but were unrelated to the presence of neutralizing antibodies or evidence of protection (44). Rhesus macaques immunized with MVA vectors encoding the SARS-CoV-1 spike protein also exhibited cellular immunopathology upon virus challenge, which was associated with a combination of IL-8 production and fewer macrophages expressing markers associated with wound healing (45). In both studies, immunopathology occurred despite the presence of high titers of virus neutralizing antibodies (44, 45). VAED after SARS-CoV-1 vaccination has been suggested to be associated with vaccine-induced T H 17 host responses, including extravasation of eosinophils from the bone marrow and infiltration of tissues (5, 49). Thus, the evidence suggests a potential role of T H 17 in coronavirus infections that differs from immune enhancement of disease due to the FI-RSV vaccine or dengue virus infection (Fig. 1).

SARS-CoV-1 vaccines comprising inactivated whole virus (with virus inactivation by formalin or ultraviolet irradiation), recombinant spike protein (expressed in baculovirus), or chimeric viral-like particles have elicited cellular immunopathology when administered to mice despite the presence of high titers of neutralizing antibodies (50). In these studies, an alum adjuvant was shown to reduce immunopathology compared with nonadjuvanted vaccines, a finding confirmed in mouse immunization experiments with the SARS-CoV-1 spike protein receptor binding domain formulated with alum (51). Other studies have highlighted the importance of inducing T H 17 responses and CD8+ T cells after vaccination of mice as a means to enhance protective immunity and prevent cellular immunopathology (45, 52–54). When MERS-CoV vaccines were tested in nonhuman primates including a DNA vaccine (55), a MERS-CoV spike protein receptor binding domain subunit vaccine with alum adjuvant, a spike protein subunit vaccine with Ribi adjuvant (56, 57), or an adenovirus vector vaccine expressing MERS-CoV spike protein, no lung immunopathology or VAED was observed after challenge with MERS-CoV.

Certain antibodies against the spike protein have been shown to enhance the uptake of SARS-CoV via immunoglobulin G (IgG) binding to FcγRII receptors expressed by cells in vitro (48, 58–60). For these studies, fluorescence microscopy and real-time quantitative reverse transcriptase polymerase chain reaction were used to measure infection of cells in vitro, rather than measuring the capacity of live viruses or pseudoviruses to replicate and produce more viruses in these cells. In vitro studies have shown ADE after infection of cultured cells with MERS-CoV or feline infectious peritonitis virus, an animal coronavirus (61, 62). For feline infectious peritonitis virus, serum antibodies can coincide with disease onset in cats, but disease may also arise due to mutations in the 3c gene of nonpathogenic feline enteric coronaviruses, leading to increased replication and transmission in the feline gut (61). In the case of MERS-CoV, one in vitro study showed that neutralizing antibodies that bound to the spike protein triggered a conformational change that facilitated virus entry into IgG Fc receptor–expressing cells (62). In a rabbit model of MERS-CoV, ADE was associated with non-neutralizing antibodies in addition to complement activation and other factors, but did not translate into clinically observable disease (58–60, 62, 63). An inactivated whole-virus MERS-CoV vaccine elicited eosinophilic immunopathology and, potentially, ADE in mice that were linked to neutralizing antibodies (64). Similarly, in a SARS-CoV-1 challenge model in African green macaques, lung immunopathology was unrelated to preexisting neutralizing antibodies (44), as was the case for a whole inactivated virus vaccine and other SARS-CoV-1 vaccines in mice (50).

