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REVIEW

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CORONAVIRUS

Prospects for a safe COVID-19 vaccine

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Rapid development of an efficacious vaccine against the viral pathogen 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 post-licensure surveillance should provide a reliable strategy to identify adverse events, including the potential for enhanced severity of COVID-19 disease, following 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 Organisation (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 pre-existing 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 to 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 1-2 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 (*1–3*, *5*, *10–12*) (Fig. 1). In these studies, the overall incidence of RSV infection was not increased when compared to 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 6-11 months of age, with two fatal cases in this age group, and, to a lesser extent, in those immunized at 12-23 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 pre-fusion conformation prior to virus entry into host cells, which changes to a post-fusion form upon host cell receptor engagement. The post-fusion 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%; 10/23) than after natural infection (75%; 12/16) (*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 to controls with natural RSV infection (*14*). 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*). However, 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 non-protective 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 due to 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: "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 one 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 fever/shock syndrome, which can occur with primary infection but also when a person who had a prior infection becomes infected with another serotype (*22*). Although immunity from a previous infection can provide protection, enhancement of disease severity under these circumstances has been suggested to be mediated by ADE, based on in vitro observations. In cell culture, antibodies against dengue virus that bind to virus particles and cells of the immune system (macrophages, monocytes or dendritic cells) that express receptors for the Fc portion of the antibody provide an alternate pathway for virus entry, in addition to binding and entry via the specific viral receptor (Fig. 1). Whereas antibody-mediated entry of host cells results in destruction of most viruses, dengue viruses can replicate after entry through this pathway. Thus, ADE of dengue disease can occur during a second infection with a different virus serotype due to cross-reactive antibodies with suboptimal neutralizing capacity against the newly incoming virus, in

combination with the Fc-mediated targeting of immune cells by the virus. ADE of dengue disease has been reported to have a 0.5% attack rate (36/6684) where it was associated with a narrow range of neutralizing antibody titers (1:20-1:80) at the time of infection (*23*).

A potential enhancing effect of pre-existing dengue antibodies was also raised as a concern in clinical trials of the quadrivalent live-attenuated dengue vaccine, Dengvaxia (Sanofi Pasteur), where immunization of dengue-naïve 2-8 year old children correlated with a lower risk of severe disease for two years, but subsequent hospitalization rates were higher in vaccinees than placebo recipients in the third year (*24*). It was not established whether the higher hospitalization rates resulted from an undefined age-related factor, failure to protect against infection with particular serotypes, cross-reactive antibodies, limited cell-mediated immunity, or a combination of factors. Post-licensure, 15 deaths from dengue disease were reported in 9-13 year old children in the Philippines (where >830,000 children received one dose and >365,000 received all three doses) and 14 of the deaths were investigated by the WHO Global Advisory Committee on Vaccine Safety. Their conclusion was that individual cases could not be attributed to vaccine failure or vaccine-related immune enhancement because there were no criteria to differentiate the two (*25*). Based on protective efficacy, Dengvaxia is now recommended for dengue seropositive individuals > 9 years old where dengue is prevalent.

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 pre-existing 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, pre-existing 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 Severe Acute Respiratory Syndrome (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 non-human 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 or not 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 following 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 to 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 to 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 re-infection 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 non-human 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 (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 re-infection model in African green macaques, alveolitis and interstitial pneumonitis associated with dysregulated cellular inflammatory and cytokine responses was observed, but was 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 following SARS-CoV-1 vaccination has been suggested to be associated with vaccine-induced Th17 host responses, including extravasation of eosinophils from the bone marrow and infiltration of tissues (*5*, *49*). Thus, the evidence suggests a potential role of Th17 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 UV 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 to non-adjuvanted 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 Th1 responses as well as 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 non-human 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 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 (RT-PCR) 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*) and (*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 non-pathogenic 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 was linked to neutralizing antibodies (*64*). Similarly, in a SARS-CoV-1 challenge model in African green macaques, lung immunopathology was unrelated to pre-existing neutralizing antibodies (*44*), as was the case for a whole inactivated virus vaccine and other SARS-CoV-1 vaccines in mice (*50*).

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 Th2 responses and the possibility of pathogenic Th17-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.

DOES IMMUNE ENHANCEMENT OF DISEASE OCCUR IN HUMAN CORONAVIRUS INFECTIONS?

There are seven coronavirus (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, one 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 SARS-CoV-1 patient who recovered from SARS-CoV-1 infection (*69*). Regarding T cell immunity to common cold CoVs, ~40-60% 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 pre-existing 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 to 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*).

