

The antibody-dependent enhancement of disease is reviewed, with an emphasis on implications for the prevention and treatment of SARS-CoV-2 infection.

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A perspective on potential antibody-dependent enhancement of SARS-CoV-2

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Antibody-dependent enhancement (ADE) of disease is a general concern for the development of vaccines and antibody therapies because the mechanisms that underlie antibody protection against any virus have a theoretical potential to amplify the infection or trigger harmful immunopathology. This possibility requires careful consideration at this critical point in the pandemic of coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Here we review observations relevant to the risks of ADE of disease, and their potential implications for SARS-CoV-2 infection. At present, there are no known clinical findings, immunological assays or biomarkers that can differentiate any severe viral infection from immune-enhanced disease, whether by measuring antibodies, T cells or intrinsic host responses. In vitro systems and animal models do not predict the risk of ADE of disease, in part because protective and potentially detrimental antibody-mediated mechanisms are the same and designing animal models depends on understanding how antiviral host responses may become harmful in humans. The implications of our lack of knowledge are twofold. First, comprehensive studies are urgently needed to define clinical correlates of protective immunity against SARS-CoV-2. Second, because ADE of disease cannot be reliably predicted after either vaccination or treatment with antibodies—regardless of what virus is

the causative agent—it will be essential to depend on careful analysis of safety in humans as immune interventions for COVID-19 move forward.

The benefit of passive antibodies in ameliorating infectious diseases was recognized during the 1918 influenza pandemic¹. Since then, hyperimmune globulin has been widely used as pre- and post-exposure prophylaxis for hepatitis A, hepatitis B, chickenpox, rabies and other indications for decades without evidence of ADE of disease² (see Box 1 for definition of terms). The detection of antibodies has also been a reliable marker of the effectiveness of the many licensed human vaccines³. The antiviral activity of antibodies is now known to be mediated by the inhibition of entry of infectious viral particles into host cells (neutralization) and by the effector functions of antibodies as they recruit other components of the immune response. Neutralizing antibodies are directed against viral entry proteins that bind to cell surface receptors, either by targeting viral proteins that are required for fusion or by inhibiting fusion after attachment^{4–6} (Fig. 1). Antibodies can cross-neutralize related viruses when the entry proteins of the viruses share epitopes—the part of a protein to which the antibody attaches. Antibodies also eliminate viruses through effector functions triggered by simultaneous binding of the antigen-binding fragment (Fab) regions of immunoglobulin G (IgG) to viral proteins on the surfaces of viruses or infected cells, and of the fragment crystallizable (Fc) portion of the antibody to Fc gamma receptors (FcγRs) that are expressed by immune cells^{7,8}. Antibodies that mediate FcγR- and complement-dependent effector functions may or may not have neutralizing activity, can recognize other viral proteins that are not involved in host-cell entry and can be protective in vivo independent of any Fab-mediated viral inhibition^{9,10}. Recent advances in FcR biology have identified four activating FcγRs (FcγRI, FcγRIIa, FcγRIIc and FcγRIIIa) and one inhibitory FcγR (FcγRIIb) that have various Fc ligand specificities and cell-signalling motifs¹⁰. The neonatal Fc receptor (FcRn) has been described to support antibody recycling and B and T cell immunity through dendritic cell endocytosis of immune complexes^{11,12}. Natural killer cells recognize IgG–viral protein complexes on infected cells via FcγRs to mediate antibody-dependent cytotoxicity, and myeloid cells use these interactions to clear opsonized virions and virus-infected cells by antibody-dependent cellular phagocytosis. The complement pathway is also activated by Fc binding to the complement component C1q, resulting in the opsonization of viruses or infected cells and the recruitment of myeloid cells. Antibody effector functions also contribute to antiviral T cell-mediated immunity in vivo¹³. Notably, new knowledge about Fc effector functions has led

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to improved passive-antibody therapies through Fc modifications that reduce or enhance interactions with FcγRs, lengthen the half-life of the antibody and potentially enhance antigen presentation to T cells, providing what is termed a vaccinal effect^{8,11,14}.

Although their importance for protection is indisputable, the concern about ADE of disease arises from the possibility that antibodies present at the time of infection may increase the severity of an illness. The enhancement of disease by antibody-dependent mechanisms has been described clinically in children given formalin-inactivated respiratory syncytial virus (RSV) or measles vaccines in the 1960s, and in dengue haemorrhagic fever due to secondary infection with a heterologous dengue serotype^{15–21}. For example, antibodies may enable viral entry into FcγR-bearing cells, bypassing specific receptor-mediated entry; this is typically followed by degradation of the virus, but could amplify infection if progeny virions can be produced. Although cytokine release triggered by interactions between the virus, antibody and FcγR is also highly beneficial—owing to direct antiviral effects and the recruitment of immune cells—tissue damage initiated by viral infection may be exacerbated²².

While recognizing that other mechanisms of immune enhancement may occur, the purpose of this Perspective is to review clinical experiences, in vitro analyses and animal models relevant to understanding the potential risks of antibody-dependent mechanisms and their implications for the development of the vaccines and antibodies that will be essential to stop the COVID-19 pandemic. Our objective is to evaluate the hypothesis that antibody-mediated enhancement is a consequence of low-affinity antibodies that bind to viral entry proteins but have limited or no neutralizing activity; antibodies that were elicited by infection with or vaccination against a closely related serotype, termed ‘cross-reactive’ antibodies; or suboptimal titres of otherwise potentially neutralizing antibodies. We assess whether there are experimental approaches that are capable of reliably predicting ADE of disease in humans and conclude that this is not the case.

Principles for assessing potential ADE of disease

The use of ADE to denote enhanced severity of disease must be rigorously differentiated from ADE of infection—that is, from the replication, binding, uptake, cytokine release or other activities of antibodies detected in vitro. The first principle is that an antibody-dependent effect in vitro does not represent or predict ADE of disease without proof of a role for the antibody in

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the pathogenesis of a more severe clinical outcome. A second principle is that animal models for the evaluation of human polyclonal antibodies or monoclonal antibodies (mAbs) should be judged with caution because FcRs that are engaged by IgGs are species-specific^{23,24}, as is complement activation. Antibodies can have very different properties in animals that are not predictive of those in the human host, because the effector functions of antibodies are altered by species-specific interactions between the antibody and immune cells. Animals may also develop antibodies against a therapeutic antibody that limit its effectiveness, or cause immunopathology. In addition, the pathogenesis of a model virus strain in animals does not fully reflect human infection because most viruses are highly species-specific. These differences may falsely support either protective or immunopathological effects of vaccines and antibodies. A third principle is that the nature of the antibody response depends on the form of the viral protein that is recognized by the immune system, thus determining what epitopes are presented. Protective and non-protective antibodies can be elicited to different forms of the same protein. A fourth principle is that mechanisms of pathogenesis in the human host differ substantially among viruses, or even between strains of a particular virus. Therefore, findings regarding the effects of passive antibodies or vaccine-induced immunity on outcomes cannot be extrapolated with confidence from one viral pathogen to another.