### Table 1. Immune enhancement of coronavirus disease in animal models.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Infection or vaccine</th>
<th>Animal model</th>
<th>Immune enhancement of disease after virus exposure</th>
<th>Virus neutralizing antibody (VNA) titers</th>
<th>Reference</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection with live virus</td>
<td>Rhesus macaques</td>
<td>No</td>
<td>83–197 by the pseudovirus neutralization assay; 35–326 by the live virus neutralization assay</td>
<td>(31, 32) After virus reinfection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SARS-CoV-2</td>
<td>DNA vaccine</td>
<td>Rhesus macaques</td>
<td>No</td>
<td>Median titer, 74</td>
<td>(35)</td>
<td></td>
</tr>
<tr>
<td>Inactivated virus vaccine with alum</td>
<td>Rhesus macaques</td>
<td>No</td>
<td>145–400</td>
<td>(5, 34)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenovirus vector vaccine</td>
<td>Rhesus macaques</td>
<td>No</td>
<td>5–40</td>
<td>(33)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

continued on the next page
Overall, the immunological mechanisms associated with cellular immunopathology in SARS-CoV and MER-CoV animal models are conflicting, with evidence pointing to both the protective and accelerating properties of T<sub>H2</sub> responses and the possibility of pathogenic T<sub>H17</sub>-derived mechanisms (6). ADE of infection has been seen in vitro for SARS-CoV-1 and MERS-CoV, but it remains unclear whether VAED occurs in animal models administered MERS-CoV or SARS-CoV-1 vaccines.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Infection or vaccine</th>
<th>Animal model</th>
<th>Immune enhancement of disease after virus exposure</th>
<th>Virus neutralizing antibody (VNA) titers</th>
<th>Reference</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>SARS-CoV-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infection with live virus</td>
<td>Ferrets</td>
<td>No</td>
<td>720–800 U</td>
<td></td>
<td>(43)</td>
<td>After virus reinfection</td>
</tr>
<tr>
<td>Infection with live virus</td>
<td>African green monkeys</td>
<td>Yes</td>
<td>10&lt;sup&gt;2&lt;/sup&gt;–10&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
<td>(44)</td>
<td>After virus reinfection</td>
</tr>
<tr>
<td>Modified virus vaccinia Ankara (MVA) vector vaccine</td>
<td>Ferrets</td>
<td>Yes</td>
<td>20–40 before challenge, up to 1280 after challenge</td>
<td></td>
<td>(46, 125)</td>
<td>No neutralizing antibody in mVA expressing N protein</td>
</tr>
<tr>
<td>MVA vector vaccine</td>
<td>Chinese rhesus macaques</td>
<td>Yes</td>
<td>10&lt;sup&gt;6&lt;/sup&gt;–10&lt;sup&gt;8&lt;/sup&gt;</td>
<td></td>
<td>(45)</td>
<td>Immunopathology associated with IL-8</td>
</tr>
<tr>
<td>Recombinant vaccinia vaccine</td>
<td>Mice</td>
<td>Yes</td>
<td>Not reported</td>
<td></td>
<td>(47)</td>
<td>Immunopathology associated with IL-6</td>
</tr>
<tr>
<td>Dendritic cell peptide immunization with or without a recombinant vaccinia virus booster</td>
<td>Mice</td>
<td>No</td>
<td>Not reported</td>
<td></td>
<td>(52, 54)</td>
<td>Protection associated with CD8&lt;sup&gt;+&lt;/sup&gt; T cell responses</td>
</tr>
<tr>
<td>Venezuelan equine encephalitis replicon</td>
<td>Mice</td>
<td>Yes/no</td>
<td>PRNT&lt;sub&gt;80&lt;/sub&gt;100–1600</td>
<td>(48, 82)</td>
<td>Conflicting results implicating viral nucleoprotein</td>
<td></td>
</tr>
<tr>
<td>Inactivated virus vaccine</td>
<td>Mice</td>
<td>Yes</td>
<td>Geometric mean neutralizing antibody log&lt;sub&gt;2&lt;/sub&gt;7–10</td>
<td>VNA detected after challenge only</td>
<td>(50, 53)</td>
<td>Immunopathology with unadjuvanted whole-virus vaccine, despite protection; reduced immunopathology with alum</td>
</tr>
<tr>
<td>Spike protein and spike protein receptor binding domain subunit vaccines</td>
<td>Mice</td>
<td>No (spike protein receptor binding domain)</td>
<td>Geometric mean neutralizing antibody log&lt;sub&gt;2&lt;/sub&gt;5–10</td>
<td>VNA detected after challenge only</td>
<td>(50, 51, 53)</td>
<td>Conflicting results with spike protein (both reduced and enhanced with alum)</td>
</tr>
<tr>
<td>DNA vaccine</td>
<td>Rhesus macaques</td>
<td>No</td>
<td>About 10&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
<td>(55)</td>
<td></td>
</tr>
<tr>
<td>Spike protein (Ribi) and receptor binding domain subunit vaccines with alum</td>
<td>Rhesus macaques</td>
<td>No</td>
<td>Pseudovirus inhibition (PI)&lt;sub&gt;50&lt;/sub&gt; = 400–1200</td>
<td></td>
<td>(56, 57)</td>
<td>Spike protein formulated with Ribi; receptor binding domain formulated with alum</td>
</tr>
<tr>
<td>Adenovirus vector vaccine</td>
<td>Rhesus macaques</td>
<td>No</td>
<td>Geometric mean titer up to 148</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MERS-CoV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infection with live virus</td>
<td>New Zealand white rabbits</td>
<td>Yes</td>
<td>Neutralizing antibodies associated with protection from viral infection and associated pathology</td>
<td></td>
<td>(63)</td>
<td></td>
</tr>
<tr>
<td>Inactivated virus vaccine</td>
<td>Mice</td>
<td>Yes</td>
<td>Geometric mean titer log&lt;sub&gt;2&lt;/sub&gt;4–6</td>
<td></td>
<td>(64)</td>
<td>Eosinophilic pathology with both unadjuvanted vaccine or vaccine with alum or MF59</td>
</tr>
</tbody>
</table>
Does immune enhancement of disease occur in human coronavirus infections?