Tam *et al.* have suggested that IgM and IgG against the SARS-CoV-2 nucleocapsid protein increased in patients with severe compared to mild COVID-19 disease (*83*). Systems analysis of serological signatures in COVID-19 disease revealed 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 to mild disease was not reported. Studies have reported higher SARS-CoV-2 neutralizing antibody titers in old compared to 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 to 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*). Indeed, studies have demonstrated that the nasopharyngeal SARS-CoV-2 viral load was higher in elderly patients and in severe disease compared to mild disease (*89*, *90*). However,

in other studies, no association was found between nasopharyngeal viral load and disease severity (*91*).

Two studies involving re-infection of non human primates with SARS-CoV-2 after a primary infection showed that the animals were resistant to re-infection 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, multi-nucleated giant cell formation, neutrophil or macrophage alveolar infiltrates and viral infection of several cell types (*7*, *93*, *94*). Viral proteins can be detected in 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, IP-10/CXCL10 and TNF-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 (CAR) 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 chimp 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, 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 US, UK, South Africa, and Latin America. These phase 3 trials will follow participants for at least one 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 following 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 US 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 COVID-19 patients 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 to the untreated group (*115*). Randomized controlled trials of convalescent serum treatment are underway (NCT04348656, NCT04342182, 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, 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 in order 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 depends on three important factors: (1) the frequency of VAED, (2) the time interval after vaccination when VAED might occur, and (3) whether the manifestation of VAED is distinct from natural disease of a similar severity. Currently, it is unknown if there would be clinical markers to distinguish VAED from natural COVID-19 disease. The inherent complexity of COVID-19 including non-respiratory manifestations such as coagulopathy in adults (*116*) 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 (approximately 100 person) phase 1 safety trials through large (~30,000 person) phase 3 efficacy trials (*117*). 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 (*118*). All phase 3 trial participants are expected to be followed for at least one year (*118*). 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, clinical trials might identify it through an increase in the rate of morbidity or mortality in the vaccinated group compared to 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. FDA guidelines for industry for emergency use authorization for vaccines to prevent COVID-19 were recently issued. These guidelines require that the trials (1) meet the prespecified success criteria for the study's primary efficacy endpoint, (2) provide all safety data from Phase 1, 2 and 3 trials, (3) conduct follow-up of phase 3 participants for a median duration of at least 2 months after completion of the full vaccination regimen, and (4) report 5 or more severe COVID-19 cases in the placebo group to assess the possibility of VAED in the vaccine group (*119*).

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 while 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 (*120*, *121*).

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 possibility that COVID-19 vaccine recipients might develop VAED after infection with SARS-CoV-2 at a frequency too low to be detected during the clinical trials or occurring after the clinical trials have ended. This possibility will need to be addressed by post-licensure surveillance. The appropriate methods for post-licensure 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 post-licensure vaccine effectiveness studies, such as a case-control design, can monitor for increased rates of severe disease following vaccination. Regulators may recommend specific types of studies to assess the potential for VAED related to COVID-19 vaccines (*118*), 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 US is the responsibility of the FDA and the CDC (*122*).

Finally, because of the unprecedented number of COVID-19 vaccines 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 convalescent plasma, hyperimmune globulin, and monoclonal antibodies, will evaluate protective efficacy and potential immune-associated enhanced 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, there will not be a way to evaluate whether any animal models are predictive of VAED in vaccinated humans. Although human challenge studies cannot be performed with SARS-CoV-2 in the absence of effective antiviral agents, infection of volunteers using minimally pathogenic coronaviruses may provide insights about immune correlates of protection against these viruses (*123*).

CONCLUSIONS

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 in order to be able to implement efficacious COVID-19 vaccines as widely, rapidly and safely as possible.

Our analysis also finds that in non-clinical 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 clearly 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 endpoints to evaluate safety and efficacy, and by post-licensure surveillance. If VAED is frequent or clinically distinctive, 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 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 post-licensure surveillance, as is necessary when any new viral vaccine is introduced for the prevention of morbidity and mortality that would otherwise be caused by a human viral pathogen. Thus, completion and full evaluation of COVID-19 vaccine phase 3 efficacy trials with long-term follow-up and post-licensure surveillance will provide the most comprehensive data on the safety of COVID-19 vaccines and the potential risk of VAED.