Observations about RSV, influenza and dengue

As background for considering the risks of ADE of disease caused by SARS-CoV-2, it is important to closely examine clinical circumstances relevant to the hypothesis that antibodies predispose to ADE of disease by amplifying infection or through damaging inflammatory responses. We focus on the clinical experiences with RSV, influenza and dengue to demonstrate the complexities of predicting from in vitro assays or animal models whether passively transferred or vaccine-induced antibodies will cause ADE of disease, and of differentiating ADE from a severe illness that is unrelated to pre-existing antibodies.

RSV

In a study of RSV in children under the age of 2 years, there were more cases requiring hospitalization for RSV-related bronchiolitis or pneumonia—especially in those aged between 6 and 11 months—in children who were immunized with a formalin-inactivated (FI)-RSV vaccine (10/101) than in children who were not immunized (control cases; 2/173)²⁵. This was also

observed in a second study (18/23 hospitalizations of immunized children, with two deaths, compared with 1/21 control cases)¹⁶ and in two smaller studies^{17,26}. This condition has been termed vaccine-associated enhanced respiratory disease. Later studies showed that the ratio of fusion protein (F) binding antibodies to neutralizing antibodies was higher in the sera of 36 vaccinated compared to 24 naturally infected children, suggesting that non-neutralizing antibodies to an abnormal F-protein conformation may have been a predisposing factor²⁷. Complement activation, detected by the presence of C4d in the lungs of the two fatal cases, suggested that antibody–F protein immune complexes led to more severe disease²⁸. However, C4d deposition can result from the lectin-binding pathway as well as from the classical pathway, and C4 can be produced by epithelial cells and activated by tissue proteases²⁹. Whether harmful RSV-specific T cells were induced was not determined: although lymphocyte transformation frequencies were higher, this early method did not differentiate antigen-specific responses from secondary cytokine stimulation or from CD4 and CD8 T cell responses, although CD4 T cell proliferation is more likely³⁰. Importantly, the FI-RSV clinical experience did not establish that vaccine-enhanced disease was antibody-dependent³¹. Subsequently, in animal studies, the production of low-avidity antibodies due to insufficient Toll-like receptor signalling and lack of antibody maturation, and the formation of immune complexes have been implicated. However, a definitive antibody-mediated mechanism of enhancement has not been documented³², and models have also identified Th2-skewing of the T cell response and lung eosinophilia with challenge after FI-RSV, raising the possibility that T cells contribute to vaccine-induced enhancement of RSV disease^{31,33}.

Experience with RSV also includes more than 20 years of successful prophylaxis of high-risk infants with palivizumab, a mAb directed against pre- and post-fusion F protein³⁴. Importantly, this experience challenges a role for low neutralizing-antibody titres in the ADE of lung disease, because RSV morbidity does not increase as titres decrease. Further, if suboptimal neutralization were a factor, the failure of suptavumab—caused by F protein drift in RSV B strains—would be associated with ADE of disease; however, infections in such cases were not more severe³⁵. Clinical trials of an RSV mAb that has an extended half-life have shown a reduction in hospitalizations of around 80%, again supporting the concept that such treatments provide protection without a secondary risk from declining titres³⁶. mAbs against RSV have been consistently safe, even as the neutralizing capacity diminishes after administration.

Influenza

Influenza is instructive when considering the hypothesis that cross-reactive antibodies predispose to ADE of disease, because almost all humans harbour antibodies that are not fully protective against antigenically drifted strains that emerge year after year. Instead, pre-existing immunity typically provides some protection against a second viral strain of the same subtype. Antibodies against neuraminidase and against the stem or head regions of haemagglutinin also correlate with protection³⁷. When an H1N1 strain with a haemagglutinin shift emerged in the 2009 H1N1 pandemic, some epidemiological studies linked a greater incidence of medically treated illness to previous vaccination against influenza, whereas others did not^{38–41}. One report correlated cross-reactive, low-avidity and poorly neutralizing antibodies with risk in middle-aged people—the demographic with a higher prevalence of severe 2009 H1N1⁴². Immunopathology and C4d were reported in the lungs of six fatal cases in this age group, indicating that antibody-dependent complement activation through immune-complex formation may have been a contributing factor. However, as noted above, other mechanisms lead to C4d deposition, and lung T lymphocytosis attributed to T cell epitopes shared by 2009 H1N1 and earlier H1N1 strains was also observed, raising the possibility that T cells played a part. Another study correlated pre-existing antibodies that mediated infected cell lysis by complement activation with protection against H1N1 in children⁴³. In a porcine model, enhanced pulmonary disease was observed after vaccination with an inactivated influenza H1N2 strain followed by heterologous H1N1 challenge⁴⁴. The animals had non-neutralizing antibodies that bound haemagglutinin in the stem region, but failed to block the binding of haemagglutinin to its cell receptor and accelerated fusion in vitro by a Fab-dependent mechanism (Fig. 2). Lung pathology was also observed in mice treated with a mAb that induced a conformational change in haemagglutinin that facilitated fusion⁴⁵. Such a mechanism was postulated to have potential clinical relevance when the infecting influenza virus has undergone antigenic shift and the infection boosts non-neutralizing haemagglutinin-stem-binding antibodies without a neutralizing antibody response. The likelihood of these circumstances occurring is unclear. Further, human influenza vaccines are not known to elicit immunodominant antibodies with this property. Importantly, as noted above, stem antibodies correlate both with resistance to infection and to severe disease in humans, indicating that this interesting mechanism is not predictive of disease causation for stem-specific antibodies³⁷. In addition, monoclonal antibodies can be screened to avoid fusion-enhancing properties, and

fusion is not intrinsically accelerated by low titres of neutralizing antibodies. Notably, infants benefit from immunization from six months of age, despite their limited capacity to produce affinity-matured, high-avidity antibodies. Overall, widespread annual surveillance of influenza does not reveal ADE of disease, even though cross-reactive strains and vaccine mismatches are common.

Dengue

There are four viral serotypes of dengue that circulate in endemic areas¹⁹. Although severe dengue haemorrhagic fever and shock syndrome occurs during primary infection, possible ADE of disease has been associated with poorly neutralizing cross-reactive antibodies against a heterologous dengue serotype. Taking into account the difficulty of classification due to the overlapping signs of severe infection and ADE of disease, clinical experience indicates that ADE of disease does occur, but is rare in endemic areas (36/6,684 participants; around 0.5%) and is correlated with a narrow range of low pre-existing antibody titres (1:21–1:80)²⁰. In the same study, high antibody titres were found to be protective. The challenge of predicting how to avoid such a rare immune-enhancing situation against the background of protection conferred by dengue neutralizing antibodies implies that it will be equally difficult for SARS-CoV-2.