There are seven CoV serotypes associated with disease in humans: four that cause the common cold (OC43, NL63, 229E, and HKU1) and three that are highly pathogenic (SARS-CoV-1, SARS-CoV-2, and MERS-CoV). Ninety percent of adults are seropositive for coronavirus strains causing the common cold (65). A clinical study where participants were experimentally infected twice, 1 year apart, with CoV 229E did not report enhanced disease; after the second exposure, the time during which virus was shed in nasal secretions was reduced, and there were no symptoms of disease (66). Both serum and nasal IgA antibodies specific for CoV 229E were associated with a decreased period of nasal virus shedding (67). Immune enhancement of SARS-CoV-2 infection attributable to cross-reactive common cold CoV antibodies has not been reported so far. Rather, prior infection with common cold CoVs has been suggested to be either potentially protective by virtue of inducing antibodies that cross-react with the SARS-CoV-2 spike protein subunit S2 (68) or to be the source of SARS-CoV-2–reactive neutralizing antibodies that arose in a patient with SARS-CoV-1 who recovered from SARS-CoV-1 infection (69). Regarding T cell immunity to common cold CoVs, ~40 to 60% of individuals who have not been exposed to SARS-CoV-2 have SARS-CoV-2–reactive CD4+ T cells, suggesting that there is cross-reactive T cell recognition between common cold CoVs and SARS-CoV-2 (70, 71). So far, there is no direct evidence suggesting that preexisting immunity to common cold CoVs is detrimental to the outcome of SARS-CoV-2 infection.