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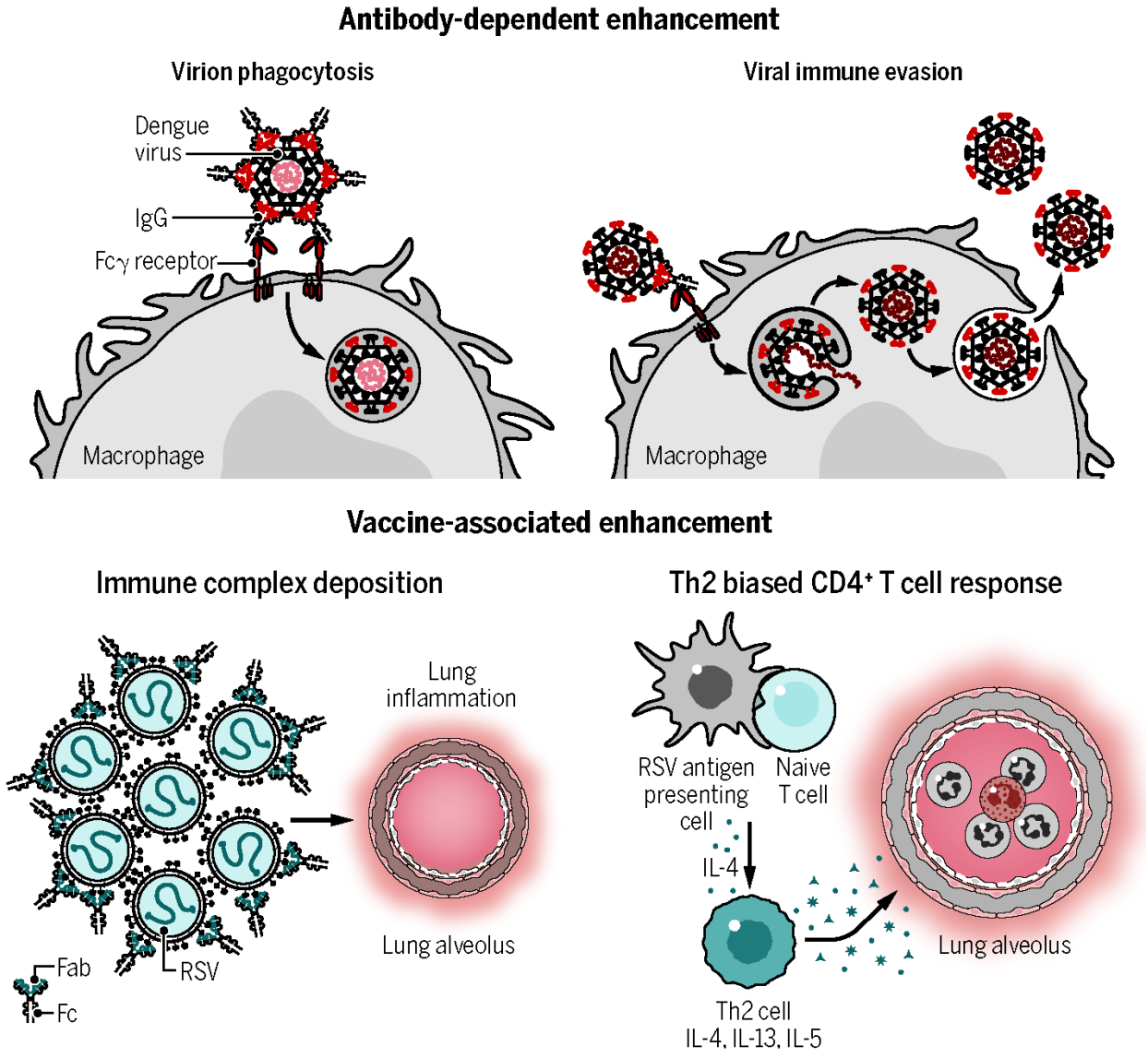

Fig. 1. Immune enhancement of human viral disease. Immune enhancement of human viral disease through viral re-infection or vaccination has been documented in (top) natural dengue virus infection and (bottom) vaccination with a formalin-inactivated vaccine for RSV. (Top) During natural dengue virus infection, 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 $Fc\gamma$ receptors expressed by macrophages and other immune cells. A second infection with a different dengue virus serotype creates a risk of antibody-dependent enhancement of disease because cross-reactive antibodies against the first serotype that have limited neutralizing capacity can mediate internalization of the virus by $Fc\gamma$ 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 peribronchiolitis and alveolitis associated with pulmonary infiltration by neutrophils and eosinophils, which is consistent with a Th2-biased $CD4^+$ T cell response.
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Table 1. Immune enhancement of coronavirus disease in animal models.

