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Competing interests
The author declares no competing interests.

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Implications of antibody-dependent enhancement of infection for SARS-CoV-2 countermeasures

To the Editor — For certain diseases, patients who have been previously infected by one strain of a virus and who are later infected by another strain can suffer outcomes that are worse than those infected only once. One explanation for this phenomenon is that differences between two viral serotypes can compromise the ability of antibodies induced by the first infection to neutralize the second one; instead, the antibodies elicited by the first infection 'bridge' the second viral strain to immunoglobulin G (IgG) antibody constant region (Fc) receptors on immune cells, such as macrophages. Because this bridging is believed to enable viral entry into immune cells, shifting the tropism of the virus[1], the outcome manifests as an antibody-dependent enhancement (ADE) of infection and a potentially more serious recurrence of disease. This phenomenon is often observed when antibody concentrations decrease as a result of waning immunity; an antibody may neutralize potently at high concentrations but cause enhancement of infection at sub-neutralizing concentrations.

ADE has been observed with dengue virus[2], Zika virus[3], Ebola virus[4] and, importantly in the context of COVID-19, coronaviruses (CoVs)[5–9]. Although no well-defined set of viral properties has been definitely established as causally linked to ADE, viruses with severe clinical manifestations of ADE show an ability to either replicate in macrophages or other immune cells or otherwise manipulate these cells' immunological state[10,11]. We believe that it is important to consider ADE in the context of efforts to develop countermeasures against the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Indeed, data from previous CoV research strongly suggest that ADE may play a role in the virus's pathology. If this is the case with SARS-CoV-2, then careful design and testing of vaccines or alternative approaches to prophylaxis will be needed to prevent ADE. Here, we highlight clinical and experimental observations from earlier CoV outbreaks and suggest strategies that may reduce ADE in treating the SARS-CoV-2 pandemic. Additionally, we outline how techniques in immunology and protein design can be leveraged to devise vaccines and biologics that avoid ADE.

Clinical samples from the 2003–2004 SARS-CoV outbreak indicate that the virus can infect immune cells, even though immune cells do not express the angiotensin-converting enzyme 2 (ACE-2) receptor, which mediates SARS-CoV entry into lung cells[12,13]. These results were corroborated by in vitro observations showing that some antibodies that bind viral spike (S) protein can facilitate uptake by human macrophages and B cells via their Fcγ receptors (FcγRs)[14,15]. In the case of Middle Eastern respiratory syndrome coronavirus (MERS-CoV), Fc-mediated targeting has been observed with neutralizing antibodies that bind directly to the receptor-binding domain of S protein[8]. For both viruses, this phenomenon is dependent on antibody concentration. Although ADE is believed to target primarily immune cell populations, it is worth noting that lung epithelial cells have also been shown to express functional neonatal Fc receptor, a non-classical FcγR[16]. RNA expression data assembled on the Human Protein Atlas[17] show that FcγRs may have complex expression patterns that may warrant further exploration for resolving ADE mechanisms.

Although efficacy data on human CoV vaccines are lacking, results from preclinical models suggest that certain vaccine designs are more likely to induce ADE immune responses than others. For example, when macaques were vaccinated with inactivated SARS-CoV and challenged with live virus, some of the animals exhibited an ADE phenotype, with extensive macrophage and lymphocyte infiltration in the lungs and edema in the alveolar cavity[18]. In addition, macaques vaccinated with a modified vaccinia Ankara virus expressing SARS-CoV spike were more likely than controls to exhibit acute diffuse alveolar damage and severe acute lung injury upon viral challenge[19]. Mice and hamsters vaccinated with trimeric spike protein vaccines were protected from SARS-CoV infection but showed evidence of developing antisera that facilitated ACE2-independent pseudovirus entry[20,21]. When four different SARS-CoV vaccines developed for human use were tested in mice (two different whole virus vaccines, a recombinant spike protein, and a virus-like particle), they all triggered pulmonary immunopathology upon viral challenge[22]. Similar effects were also seen in mice vaccinated with virus replicon particles expressing SARS-CoV nucleocapsid protein[23] and inactivated MERS-CoV[24].

Circumstantial clinical evidence for ADE in SARS-CoV patients includes the observation that macrophages treated with the virus and sera from patients who succumbed to the infection showed a cytokine profile that was similar to that observed in macaques that experienced fatal acute lung injury following vaccination and viral challenge[19]. This effect could be diminished by blocking FcγR, suggesting that, as in the macaque model, this effect is mediated by antibodies. More indirectly, antisera from SARS-CoV patients were protective in viral infectivity assays when used at a high concentration but enhanced infection when highly diluted[25].