Reports correlating antibody responses and disease severity are conflicting and confounded by higher viral loads and the potential for more immune stimulation with severe SARS-CoV-2 infection. Studies of MERS-CoV have shown increased neutralizing antibodies (72–74) or an increased duration of spike protein–binding antibody (75) in severe disease. Among 128 SARS-CoV-1–infected individuals, the amount of neutralizing antibodies was not associated with disease severity (76). However, one report suggested that increased antibody production correlated with increased respiratory failure in humans infected with SARS-CoV-1 (77). In contrast, another study showed no difference in time to seroconversion in SARS-CoV-1–infected individuals who survived compared with those who died (78). The presence of SARS-CoV-1–specific IgG 10 days after onset of symptoms was associated with a decrease in nasopharyngeal viral load and with worsening of clinical disease in ~20% of individuals with respiratory failure requiring ventilator support (79). Use of a pseudovirus and a plaque reduction neutralization test (PRNT) assay to study acutely ill and recovered SARS-CoV-1–infected patients showed a decrease in viral load coincident with the time of seroconversion, suggesting that the neutralizing antibody response may play a role in clearance of virus (80, 81). In the setting of SARS-CoV-1 infection, it has been reported that CD4+ T cell responses correlated with positive outcomes in mice (82), but more severe disease in humans (76).

Tan et al. (83) have suggested that IgM and IgG against the SARS-CoV-2 nucleocapsid protein increased in patients with severe compared with mild COVID-19 disease. Systems analysis of serological signatures in COVID-19 disease revealed that functional antibody responses to SARS-CoV-2 nucleocapsid protein were elevated in those who died, whereas spike-specific antibody responses were enriched among convalescent individuals (84). A clinical study of 175 patients with COVID-19 reported that higher serum neutralizing antibody titers may be associated with lower lymphocyte counts and higher C-reactive protein (85), but the amount of neutralizing antibodies in severe compared with mild disease was not reported. Studies have reported higher SARS-CoV-2 neutralizing antibody titers in old compared with young patients with COVID-19 (85, 86). One study reported higher IgM and IgG antibodies against SARS-CoV-2 spike and nucleocapsid proteins in patients with severe compared with mild COVID-19 disease (87). A second study of mild versus severe COVID-19 disease in SARS-CoV-2–infected individuals demonstrated elevated serum IgA and IgG antibodies against virus spike protein associated with severe disease. In individuals who had recovered from SARS-CoV-2 infection, spike protein–specific CD4+ T cell responses correlated with the magnitude of IgG and IgA antibody titers against the spike protein receptor binding domain (71). The reason for higher anti-spike protein antibody responses in severe COVID-19 disease is not clear, but may be due to higher viral loads in severe disease (88). Studies have demonstrated that the nasopharyngeal SARS-CoV-2 viral load was higher in elderly patients and in severe disease compared with mild disease (89, 90). However, in other studies, no association was found between nasopharyngeal viral load and disease severity (91).

Two studies involving reinfection of nonhuman primates with SARS-CoV-2 after a primary infection showed that the animals were resistant to reinfection with no evidence of enhanced disease (31, 32). Recently, one patient in the United States was reported to have a more severe clinical course when infected with SARS-CoV-2 a second time (92). While it is difficult to interpret data from a single case report, it will be important to monitor the frequency of repeat infections with SARS-CoV-2 and the clinical course of disease to determine if this finding is relevant more broadly.

Lung pathology in COVID-19 disease is characterized by diffuse alveolar damage, with hyaline membrane formation, pneumocyte desquamation, multinucleated giant cell formation, neutrophil or macrophage alveolar infiltrates, and viral infection of several cell types (7, 93, 94). Viral proteins can be detected in the upper airway and bronchiolar epithelium, submucosal gland epithelium and in type I and type II lung pneumocytes, alveolar macrophages, and the hyaline membranes of the lung (94).

In COVID-19, disease severity and death have been associated with higher amounts of inflammatory markers in the blood and increased concentrations of serum inflammatory cytokines and chemokines (95). Predictors of severe COVID-19 disease are emerging, with lymphopenia, elevated serum C-reactive protein, ferritin, and D-dimers, and high serum concentrations of IL-6, IL-10, interferon-γ–induced protein-10 (IP-10)/CXC motif chemokine 10 (CXCL10), and tumor necrosis factor–α (96, 97) in some patients (95, 97). Dysregulated cytokine induction has also been reported in acute respiratory distress syndrome in patients infected with SARS-CoV-1 or MERS-CoV (98–101). Recently, the similarity between acute respiratory distress syndrome associated with severe CoV respiratory infections and acute respiratory distress syndrome that occurs during immunotherapy with chimeric antigen receptor T cells has been pointed out (102).