Virus	Infection or Vaccine	Animal Model	Immune enhancement of disease after virus exposure	Virus neutralizing antibody (VNA) titers	Reference	Notes
SARS-CoV-2	Infection with live virus	Rhesus macaques	No	83-197 by the pseudovirus neutralization assay; 35-326 by the live virus neutralization assay	(*31*, *32*)	Following virus reinfection
	DNA vaccine	Rhesus macaques	No	Median titer, 74	(*35*)	
	Inactivated virus vaccine with alum	Rhesus macaques	No	145-400	(*5*, *34*)	
	Adenovirus-vector vaccine	Rhesus macaques	No	5-40		
SARS-CoV-1	Infection with live virus	Ferrets	No	720-800 U	(*43*)	Following virus reinfection
	Infection with live virus	African green monkeys	Yes	10^2-10^4	(*44*)	Following virus reinfection
	Modified virus vaccinia Ankara (MVA) vector vaccine	Ferrets	Yes	20-40 Pre-challenge, up to 1,280 Post-challenge	(*46*, *124*)	No neutralizing antibody in rMVA expressing N protein
	Modified virus vaccinia Ankara (MVA) vector vaccine	Chinese Rhesus macaques	Yes	10^3-10^4	(*45*)	Immunopathology associated with IL-8
	Recombinant vaccinia vaccine	Mice	Yes	Not reported	(*47*)	Immunopathology associated with IL-6
	Dendritic cell peptide immunization with or without a recombinant vaccinia virus booster	Mice	No	Not reported	(*52*, *54*)	Protection associated with CD8+ T cell responses
	Venezuelan equine encephalitis replicon	Mice	Yes/No	$PRNT_{80}$100-1,600	(*48*, *82*)	Conflicting results implicating viral nucleoprotein
	Inactivated virus vaccine	Mice	Yes	Geometric mean neutralizing antibody $Log_2$7-10 VNA detected post-challenge only	(*50*, *53*)	Immunopathology with unadjuvanted whole virus vaccine, despite protection; reduced immunopathology with alum
	Spike protein and spike protein receptor binding domain	Mice	Yes (spike protein) No (spike protein receptor binding domain)	Geometric mean neutralizing antibody $Log_2$5-10	(*50*, *51*, *53*)	Conflicting results with spike protein (both reduced and enhanced with alum)

	subunit vaccines			Geometric mean neutralizing antibody $Log_2$4-6 VNA detected post-challenge only		Reduced immunopathology with spike protein receptor binding domain with alum
MERS-CoV	DNA vaccine	Rhesus macaques	No	Approximately 10^2	(*55*)	
	Spike protein (Ribi) and receptor binding domain subunit vaccines with alum	Rhesus macaques	No	Pseudovirus inhibition $(PI)_{50}$ = 400-1,200	(*56*, *57*)	Spike protein formulated with Ribi; receptor binding domain formulated with alum
	Adenovirus vector vaccine	Rhesus macaques	No	Geometric mean titer up to 148		
	Infection with live virus	New Zealand white rabbits	Yes	Neutralizing antibodies associated with protection from viral infection and associated pathology	(*63*)	Immunopathology after virus reinfection associated with non-neutralizing antibodies, complement activation, and CD3+ T cells, but no clinically discernable disease
	Inactivated virus vaccine	Mice	Yes	Geometric mean titer $Log_2$4-6	(*64*)	Eosinophilic pathology with both unadjuvanted vaccine or vaccine with alum or MF59

Table 2. Power calculation to detect an elevated rate of severe COVID-19 disease in vaccine vs. placebo recipients over 12 months, with 20,000 enrolled vaccine recipients and 10,000 enrolled placebo recipients*

	Hazard Ratio (Vaccine/Placebo) of Severe COVID-19				Results reported if an Elevated Rate of Severe COVID-19 Disease was Just Detected ***			
Annual Incidence in Placebo Arm**	1.25	1.5	2.0	3.0	Expected # Placebo Cases	# Vaccine Cases	Est. HR	95% CI
[a]0.0010	0.083	0.183	0.537	0.959	10	40	2.00	1.01-4.00
[b] 0.0020	0.141	0.367	0.851	>0.999	20	66	1.65	1.01-2.72
[c] 0.0040	0.233	0.629	0.991	>0.999	40	115	1.44	1.00-2.06
[d] 0.0050	0.264	0.732	0.997	>0.999	50	139	1.39	1.01-1.92
[e] 0.01	0.479	0.949	>0.999	>0.999	99	251	1.27	1.01-1.60

*Power calculated based on a 1-sided 0.025-level log-rank test comparing the rate of severe COVID-19 disease in vaccine vs. placebo groups; participants were followed for an average of 12 months with 2% annual dropout; all events post enrollment were counted.
**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; (e) 4% annual COVID-19 incidence and 25% severe cases.
***Expected numbers of observed placebo group cases of severe COVID-19 disease (Expected # Placebo Cases) are calculated based on the incidence assumed in the first column, with 2% annual dropout. Estimated (Est.) hazard ratio (HR) is the smallest estimated HR of severe COVID-19 disease (vaccine/placebo) such that the Wald 2-sided 95% confidence interval (CI) in a Cox proportional hazards model just lies above 1.0, where # Vaccine Cases and 95% CI correspond to this estimate.

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