When considering conditions that may result in ADE of disease, it is important to emphasize that dengue differs from other viruses because it targets monocytes, macrophages and dendritic cells and can produce progeny virus in these cells, which abundantly express both viral entry receptors and FcγRs. ADE of infection can be demonstrated in vitro with FcγR-expressing cells—typically with cross-reactive antibodies that have low or no neutralizing activity, have low affinity, or target non-protective epitopes, or if a narrow range of antibody and infectious virus concentrations is tested^{46,47}. The mechanism of ADE of disease associated with dengue therefore depends on three factors: the circulation of multiple strains of a virus that have variable antigenicity, a virus that is capable of replication in FcγR-expressing myeloid cells, and sequential infection of the same person with these different viral serotypes. Despite these pre-disposing conditions and the fact that dengue is an increasingly common infectious disease, severe dengue disease is rare.

The role of pre-existing immunity has also been a concern for the quadrivalent live attenuated dengue vaccine (Dengvaxia), because higher hospitalization rates were observed

among vaccine recipients who were initially seronegative—especially children aged between two and eight years⁴⁸. Other explanations for this outcome include poor efficacy against serotypes 1–3, or the failure to induce cell-mediated immunity because T cells primarily recognize non-structural proteins that are not present in the chimeric vaccine. Importantly, the cause of death in 14 fatal cases of dengue could not be determined by the WHO (World Health Organization) Global Advisory Committee on Vaccine Safety, because a failure of vaccine protection could not be distinguished from immune enhancement by clinical or laboratory criteria⁴⁹. This experience underscores how difficult it is to predict the potential for vaccine-induced antibodies or a therapeutic antibody to enhance the severity of disease, because other mechanisms of pathogenesis that result in severe disease are potentially involved—even for the well-studied case of dengue.

In other assessments of the risks and benefits of cross-reactive antibodies, infection with Zika—which, like dengue, is a flavivirus—was less common in individuals who had previously been infected with dengue⁵⁰. In addition, the presence of cross-reactive antibodies has been associated with improved efficacy, as measured by the responses to a yellow fever vaccine in recipients who had received a Japanese encephalitis vaccine⁴⁷, and by association of the effectiveness of Dengvaxia with seropositivity for dengue at the time of immunization⁵¹.

In summary, these clinical experiences with RSV, influenza and dengue provide strong evidence that the circumstances that are proposed to lead to ADE of disease—including low affinity or cross-reactive antibodies with limited or no neutralizing activity or suboptimal titres—are very rarely implicated as the cause of severe viral infection in the human host. Furthermore, clinical signs, immunological assays or biomarkers that can differentiate severe viral infection from a viral infection enhanced by an immune mechanism have not been established^{49,52}.

Assessing the risk of ADE of disease with SARS-CoV-2

Given the complexities described above, it is sobering to take on the challenge of predicting ADE of disease caused by SARS-CoV-2. Here we consider whether clinical circumstances point to a role for antibodies with poor or no neutralizing activity in severe COVID-19, incorporating relevant experience from disease caused by the common human coronaviruses, as well as by severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome-related coronavirus (MERS-CoV).

Infection by SARS-CoV-2 is initiated by the binding of its fusion protein, the spike (S) protein, to the entry receptor, angiotensin-converting enzyme 2 (ACE2)^{53–55}. Other receptors for SARS-CoV-2, such as CD147, have also been reported⁵⁶. ACE2 is expressed on alveolar type II pneumocytes, airway epithelial cells, nasal tract goblet cells and ciliated cells, as well as on intestinal and other non-respiratory tract cells, as assessed by RNA expression⁵⁷. On most such cells, ACE2 seems to be expressed at low levels; however, it can be upregulated by interferons⁵⁸, which could theoretically promote infection if the virus overcomes interferon-induced barriers. FcγRIIa and FcγRIIIa were detected in alveolar, bronchial and nasal-cavity epithelial cells by single-cell RNA sequencing, but both fractions of positive cells and levels of expression per cell were considerably lower than for resident myeloid and natural killer cells^{59,60}. The moderate prevalence of both ACE2 and FcγRs results in poor co-occurrence, although this might be underestimated because of the dropout effect in single-cell transcriptomics. Co-expression of ACE2 and FcγRs therefore seems to be limited, which would mitigate against antibody-enhanced disease caused by SARS-CoV-2 via the dual-receptor mechanism proposed in dengue infection.

When considering potential detrimental effects of antibodies, the presence or absence of cross-reactive antibodies against other human coronavirus (HCoV) strains has not been linked to whether SARS-CoV-2 infection is more severe, mild or asymptomatic, although antibodies that recognized the SARS-CoV-2 S2 subunit were detected in 12 out of 95 uninfected individuals⁶¹. In two reports, 30–50% of SARS-CoV-2 seronegative or unexposed individuals had CD4 T cells that recognized the SARS-CoV-2 S protein^{62,63}. Previous infection with HCoV-HKU1 and HCoV-OC43 betacoronaviruses, or HCoV-NL63 and HCoV-229E alphacoronaviruses, is not known to predispose to more severe infection with the related virus from the same lineage^{64–67}. Conversely, the endemic nature of coronavirus infections indicates that infection in the presence of low levels of antibodies is common, providing a theoretical opportunity for ADE of disease—although these illnesses are mild—and suggesting that cross-protection may be transient⁶⁸. It is of interest that neither low neutralizing-antibody titres nor heterologous virus challenge were associated with enhanced disease in human studies of HCoV-229E^{64,65}. Although HCoV-NL63 also uses the ACE2 entry receptor, the receptor-binding domain (RBD) of HCoV-NL63 is structurally very different from that of SARS-CoV-2, which would limit antibody cross-reactivity.

Antibodies to the S proteins of SARS-CoV and SARS-CoV-2—and, to a much lesser extent, MERS-CoV—can cross-react, and both high-potency neutralizing antibodies that also mediate antibody-dependent cytotoxicity and antibody-dependent cellular phagocytosis⁶⁹, as well as non-neutralizing antibodies, can be elicited against conserved S epitopes^{70,71}. However, the limited spread of SARS-CoV and MERS-CoV means that it is not feasible to assess whether there is any ADE of disease due to SARS-CoV-2 attributable to cross-reactive antibodies⁷². A finding that pre-existing antibodies for other coronaviruses correlate with the low incidence of symptomatic SARS-CoV-2 infection in children would support protection rather than a risk of disease enhancement⁷³. To answer this question, the broad application of serological assays that quantify antibodies to virus-specific and cross-reactive epitopes of human coronaviruses in relation to the outcomes of natural infection and of vaccine and antibody trials is required.