What vaccine trials and convalescent plasma reveal about immune enhancement of disease

In phase 1 clinical trials, a MERS-CoV DNA vaccine was well tolerated (NCT03721718) (103) as was an MVA vector–spike protein vaccine (NCT03615911) (104). A chimpanzee adenovirus vector (ChAdOx1) vaccine expressing the MERS-CoV spike protein did not result in any severe adverse events over a 12-month follow-up period in 24 trial
participants, and all mild or moderate adverse events resolved within 6 days (NCT03399578) (105). Moreover, no evidence of immune enhancement of disease was noted in a clinical trial of an inactivated whole-virus SARS-CoV-1 vaccine (106) or a DNA vaccine expressing SARS-CoV-1 spike protein in 10 individuals (NCT00099463) (107). Infection was not reported after vaccination in any of these trials.

To date, five different phase 1 studies of vaccines against SARS-CoV-2 have been published (NCT04368728, NCT04313127, NCT04324606, and NCT04283461) (108). Mild to moderate adverse events were commonly reported with low rates of severe adverse events (108–111). However, these early phase 1 trials are not sufficiently powered to be able to definitively demonstrate that serious adverse events including VAED are not associated with COVID-19 vaccines. Phase 3 efficacy trials for COVID-19 candidate vaccines have begun in regions of ongoing SARS-CoV-2 transmission, including the United States, United Kingdom, South Africa, and Latin America. These phase 3 trials will follow participants for at least 1 year to monitor efficacy outcomes and safety in the context of ongoing SARS-CoV-2 infection and will provide direct data for these vaccine candidates regarding disease enhancement after vaccination. Importantly, in phase 2 and 3 trials using the chimp adenovirus vector vaccine (ChimpAdOx-1), there have been early reports of two possible cases of inflammatory neurological disease (transverse myelitis) in trial participants, and this phase 3 trial has been paused in the United States at this time (112, 113).

Another approach for elucidating potential complications caused by neutralizing antibodies or other antibodies to SARS-CoV-2 during ongoing infection is to determine whether administration of convalescent plasma from patients with COVID-19 enhances disease in recipients. Uncontrolled studies of convalescent plasma administration to more than 35,000 severely ill patients with COVID-19 have shown that antibody administration in the form of plasma transfusions is not associated with worsening of disease (114). A matched-control trial of convalescent serum administration to 45 patients with COVID-19 demonstrated a decrease in oxygen supplement requirements and an overall survival benefit in the treated group compared with the untreated group (115, 116). Randomized controlled trials of convalescent serum treatment are underway (NCT04348656, NCT04342182, and NCT04338360). To date, there is no consistent evidence of immune enhancement of SARS-CoV-2 infection in humans from data from natural infection, various vaccine candidates, or convalescent plasma treatment.

Implications of immune enhancement of disease for vaccine development

A key question is why VAED is raised as a possibility for COVID-19 vaccines. Fundamentally, this question should be asked of all vaccine candidates under development, despite the rarity of the phenomenon. If judged safe and effective by regulatory authorities based on efficacy clinical trials that could include up to 30,000 participants per trial, then COVID-19 vaccines could be made rapidly available to far larger numbers of people. Although determinations of vaccine safety and efficacy will be based on well-established requirements of regulatory authorities in the United States, the European Union, and other global regions, the capacity to produce and deliver millions of vaccine doses has been accelerated to gain control of the pandemic. As a result, many people may be vaccinated before longer-term follow-up is possible. In addition, COVID-19 vaccines will be administered to older individuals who are naïve to this pathogen, whereas knowledge about vaccine responses in this age group has often come from vaccines designed to boost waning immunity. However, age-related differences in immune responses are being evaluated in phase 3 COVID-19 vaccine trials.