The ADE mechanisms driving viral replication or T-helper type 2 cell–mediated pathology of SARS-CoV and MERS-CoV remain unresolved. In the case of SARS-CoV, infected macrophages exhibit little or no induction of interferon-β, leading to the hypothesis that viral suppression of the immune response results in unchecked viral replication in respiratory epithelial cells[26]. This may result in high viral loads that lead to further tissue damage and drive pathologic adaptive immune responses. Antiserum against the S protein can enhance viral load in macrophages[27], suggesting a model in which antibody-mediated CoV–FcγR binding increases virus uptake by macrophages, functionally inactivating them by virally mediated immunosuppression.

Whether SARS-CoV-2 can cause ADE effects remains an open question. Epidemiological studies investigating ADE in individuals with multiple SARS-CoV-2 infections or cross-reactivity to common-cold-causing CoVs will likely take several years. However, given that ADE has been observed with the closely related SARS-CoV, we believe that the question of ADE effects in SARS-CoV-2 should be urgently resolved using experimental immunology. For example, measuring cross-reactivity of antibodies of unexposed individuals to SARS-CoV-2 could help identify related viruses capable of generating ADE-causing antibodies. Similar characterization of cellular responses, some of which have already been published[28], would help reveal cross-reactive T cell memory responses that may be involved in amplifying common cold B cell responses. Such cross-reactive T cell responses have been linked to plasma leakage syndrome in dengue fever[29].

Titration experiments using pseudotyped viruses carrying S protein should be used to analyze sera from recovered patients. If antibodies against SARS-CoV-2 with ADE potential are detected, vaccine development efforts can leverage the full suite of modern technologies around epitope mapping, protein design, adjuvant design and delivery to maximize safety. Specifically, patient sera can be used to build probabilistic maps of ADE-associated epitopes using high-throughput peptide-based scanning methods[30–32] and hydrogen–deuterium exchange mass spectrometry[33,34]. Sequencing of patient antibody repertoires[35,36] and computational approaches[37,38] can then extend epitope mapping data to structural predictions of antibody–epitope interactions. As a cautionary note, no effort has yet been able to identify epitopes that can fully avoid the problem of ADE for any single viral pathogen associated with the phenomenon. However, studies on mouse antibodies raised against immunogenic SARS-CoV epitopes suggest that regions of the spike protein do vary in their propensity to cause ADE[18]. If it turns out that no SARS-CoV-2 antigens fully avoid ADE, then vaccine development efforts should aim at eliciting a T cell response against non-surface proteins, perhaps by borrowing approaches from neoantigen vaccines[39].

Assuming that antibodies with a low risk for ADE can be identified, the candidate molecules can then be further filtered for their ability to elicit T cell immunity, an important aspect of vaccine design. To predict epitopes that are recognized by T helper cells, state-of-the-art major histocompatibility complex (MHC) prediction methods can rank candidates likely to be presented on MHC-II molecules[40–42]. These predictions could be complemented with screens that use DNA-barcoded tetramer libraries to identify viral peptides that bind to patient-derived T cells[43–45], or using the T-Scan technology[46]. The chosen epitopes known to have a low risk of eliciting ADE antibodies will need to fold properly, mimicking the native conformation in the virus, to elicit productive B cell responses in vaccinated individuals. Computational tools for de novo protein folding can help identify minimal fragment sizes containing properly folded epitopes[47,48] or can help stabilize the fragments by introducing disulfide bonds or scaffold domains[49,50]. For example, prolines have been introduced in the fusion domain to stabilize the pre-fusion conformation of S protein[51,52].

Because there is no guarantee that epitopes that exhibit low ADE risk are also immunogenic, vaccine programs may have to boost titers by using adjuvants and/or maximizing the intrinsic immunogenicity of the various vaccine platforms. Gene-based vaccination platforms, such as plasmid DNA or synthetic mRNA, should allow a greater immune response to weakly immunogenic antigens by delivering them with or fusing them to protein-based adjuvants[53,54]. Furthermore, one such adjuvant, CD40L, has been shown to reduce the pulmonary pathology otherwise associated with an adenoviral MERS-CoV vaccine[55]. Although the mechanism for this observation has yet to be elucidated, the data underscore the need for testing specific adjuvants that may reduce the risk of ADE — perhaps by shifting either the isotype or the breadth of the resulting immune response.

Animal models often serve as invaluable tools for determining the safety and efficacy of vaccines. In fact, preclinical studies employing various animals, including mice, hamsters, ferrets and macaques, provided evidence that SARS-CoV vaccines are capable of causing an ADE response[18–23,56,57]. However, the immune system of each of these animals differs compositionally and functionally from the human immune system. Specifically, Fc receptors differ in sequence, structure and function between animal models and humans[58,59]. Such differences make it difficult to study human antibodies in animal models and to translate the effects of a vaccine in a model to human use. However, the use of transgenic models, such as human FcγR-expressing mice[60], may more faithfully model human immune responses to candidate vaccines and antibody therapeutics. Ultimately, non-human primates represent the best option for building models of human ADE responses, as evidenced by the ability of SARS-CoV Chinese macaque vaccination/challenge model to recapitulate certain aspects of sera from patients with SARS[19]. However, failures of some HIV vaccine candidates have highlighted the need for caution even with non-human primate models[59].