The administration of passive antibodies could also reveal whether antibodies predispose to ADE of disease. In small studies, patients infected with SARS or MERS received polyclonal antibodies without apparent worsening of their illness^{74–77}, and from a meta-analysis it was concluded that early treatment with plasma from patients that had recovered from SARS-CoV infection correlated with a better outcome⁷⁶. In 10 patients with severe COVID-19 that were given plasma with neutralizing titres greater than 1:640 (200 ml) at a median of 16.5 days after disease onset, viremia was no longer detected and clinical parameters improved within 3 days⁷⁸. Similar findings were reported for 5 severely ill patients treated with plasma with neutralizing titres greater than 1:40⁷⁹; however, another study found no difference in outcome between 52 treated and 51 untreated patients⁸⁰. The evidence that COVID-19 does not worsen after treatment with plasma from convalescent patients has been substantially reinforced by a study of 20,000 patients who were severely ill with the disease, showing an adverse event incidence of 1–3%⁸¹. If further substantiated, these findings will markedly diminish the concern that clinically relevant amplification of infection, release of immunopathogenic cytokines, or immune-complex deposition in the presence of a high viral load is mediated by SARS-CoV-2 antibody-dependent mechanisms^{82,83}.

High-dose intravenous polyclonal IgG (IVIg)—which is used to treat systemic lupus erythematosus (SLE), idiopathic thrombocytopenia and Kawasaki syndrome⁸⁴—is thought to exert its beneficial effects through the activation of FcγR inhibitory signalling. Because severe COVID-19 could reflect immune dysregulation, a benefit and/or lack of adverse effects in

patients receiving plasma from convalescent individuals might reflect the suppression of inflammation induced by IgG, rather than supporting the conclusion that passive antibodies do not trigger ADE of disease through Fab- or Fc-dependent mechanisms. However, the dose of IgG administered to patients with SLE (2 g per kg over 5 days)⁸⁵ is much higher than the dose received from convalescent plasma, based on the expected IgG concentrations in plasma (around 500–800 mg per 100 ml) and the amount of convalescent plasma received (200 ml)^{78,79}. Assuming a concentration of 1,600 mg per 200 ml, the IgG levels after receiving convalescent plasma (1.6 g per 80 kg) would be approximately 100-fold less than after receiving IVIg (160 g per 80 kg). It is therefore unlikely that the immunomodulatory effects of polyclonal non-antigen-specific IgG dampened possible manifestations of enhanced illness.

Clinically, infections with SARS-CoV, MERS-CoV and SARS-CoV-2 are often biphasic, with more severe respiratory symptoms developing after a week or more and, in some patients, in association with the release of pro-inflammatory cytokines. This pattern has led to the hypothesis that an emerging immune response—including low-avidity, poorly neutralizing antibodies—could exacerbate the disease. However, reports that relate antibody titres to disease progression involve relatively few patients^{86–88}, and are confounded by the higher levels of antigen seen in severe infections that are predicted to drive a stronger immune response or a heightened innate inflammatory response. One report of three cases of fatal SARS-CoV infection reported that high neutralizing anti-S antibodies and a prominent CD163⁺ monocyte/macrophage pulmonary infiltrate of cells were associated with reduced expression of TGF- β and CD206⁺, which are proposed to be markers of macrophages with beneficial functions⁸⁹. However, quantitative analysis of these changes and evidence of an antibody-mediated pathology that is dependent on these cells were not reported. A recent meta-analysis found no relationship between the kinetics of antibody responses to SARS-CoV, MERS-CoV or SARS-CoV-2 and clinical outcomes⁹⁰. At present, there is no evidence that ADE of disease is a factor in the severity of COVID-19. Instead, lung pathology is characterized by diffuse alveolar damage, pneumocyte desquamation, hyaline membranes, neutrophil or macrophage alveolar infiltrates and viral infection of epithelial cells and type II pneumocytes⁹¹. Further, if instances of ADE of disease occur at all, the experience with dengue suggests that this or other types of immune enhancement will be rare and will occur under highly specific conditions. The aetiology of the

inflammatory, Kawasaki-like syndrome that has been associated with SARS-CoV-2 infection in children is unknown, but has not been associated with antibody responses so far⁹².

In summary, current clinical experience is insufficient to implicate a role for ADE of disease, or immune enhancement by any other mechanism, in the severity of COVID-19. Prospective studies that relate the kinetics and burden of infection and the host response—including the magnitude, antigen-specificity and molecular mechanisms of action of antibodies, antibody classes and T cell subpopulations—to clinical outcomes are needed to define the characteristics of a beneficial compared with a failed or a potentially detrimental host response to SARS-CoV-2 infection. Although it will probably continue to be difficult to prove that ADE of disease is occurring, or to predict when it might occur, it should be possible to identify correlates of protection that can inform immune-based approaches to the COVID-19 pandemic.

Effects of antibodies on SARS-CoV and MERS-CoV

In vitro studies of the effects of antibodies on viral infection have been used extensively to seek correlates or predictors of ADE of disease. These efforts are complicated by the fact that the same antibody mechanisms that are often proposed to result in ADE of infection are responsible for protection from viral disease in vivo. Although infection was most often blocked by anti-S antibodies, several reports have shown antibody-dependent uptake of SARS-CoV or SARS-CoV S-pseudoviruses that was mediated by binding of the Fab component to the virus and the Fc component to FcγR on the target cell (Fig. 2) using in vitro methods^{93–98}. Importantly, viral uptake did not result in productive infection. An antibody that binds the S protein and mimics receptor-mediated entry to facilitate viral uptake has been described for MERS-CoV⁹⁹, but not for SARS-CoV or SARS-CoV-2. Although SARS-CoV and SARS-CoV-2 do not infect myeloid cells^{100–103}, the productive infection of macrophages by MERS-CoV has been reported, albeit at low levels¹⁰⁴. It is notable that higher production of immune-cell-attracting chemokines was observed in myeloid cells infected by MERS-CoV but not in cells exposed to SARS-CoV, suggesting that productive infection has a greater effect on this response¹⁰⁴. The biology of the interactions of coronaviruses with cells expressing FcγRs is therefore very different from the targeting of FcγR-expressing myeloid cells by the dengue viruses. Conversely, in vitro methods can reliably define the properties of mAbs or of vaccine-induced antibodies—including their epitope specificity, binding affinity and avidity, and maturation as well as any potential to

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enhance fusion, together with their capacities for neutralization and antiviral Fc-dependent effector functions (Fig. 2).

Antibody effects in coronavirus-infected animals

Small-animal models

Several mouse, rat and other small-animal models of SARS-CoV infection have used passive-antibody administration or immunization to investigate whether pre-existing antibodies protect against or enhance disease. Although vaccine enhancement of disease in these models could occur through other mechanisms, such studies can directly assess the protective or enhancing properties of passive antibodies.