Given current knowledge, the main opportunity to identify whether a COVID-19 vaccine candidate has a risk of VAED will be in randomized, placebo-controlled phase 3 clinical trials. Whether and when such a risk would be identified in clinical trials depend on three important factors: (i) the frequency of VAED, (ii) the time interval after vaccination when VAED might occur, and (iii) whether the manifestation of VAED is distinct from natural disease of a similar severity. Currently, it is unknown whether there would be clinical markers to distinguish VAED from natural COVID-19 disease. The inherent complexity of COVID-19, including nonrespiratory manifestations such as coagulopathy in adults (117) and multisystem inflammatory syndrome in children, may make this distinction particularly difficult. Nonetheless, the occurrence of severe disease with a higher than expected frequency in a particular age group may be important as a potential signal of VAED.

The design of COVID-19 vaccine clinical trials takes these points into account by progressing from small (about 100 person) phase 1 safety trials through large (~30,000 person) phase 3 efficacy trials (118). The primary efficacy analysis in a phase 3 trial may occur less than 12 months after the start of the phase 1 trial, and phase 3 trials are expected to include enough incident COVID-19 cases (e.g., 150 infection events) at that point to confidently assess whether a vaccine candidate is reducing disease incidence by a factor of 2 or greater (119). All phase 3 trial participants are expected to be followed for at least 1 year (119). Thus, it is critical to implement and complete phase 3 efficacy studies to ensure that the vaccine is both safe and efficacious. Given the duration of the clinical trials, VAED will be identified if there is little delay after vaccination before the putative risk of VAED develops. If VAED occurred during a trial and was not distinguishable from natural disease, then clinical trials might identify it through an increase in the rate of morbidity or mortality in the vaccinated group compared with the control group (Table 2). Alternatively, if VAED occurred and was distinguishable from natural disease, then clinical trials might be able to identify much lower rates of VAED. U.S. Food and Drug Administration (FDA) guidelines for industry for emergency use authorization for vaccines to prevent COVID-19 were recently issued. These guidelines require that the trials (i) meet the prespecified success criteria for the study’s primary efficacy end point, (ii) provide all safety data from phase 1, 2, and 3 trials, (iii) conduct follow-up of phase 3 participants for a median duration of at least 2 months after completion of the full vaccination regimen, and (iv) report five or more severe COVID-19 cases in the placebo group to assess the possibility of VAED in the vaccine group (120).

Participants in phase 3 vaccine trials are monitored to detect adverse events ranging from mild to severe. A severe adverse event triggers a pause in the trial, whereas a comprehensive assessment of causality for relatedness to vaccine administration is completed by an independent review committee, as occurred in the chimp adenovirus vector vaccine study (121, 122).

If data from phase 3 efficacy trials demonstrate that vaccine candidates meet the safety, efficacy, and quality standards set by regulators, then vaccine candidates may be licensed for use. The possibility of adverse events too rare for identification in clinical trials is assumed for all licensed vaccines. There remains the theoretical
However, unless immune enhanced disease is observed in humans, as researchers attempt to identify models in young and aged animals have notable differences from antibody-based interventions given the caveat that vaccine-induced immune responses are expected to evidence about mechanisms of protection and, if present, VAED, with vaccines elicit similar antibody responses, these data will provide enhanced disease as described for vaccine clinical trials. To the extent that will evaluate protective efficacy and potential immune-associated en-
cer for different vaccines and the placebo groups. This will provide the immunopathology of COVID-19 disease in different age groups
and the lack of VAED in clinical trials would provide important
correction based on the immunological principles or the available preclinical data. We conclude that the available data do not support more concern about VAED for COVID-19 vaccines than is appropriate for the development of any viral vaccine. Convalescent plasma studies suggest potential benefit rather than a risk of more severe disease. In addition, no serious safety signals have been reported from initial phase 1 trials of COVID-19 vaccine candidates, with the caveat that the number of vaccinees who have been subsequently exposed to SARS-CoV-2 infection is unknown but probably low. Nevertheless, an abundance of caution to exclude such a concern is warranted to be able to implement efficacious COVID-19 vaccines as widely, rapidly, and safely as possible.