While vaccine testing is ongoing, monoclonal antibodies will be a compelling option for protecting immunocompromised populations, including the elderly, or healthcare workers who may be exposed to high viral loads. Monoclonal antibodies and associated approaches, such as convalescent sera, should be carefully tested for ADE effects. One powerful potential safeguard could involve mutating the Fc-binding domain of the monoclonal antibody to retain its neutralizing potential while preventing uptake in immune cells[61,62]. There are known mutations that abrogate the binding of antibodies to Fcγ receptors, including LALA (L234A L235A), LALA-PG (L234A L235A P329G), and elimination of the glycosylation site at N297[63–65]. Notably, introduction of LALA-PG and elimination of the glycosylation binding site have been demonstrated to completely eliminate the effector functions of the Fc region, whereas introduction of the LALA mutation leaves minimal, but sometimes detectable, activity. Additionally, recent efforts have suggested that alteration of the Fc portion can be performed not just to eliminate binding to FcγRs, but conversely, to allow enhanced immune responses to viruses. For example, the F241A mutation in the Fc region results in an antibody with a more robust endogenous immune response caused by more efficient CD23 uptake and greater immunogen formation in situ[66]. Whether such immune-enhancing mutations alter the risk of ADE is unknown.

Although the development of vaccines and therapeutics for SARS-CoV-2 remains urgent, we must proceed with caution, using the full armory of vaccine and protein design tools at our disposal to rationally minimize the risk of ADE. ❐

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Nikolai Eroshenko[1], Taylor Gill[1], Marianna K. Keaveney[1], George M. Church[2,3], Jose M. Trevejo[4] and Hannu Rajaniemi[1]✉

[1]*Helix Nanotechnologies, Cambridge, MA, USA.* [2]*Department of Genetics, Harvard Medical School, Boston, MA, USA.* [3]*Wyss Institute for Biologically Inspired Engineering, Boston, MA, USA.* [4]*SmartPharm Therapeutics, Cambridge, MA, USA.* ✉*e-mail: hannu@helixnano.com*

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Competing interests

N.E, T.G, M.K.K, G.M.C., J.M.T. and H.R. are shareholders in and/or employees of Helix Nanotechnologies Inc., which is developing gene-encoded vaccines for SARS-CoV-2. A full list of G.M.C.'s technology transfer, advisory roles and funding sources can be found at http://arep.med.harvard.edu/gmc/tech.html. J.M.T. is a shareholder and employee of SmartPharm Therapeutics, which is developing gene-encoded antibody vaccines for SARS-CoV-2.

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Blueprint for a pop-up SARS-CoV-2 testing lab

To the Editor — On 11 March 2020, the World Health Organization declared the 2019 coronavirus disease (COVID-19) a global pandemic[1]. As of 29 May, the virus that causes the disease, SARS-CoV-2, has infected over 5,813,000 people and killed more than 360,000 worldwide (https://coronavirus.jhu.edu/map.html). The virus continues to spread around the world, and at the time of writing there are no clinically validated medical interventions to prevent or cure COVID-19. Public health measures in the United States and elsewhere focus on mitigating spread through diagnostic testing, self-isolation and shelter-in-place orders[2].

The presence of presymptomatic and mildly symptomatic individuals in the general population is a major driver in the accelerated and widespread outbreaks that have overwhelmed healthcare infrastructures worldwide, causing more deaths[2–5]. Extensive testing in countries such as Iceland, New Zealand, Germany and South Korea, among others, has proven an effective tool in controlling the spread of the disease[3–7].

At the start of our effort, on 14 March 2020, the turnaround time for testing for University of California (UC) Berkeley students through commercial labs exceeded seven days (UC Berkeley Tang Center, personal communication), and no rapid or surveillance testing was available to City of Berkeley first responders (City of Berkeley Fire Department Chief David Brannigan, personal communication) or to vulnerable populations in Berkeley, including those living in congregated settings and the unsheltered.

To address the need for expanded testing capacity, the Innovative Genomics Institute (IGI) at UC Berkeley established a clinical testing laboratory for SARS-CoV-2 in three weeks (see "Timeline of SARS-CoV-2 IGI Laboratory Establishment" on Figshare). Timely setup presented formidable challenges, including navigating state and federal regulations, supply-chain and logistic obstacles, and challenges related to serving populations beyond UC Berkeley (Table 1). To tackle these hurdles, we partnered with UC Berkeley's University Health Services (UHS) and created specialized teams to execute the technical, operations, regulatory, human resources, data management, physician interface, sample collection and sample reporting processes for the IGI laboratory (see "IGI SARS-CoV-2 Testing Organizational Chart" on Figshare).

When we began, our campus did not have a clinical testing facility that would allow our testing lab volunteers to work at the level of biosafety required by our campus for SARS-CoV-2 diagnostics, and without a medical school with an affiliated medical center, our campus had no mechanism to