In the ferret model of SARS-CoV infection, a human mAb was found to protect the animals from infection¹⁰⁵; however, modified vaccinia Ankara expressing S protein (MVA-S) was not protective and liver inflammation was noted in this model¹⁰⁶. Pre- and post-exposure administration of a mAb against MERS-CoV protected mice from challenge, as assessed by lung viral load, lung pathology and weight loss¹⁰⁷. Three mAbs against SARS-CoV, given at a high dose before challenge, protected young and old mice against lung viral spread and inflammation, but had no effect when given after infection¹⁰⁸. Low doses were less protective, but no ADE of disease was observed. A caveat is that human mAbs were tested in the context of mouse FcγRs; however, this can be addressed using human FcγR transgenic animals¹⁰⁹. Both previous infection and passive transfer of mouse neutralizing Abs partially protected 4–6 week old mice against secondary infection with SARS-CoV¹¹⁰, and no ADE of disease was observed despite low neutralizing titres. In another mouse study¹¹¹, passive transfer of SARS-CoV-immune serum was found to mediate protection by Fc-dependent monocyte effector function through antibody-dependent cellular phagocytosis; however, natural killer cells, antibody-dependent cytotoxicity or complement-Ab complexes did not contribute to protection. In a mouse model of vaccination, which used SARS-CoV in which the E protein had been deleted as a live attenuated vaccine, induction of antibodies and T cell immunity and protection against lethal viral challenge was observed in mice from three age groups¹¹². By contrast, enhanced disease was observed in mice that were immunized with formalin- or ultraviolet-inactivated SARS-CoV. Whereas younger mice were protected, older mice developed pulmonary pathology with an eosinophil infiltrate; this suggests a detrimental Th2 response related to age, rather than ADE of disease¹¹³. In some

models, cellular immunopathology might be linked to Th17-mediated activation of eosinophils¹¹⁴. In another report, mice given formalin- or ultraviolet-inactivated SARS-CoV or other vaccine formulations developed neutralizing antibodies and were protected from challenge, but also developed eosinophilic pulmonary infiltrates¹¹⁵. This type of immunopathology has not been reported in fatal human coronavirus infections.

Small-animal studies of SARS-CoV-2 infection are being reported rapidly. Neutralizing antibodies to SARS-CoV-2 were induced by immunizing rats with the RBD of the S protein and adjuvant⁹⁴. In vitro evaluation of the potential for enhanced uptake of SARS-CoV-2 using HEK293T cells expressing rat FcγRI in the presence or absence of ACE2 expression showed neutralization but no enhancement of viral entry. Mice that were given an mRNA vaccine expressing pre-fusion SARS-CoV-2 S protein developed neutralizing antibodies and S-protein-specific CD8 T cell responses that were protective against lung infection without evidence of immunopathology¹¹⁶, and neutralizing mAbs against the RBD of the S protein of SARS-CoV-2 reduced lung infection and cytokine release¹¹⁷.

Passive transfer of a neutralizing antibody protected Syrian hamsters against high-dose SARS-CoV-2, as demonstrated by maintained weight and low lung viral titres¹¹⁸. Similarly, hamsters immunized with recombinant SARS-CoV S protein trimer developed neutralizing antibodies and were protected against challenge¹¹⁹. Whereas serum from vaccinated hamsters mediated FcγRIIb-dependent enhancement of SARS-CoV entry into B cell lines, virus replication was abortive in vitro and viral load and lung pathology were not increased in vaccinated animals⁹⁸. These data underscore that enhancement of viral entry into cells in vitro does not predict negative consequences in vivo, further highlighting the important gap between in vitro findings and the causes of ADE of disease in vivo.

Unlike SARS-CoV, MERS-CoV and SARS-CoV-2, feline infectious peritonitis virus is an alphacoronavirus that, like dengue, has tropism for macrophages. Infection with this virus has been shown to be enhanced by pre-existing antibodies, especially those against the same strain¹²⁰.

Non-human primate models

In non-human primates (NHPs), infection with SARS-CoV, MERS-CoV or SARS-CoV-2 results in viral spread to multiple tissues, including lungs^{121–123}. Rhesus macaques that were

administered a high inoculum of SARS-CoV-2 by nasal, tracheal, ocular and oral routes had increased temperatures and respiratory rates for 1 day, and reduced appetite and dehydration for 9–16 days¹²². Macaques that were euthanized at 3 days and 21 days had multifocal lung lesions, with alveolar septal thickening due to oedema and fibrin, small to moderate numbers of macrophages, a few neutrophils, minimal type II pneumocyte hyperplasia and some perivascular lymphocyte cuffing. SARS-CoV-2 viral proteins were detected in a few type I and type II pneumocytes, and alveolar macrophages and virions were found in type I pneumocytes. Although these foci of lung pathology have some similarities to those observed in human infection⁹¹, NHPs develop minimal or no signs of respiratory or systemic betacoronavirus disease.

After the outbreaks of SARS-CoV and MERS-CoV disease, NHPs were used in the evaluation of several vaccine and antibody interventions (Supplementary Table 1). In one study, FI-SARS-CoV reduced viremia and protected against lung pathology in rhesus macaques¹²⁴, whereas in another study macaques given FI-SARS-CoV developed macrophage and lymphocytic infiltrates and alveolar oedema with fibrin deposition after challenge, indicating the difficulties of establishing consistent NHP models¹²⁵. Synthetic peptide vaccines have also been prepared using sera from convalescent patients to define immunodominant epitopes of SARS-CoV S protein¹²⁵. The vaccines were found to reduce pathology after SARS-CoV challenge unless the S protein of the vaccine included amino acids 597–603, suggesting an epitope-specific basis for the induction of lung pathology. However, these peptide constructs would not be expected to fully mimic antibody or T cell responses that would be elicited to the intact S protein.