Our analysis also finds that in nonclinical reports where immune-associated enhanced disease, cellular immunopathology, and ADE of disease have been observed, no consistent mechanism or immune markers of disease enhancement are apparent. Also, importantly, there is no evidence that any of the in vitro or animal models of coronavirus infection reliably predict the human experience. Thus, it is not possible to prioritize or down-select vaccine antigens, adjuvants, biotechnology platforms, or delivery mechanisms based on general immunological principles or the available preclinical data. Ultimately, the only way to address the theoretical risk of VAED is in phase 3 efficacy trials with sufficient numbers of end points to evaluate safety and efficacy, and by postlicensure surveillance. If VAED is frequent or clinically distinctive, then it should become apparent when clinical trial participants experience natural infection with SARS-CoV-2. The combination of protection against COVID-19 and the lack of VAED in clinical trials would provide important

<table>
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<tr>
<th>Annual incidence in placebo arm†</th>
<th>HR (vaccine/placebo) of severe COVID-19</th>
<th>Results reported if an elevated rate of severe COVID-19 disease was just detected‡</th>
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<tbody>
<tr>
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<td>1.25</td>
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*Power calculated on the basis of a one-sided 0.025-level log-rank test comparing the rate of severe COVID-19 disease in vaccine versus placebo groups; participants were followed for an average of 12 months with 2% annual dropout; all events after enrollment were counted; calculations assume a constant rate of the severe COVID-19 endpoint over time. †The five placebo arm incidence scenarios correspond to (a) 2% annual COVID-19 incidence and 5% severe cases, (b) 4% annual COVID-19 incidence and 5% severe cases, (c) 4% annual COVID-19 incidence and 10% severe cases, (d) 2% annual COVID-19 incidence and 25% severe cases, and (e) 4% annual COVID-19 incidence and 25% severe cases. ‡Expected numbers of observed placebo group cases of severe COVID-19 disease (expected # of placebo cases) are calculated on the basis of the incidence assumed in the first column, with 2% annual dropout. Estimated HR is the smallest estimated HR of severe COVID-19 disease (vaccine/placebo) such that the Wald two-sided 95% CI in a Cox proportional hazards model just lies above 1.0, where # of vaccine cases and 95% CI correspond to this estimate.

possibility that COVID-19 vaccine recipients might develop VAED after infection with SARS-CoV-2 at a frequency too low to be de-
tected during the clinical trials or occurring after the clinical trials have ended. This possibility will need to be addressed by postlicensure surveillance. The appropriate methods for postlicensure surveillance will depend on whether the manifestations of VAED are distinct from those of COVID-19 disease, which would allow the development of a case definition of VAED. Established methods for postlicensure vaccine effectiveness studies, such as a case-control design, can monitor for increased rates of severe disease after vaccination. Regulators may recommend specific types of studies to assess the potential for VAED related to COVID-19 vaccines (119), and sponsors of licensed vaccines may be required by regulatory authorities to monitor for known and unidentified risks after licensure. Implementing post-
vaccination surveillance procedures in the United States is the responsibility of the FDA and the U.S. Centers for Disease Control and Prevention (CDC) (123).

Last, because of the unprecedented number of COVID-19 vac-
cines in development, there will be a very large body of clinical data available for different vaccines and the placebo groups. This will provide the opportunity for meta-analyses across many studies to better understand the immunopathology of COVID-19 disease in different age groups and to look for severe adverse events such as VAED that may be rare.

Clinical trials of other prophylactic interventions, such as conva-
lescent plasma, hyperimmune globulin, and monoclonal antibodies, will evaluate protective efficacy and potential immune-associated en-
hanced disease as described for vaccine clinical trials. To the extent that vaccines elicit similar antibody responses, these data will provide evidence about mechanisms of protection and, if present, VAED, with the caveat that vaccine-induced immune responses are expected to have notable differences from antibody-based interventions given that vaccines will likely induce both antibodies and T cell responses.

Animal models of SARS-CoV-2 infection will continue to evolve as researchers attempt to identify models in young and aged animals that recapitulate more severe human COVID-19 disease presentation. However, unless immune enhanced disease is observed in humans,
assurances of the efficacy and safety of the vaccine and the justification for vaccine use. However, the detection of low rates of VAED, associated with a later exposure to SARS-CoV-2 in people who have been vaccinated, will depend on rigorous postlicensure surveillance, as is necessary when any new viral vaccine is introduced for the prevention of respiratory syncytial virus disease in RSV-naïve infants. Vaccine 38, 101–106 (2020).


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10.2. Biomedical Advanced Research and Development Authority; N. Santos, Biomedical Advanced Research and Development Authority; T. Villafana, AstraZeneca; T. Zaks, Moderna Inc. Competing interests: B.F.H is a co-inventor on patent applications for COVID-19 vaccine designs. P.J.H. is developing a low-cost COVID-19 vaccine that has been licensed to a pharmaceutical company for manufacture. S.R. is currently employed by Sanofi, which is pursuing therapeutic vaccine that has been licensed to a pharmaceutical company for manufacture. S.R. is currently employed by Moderna Inc., which is pursuing therapeutic vaccine that has been licensed to a pharmaceutical company for manufacture.

Acknowledgments: We acknowledge T. Shimabukuro and N. Messonnier for the U.S. Centers for Disease Control and Prevention for the helpful discussions, and W. Edwards of the Duke Human Vaccine Institute and B. Tolman of Deloitte Consulting LLP for editorial assistance. We thank the ACTIV Working Group whose nonauthor members include P. Kunstman, Merck & Co. Inc.; B. Bell, University of Washington; S. Buchbinder, University of California, San Francisco and San Francisco Department of Public Health; M. Cavaletti, European Medicines Agency; M. Davis, Stanford University School of Medicine; E. Emns, Bill & Melinda Gates Foundation; G. Glenn, Novavax Inc.; E. Hanon, GlaxoSmithKline; K. Jansen, Pfizer; A. Lanzavecchia, Vir Biotechnology Inc. and Institute for Research in Biomedicine; D. Lowy, National Cancer Institute, NIH; P. Marks, FDA; J. Mascia, National Institute of Allergy and Infectious Diseases, NIH; N. Messonnier, U.S. Centers for Disease Control and Prevention; N. Michael, Walter Reed Army Institute of Research; P. Offit, University of Pennsylvania; J. Seals, Biomedical Advanced Research and Development Authority; Health and Human Services; J. Tartaglia, Sanofi Pasteur; T. Villafana, AstraZeneca; T. Zaks, Moderna Inc. Competing interests: B.F.H. is a co-inventor on patent applications for COVID-19 vaccine designs. P.J.H. is developing a low-cost COVID-19 vaccine that has been licensed to a pharmaceutical company for manufacture. S.R. is currently employed by Sanofi, which is pursuing therapeutic vaccine and vaccine development to treat COVID-19. H.S. is an employee and shareholder of Johnson & Johnson, which is developing a COVID-19 vaccine. M.W. is currently employed by Moderna Inc., which is pursuing therapeutic and vaccine development to treat COVID-19. A.A. is a consultant for Vir Biotechnology. The authors declare that they have no competing interests.

Data and materials availability: All data associated with this study are present in the paper.

Submitted 30 July 2020
Accepted 16 October 2020
Published 4 November 2020
10.1126/scitranslmed.abe0948