Two studies have reported the immunization of rhesus macaques with MVA expressing SARS-CoV S protein or an MVA control. In the first report, three out of four immunized macaques had no detectable shedding or enhanced lung infection 7 days after challenge¹²⁶. In the second report, immunization elicited polyclonal anti-S antibodies with neutralizing activity and reduced infection in three out of eight macaques after challenge⁸⁹. However, although the challenge inoculum was the same as in the first study, areas of diffuse alveolar damage were detected in six out of eight vaccinated macaques compared with one out of eight control animals euthanized at 7 days, as well as at 35 days. Immunization with MVA-S was associated with an accumulation of monocytes and macrophages, and with the detection of activated alveolar

macrophages that produced pro-inflammatory MCP-1 and IL-8, which were not observed in control animals. In a second cohort that was given polyclonal IgG from vaccinated macaques or control animals, loss of TGF- β and increased IL-6 production by activated pulmonary macrophages was observed in macaques that were pre-treated with anti-S IgG, and lung pathology was described as skewed towards immunopathological inflammation. However, it was not stated whether the histopathology was focal or widespread in the lungs, and immunopathology was not associated with impaired respiratory function in macaques evaluated for 21 days (passive anti-S) or for 35 days (MVA-S). Although differences in macrophage markers were associated with changes in the lungs, a causal relationship between anti-S antibodies and an antibody-dependent macrophage-mediated mechanism of more severe pathological changes was not explored, and whether MVA-S might have generated non-neutralizing antibodies that enhanced lung pathology was not assessed. It will therefore be important to define the epitope specificity and serum neutralization activity in these animal models, and potential T cell mechanisms will need to be excluded before enhanced immunopathology can be attributed to antibody mechanisms.

The second study reporting immunization of rhesus macaques with MVA-S⁸⁹ also described in vitro experiments using sera from patients who had recovered from SARS-CoV infection. However, only one out of eight sera samples elicited enhanced cytokine production by human macrophages in vitro. Because IL-8 production by macrophages treated with one of the serum samples was lower in the presence of Fc γ R-blocking antibody (no control serum), it was concluded that blocking Fc γ Rs might be necessary to reduce lung damage caused by SARS-CoV. However, the finding was not confirmed with sera from other severe cases of SARS, and is subject to the caveat that in vitro studies cannot be taken as evidence of ADE of disease.

In contrast to the immunopathology observed after immunization with MVA-S, other studies of SARS-CoV have suggested a protective effect of vaccine-induced antibodies. Using a purified SARS-CoV-infected cell lysate as a vaccine, cynomolgus macaques were protected from challenge, and low neutralizing antibody titres were not associated with ADE of disease¹²⁷. Further, African green monkeys with pre-existing antibody and/or T cells after primary SARS-CoV infection were protected from homologous re-challenge as assessed by lung virus titres, although the pulmonary inflammatory response was not different from that of primary infection¹²⁸.

In additional studies, rhesus macaques immunized with a chimpanzee adenovirus (ChAdOx1 MERS) expressing MERS-CoV S protein, a recombinant S-RBD protein or a synthetic MERS-CoV S DNA vaccine, had decreased infection and no enhanced lung pathology upon challenge^{129–131}.

The potential for immune enhancement of SARS-CoV-2 infection by antibody-dependent or other mechanisms has been assessed by infection and re-challenge of rhesus macaques. Out of two rhesus macaques that were re-challenged 28 days after initial infection—when neutralizing antibody titres were low (1:8–1:16)—neither exhibited viral shedding and one had no lung pathology. Immunity to SARS-CoV-2 in nine rhesus macaques—including the presence of neutralizing antibodies, antibody-mediated effector functions and antiviral CD4 and CD8 T cells—was associated with protection upon re-challenge at 35 days¹²³. When vaccines were tested, rhesus macaques immunized with purified β -propiolactone-inactivated SARS-CoV-2 in alum showed complete or partial protection against high-inoculum SARS-CoV-2 challenge, and histopathological analyses of lungs and other organs at 29 days showed no evidence of ADE of disease compared with control macaques¹³². A large study involving 35 rhesus macaques, which were given prototype DNA vaccines expressing either full-length SARS-CoV-2 S protein or components of this protein, found that protection was correlated with the presence of neutralizing antibodies—and, notably, with Fc-dependent antibody effector functions—and there were no adverse outcomes after challenge¹³³.

In studies of neutralizing mAbs (Supplementary Table 1), viral titres and lung pathology after nasal challenge were reduced in rhesus macaques that were administered a mAb directed against a proteolytic cleavage site in the SARS-CoV S protein that is required for host-cell entry¹³⁴. Macaques given mAbs against MERS-CoV showed less pulmonary involvement and no worsening of disease with challenge¹³⁵. The prophylactic administration of mAbs against MERS-CoV to marmosets one day before challenge was associated with reduced lung pathology compared with the administration of control mAbs^{136–138}; mAbs were found to be protective when administered 2–12 h after challenge but not when given 1 day after challenge^{137,138}. These animal studies of coronavirus infections parallel the observation that the passive transfer of mAbs against RSV that have selected properties can be protective, whereas a particular vaccine formulation (FI-RSV) that is directed to the same viral protein can enhance disease.

In summary, in most animal models—including NHPs—vaccination or the administration of passive mAbs have demonstrated protection against challenge with SARS-CoV, MERS-CoV or SARS-CoV-2, although reports on SARS-CoV-2 are limited. However, studies of an FI-SARS-CoV vaccine, one of two studies of an MVA vaccine expressing SARS-CoV S protein, and vaccination with one S-derived peptide showed enhanced lung pathology in NHPs. Thus, there are limited data to indicate that immune responses that include antibodies (and probably also T cells) induced by some vaccine formulations may be associated with more extensive lung pathology compared with infection alone, whereas the transfer of mAbs with specific properties have, so far, provided protection in animals.

Overall, the lack of a link between clinical measures of disease severity in NHPs and the experimental conditions associated with exacerbated lung pathology is a limitation to their utility in predicting the risks of ADE associated with passive-antibody or vaccine interventions in humans. So far, the models do not emulate the severe respiratory disease observed in COVID-19. Evaluation of T cell responses will also be needed to draw conclusions regarding mechanisms if immunopathology is observed. For example, a strong T cell response has been described as ameliorating ADE of disease in a dengue model¹³⁹ and animal studies have suggested an aberrant T cell response to FI-RSV vaccination^{29,30}. Quantitative assessments of the extent of lung involvement, and histopathological scoring of the characteristics and severity of lesions using validated markers of infected cells, patterns of cell subtype infection and quantification of infiltrating immune cells will be also be necessary before these models can be used to better understand either protective immunity or immune enhancement—whether mediated by antibodies, T cells, intrinsic responses or a combination of factors. A critical point is that the identification of correlates of protection in humans will be necessary to understand how studies in small- and large-animal models can be designed to support or question the benefits of particular immune interventions for SARS-CoV-2 infection.

Conclusions

It is clear that after many years, and considerable attention, the understanding of ADE of disease after either vaccination or administration of antiviral antibodies is insufficient to confidently predict that a given immune intervention for a viral infection will have negative outcomes in humans. Despite the importance that such information would have in the COVID-19 pandemic,

in vitro assays do not predict ADE of disease. Most animal models of vaccines and antibody interventions show protection, whereas those that suggest potential ADE of disease are not definitive and the precise mechanisms have not been defined. Although ADE is a concern, it is also clear that antibodies are a fundamentally important component of protective immunity to all of the pathogens discussed here, and that their protective effects depend both on the binding of viral proteins by their Fab fragments and on the effector functions conferred by their Fc fragments. Even when vaccine formulations such as formalin inactivation have shown disease enhancement, neutralizing antibodies with optimized properties have been protective. Further, the potential mechanisms of ADE of disease are probably virus-specific and, importantly, clinical markers do not differentiate severe infection from immune enhancement. Additional mechanism-focused studies are needed to determine whether small-animal and NHP models of virus infection, including for SARS-CoV-2, can predict the probable benefits or risks of vaccines or passive-antibody interventions in humans. Optimizing these models must be informed by understanding the correlates of protection against SARS-CoV-2 in natural human infection and as vaccines and antibodies are evaluated in humans. Such mechanistic and in vivo studies across viral pathogens are essential so that we are better prepared to face future pandemics. In the meantime, it will be necessary to directly test safety and define correlates of protection conferred by vaccines and antibodies against SARS-CoV-2 and other viral pathogens in human clinical trials.

Online content Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability [are available at](#)

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Fig. 1 | Neutralization of viruses by functions of the IgG Fab fragment. Mechanisms of antibody-mediated neutralization of viruses by functions of the IgG Fab fragment that block binding to cell surface receptors and inhibit infectivity by aggregating viral particles and inhibiting steps in the viral life cycle, such as fusion. Binding of antibodies with certain properties may enable changes in the viral entry protein that accelerate fusion.

Fig. 2 | Antibody effector functions of the IgG Fc fragment. Antibody effector functions are mediated by binding of the IgG Fc domain to FcγRs on myeloid cells or to components of the complement system. These activities occur when the antibody binds the target virus protein either on virions or on infected cells. **a**, Viral particles are internalized and degraded and local cytokine release recruits immune cells. **b**, If cells are permissive, progeny virions could be produced. When virus–antibody complexes are taken up by the cell, a detrimental cytokine response may be generated. **c**, Binding of the IgG Fc fragment to C1q leads the activation of complement components C3, C3a and C5a and of the complement membrane attack complex (MAC) that disrupts membranes. C3 and C5a facilitate phagocytosis by myeloid cells. C3a and C5a are anaphylatoxins that attract inflammatory cells, which can secrete cytokines that enhance antiviral immunity but could be detrimental if produced in excess. **d, e**, The IgG Fc domain binds to multiple types of FcγRs on myeloid cells to trigger effector functions. The specific consequences of this interaction are dependent on the FcγR that is involved and are not detailed here. **d**, Antibody-dependent phagocytosis by macrophages and dendritic cells. **e**, Antibody-dependent cytotoxicity mediated by natural killer cells. **f**, Antibody-mediated antigen

presentation after the uptake of virus or virus-infected cells by phagocytic cells leads to the activation of antiviral T cells.

Table 1 | Information provided by and limitations of approaches for the assessment of antibody-mediated protection against SARS-CoV-2 and the potential for antibody-dependent enhancement of disease

Test modality	Information provided	Limitations
In vitro: cell culture Infect relevant human cells with or without antibodies	Virus neutralization Virus uptake, productive infection or cytokines	Cell lines lack primary cell receptor characteristics Primary human cells are difficult to culture and have donor variability <ul style="list-style-type: none"> • Receptor expression must be maintained
In vivo: animal models Infection of animals with or without antibody or vaccine intervention	Protection against or increase of viral replication or disease	Lack of disease models of human illness Lack of models predictive of enhanced disease in humans Viral replication as a proxy of disease requires clinical validation Need to assess T cells for contribution to pathology or reducing ADE With human mAbs: <ul style="list-style-type: none"> • Differential engagement of animal FcγRs • Different expression patterns of FcγRs in humans and animals • Potential generation of anti-human antibodies
Human: Clinical and epidemiological studies	Correlations of outcomes with <ul style="list-style-type: none"> • Previous HCoV infection • Treatment with plasma from convalescent patients • Kinetics of adaptive immune responses 	No markers to differentiate severe disease from enhanced disease Limited knowledge of antibody or T cell epitope specificities during natural SARS-CoV-2 or other HCoV infection, and of outcomes of infection with new coronaviruses

Box 1 | Definitions

ADE of disease: Enhancement of disease severity in an infected person or animal when an antibody against a pathogen—whether acquired by an earlier infection, vaccination or passive transfer—worsens its virulence by a mechanism that is shown to be antibody-dependent.

Vaccine enhancement of disease: Enhancement of disease severity in an infected person or animal that had been vaccinated against the pathogen compared to unvaccinated controls. This results from deleterious T cell responses or ADE of disease and is usually difficult to link to one or the other.

Neither ADE of disease nor vaccine enhancement of disease have established, objective clinical signs or biomarkers that can be used to distinguish these events from severe disease caused by the pathogen. Carefully controlled human studies of sufficient size enable the detection of an increased frequency of severe cases in cohorts

given passive antibodies or vaccines compared to the control group, and atypical manifestations of infection can be identified should they occur.

Mechanisms of antibody-mediated protection and the potential for ADE of infection

The essential benefits of antibodies are mediated by several well-defined mechanisms that also have the potential for ADE of infection. Protection as well as ADE of infection can be observed in various assays of virus–cell interactions. An observation of ADE of infection *in vitro* does not predict ADE of disease in humans or animals.

Virus entry: Antibodies block viruses by interfering with their binding to receptors on host cells or inhibiting changes in the viral protein needed for entry.

Virus binding and internalization: Antibodies bind viruses to cells of the immune system via Fc γ receptors on the cell surface and internalization of viruses typically results in their degradation.

Instead of protection, ADE of infection may occur if antibody binding improves the capacity of the viral protein to enable entry of the virus into its target cell, or if the virus has the capacity to evade destruction and produce more viruses after Fc γ receptor-mediated entry.

Cytokine release: Antibodies that bind viruses and Fc γ receptors on cells of the immune system trigger the release of cytokines that inhibit viral spread and recruit other immune cells to eliminate infected cells. Although a part of the normal protective immune response, this can result in ADE of disease if excessive.

Complement activation: Antibodies binding to virus or viral proteins on host cells may activate the complement cascade, a series of plasma proteins that together have a role in protective immunity through multiple mechanisms. Formation of large complexes of antibodies and viral proteins (antigens) can lead to immune complex deposition that activates complement. When excessive, antibody-dependent activation of complement may result in tissue damage and potential ADE of disease.

Antibody-mediated mechanisms in the development of memory immunity: Antibodies bound to viruses or viral proteins can be taken up Fc γ receptors into immune system cells that process the antigens for activation and expansion of B cells and T cells. These mechanisms, which are critical for the establishment of memory immunity against future encounters with the virus, balance the potential risk of amplification of infection after viral uptake by some immune system cells.

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Author contributions A.M.A. and H.W.V. drafted the manuscript, K.F., M.A.S., A.C., R.S., C.H.-D., A.L. and D.C. edited the draft, and A.M.A. and H.W.V. prepared the final manuscript, which was reviewed and approved by all authors.

Competing interests The authors of this manuscript are employees of, or have other affiliations with, Vir Biotechnology. Vir had to choose how to proceed with monoclonal antibodies to treat or prevent COVID-19 disease in the light of the evidence surrounding the possibility of ADE as detailed in this review. This review reflects the result of the team of authors carefully reviewing the literature to assess these choices and is provided as a service to the community. Vir has elected to test human monoclonal antibodies with Fc activity preserved or enhanced, based on the lack of consistent evidence for ADE of disease noted above and evidence that the protective activities and potency of antibodies often involves antibody effector functions. We could have elected to take forward monoclonal antibodies engineered to lack effector function, and so our antibody-related clinical programmes for SARS-CoV-2 could have moved forward regardless of the outcome of our review. A.M.A.'s contributions were part of her personal outside consulting arrangement with Vir Biotechnology and were not associated with Stanford University.

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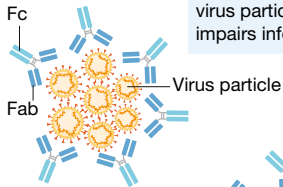
Additional information

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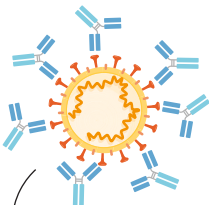
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Virus particle

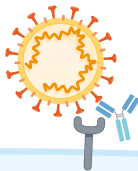


Interference with viral protein binding to the cell receptor

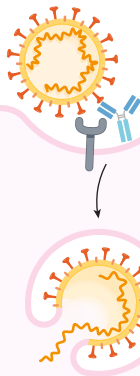
Viral protein receptor

Host cell

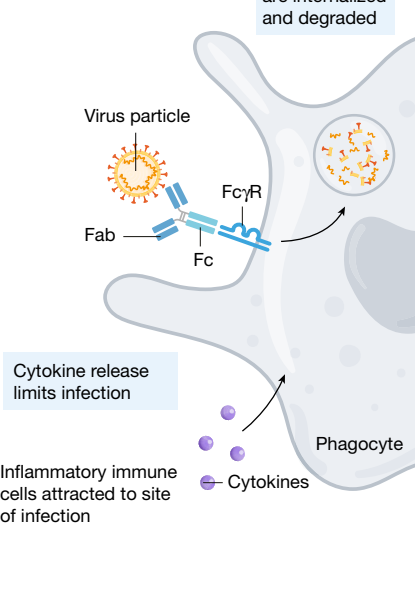
Interference with viral protein fusion function after cell receptor binding



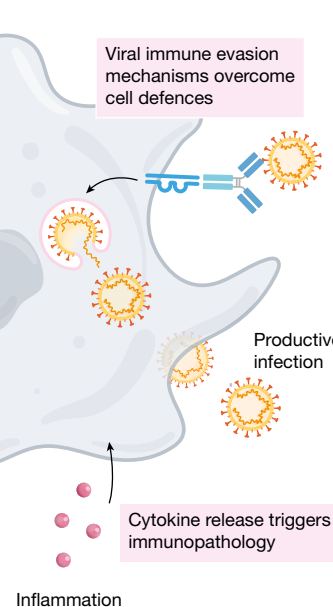
Antibody binding can enhance viral fusion



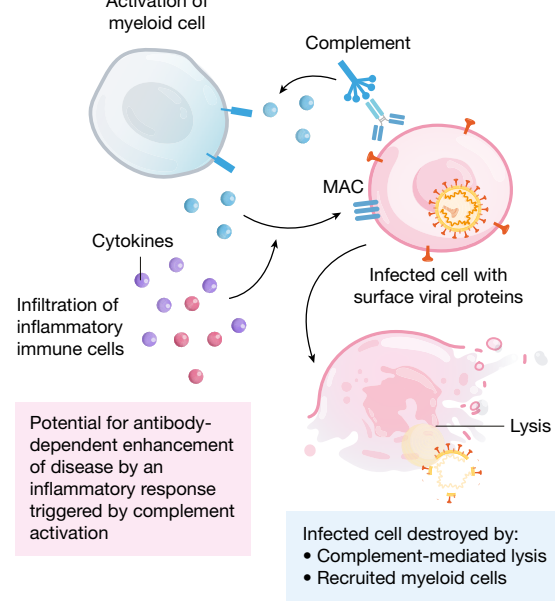
a Mechanisms of antiviral antibodies



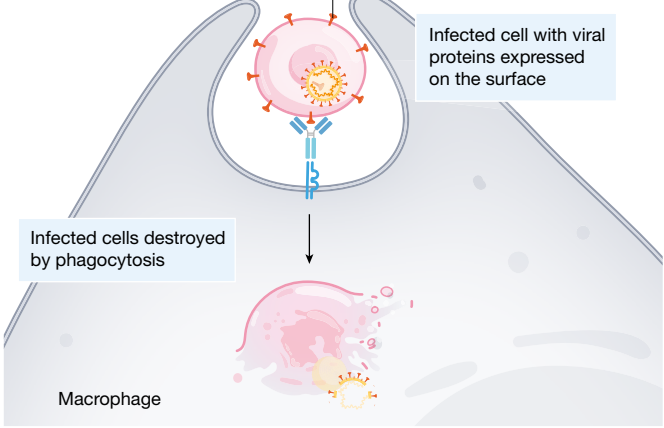
b Antibody-dependent enhancement of infection



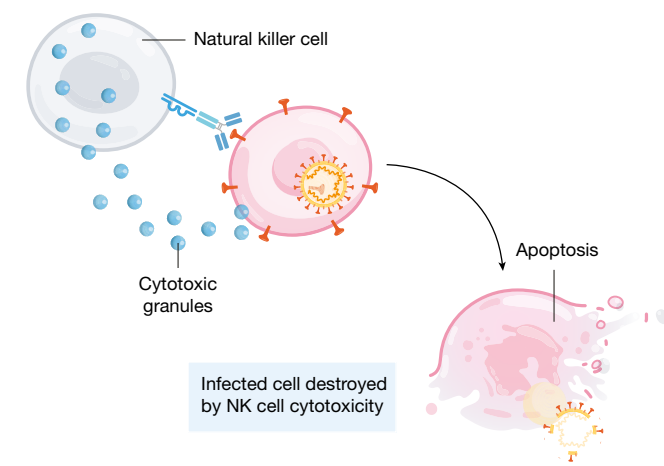
c



d Antibody-dependent cellular phagocytosis



e Antibody-dependent cell-mediated cytotoxicity



f Antibody-mediated viral antigen presentation activates T cells

