POTENTIAL MECHANISMS OF AGE RELATED SEVERITY OF COVID-19 INFECTION: IMPLICATIONS FOR DEVELOPMENT OF VACCINES, CONVALESCENT SERUM, AND ANTIBODY THERAPIES

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ABSTRACT

There is an urgent need for vaccines to induce immunity to the 2019 coronavirus strain (COVID-19; CoV-SARS-2). Vaccine development may not be straightforward, in part due to the the phenomenon of antibody-dependent enhancement (ADE). The immune response to coronavirus infection or vaccination generates a mixture of IgG antibodies against viral surface proteins. Many of these antibodies block viral infection. However, in some cases IgG:virus complexes can facilitate viral entry and infection of cells by ADE, increasing the the risk and severity of infection. This phenomenon occurs in SARS, MERS, HIV, Zika and dengue virus infection and vaccination; it has been a serious barrier to vaccine development for these infections. Lack of high-affinity anti-COVID-19 IgG antibodies in children and younger adults may explain, in part, the decreased severity of infection in these groups. Here, we discuss the evidence for ADE in the context of COVID-19 infection, and how it may affect development of a vaccine and convalescent serum therapies. Here we discuss ADE in the context of COVID-19 infection, and how this may affect vaccine development, convalescent serum, and targeted monoclonal antibody therapies.

Keywords COVID-19 · coronavirus · antibody-dependent enhancement · Fc receptor · vaccine · antibody

Introduction

The coronavirus infectious disease 2019 (COVID-19, CoV-SARS-2) pandemic is currently an immense global health threat[1]. Estimates are that 14-20% of infected patients develop severe illness requiring hospitalization[2, 3, 4]. There are currently no effective treatments available, although there is a global rush to develop vaccines, small molecule inhibitors, and to test a variety of existing compounds for anti-viral activity. Approximately ~5% of those infected develop acute respiratory distress syndrome(ARDS), with high mortality. COVID-19 infection appears to occur at similar rates across age groups, al-

though the severity of disease is less for those <19 years of age[3, 5]. Interestingly, younger individuals are also known to lack or have a lower incidence of high affinity anti-CoV IgG. This is relevant as certain antibodies can potentiate, rather than protect against, CoV infection through antibody-dependent enhancement (ADE), wherein normal mechanisms of antigen-antibody complex clearance fail, and instead provide an alternate route for host cell infection[6]. These observations have serious implications for the development strategy of vaccines that induce anti-COVID-19 IgG antibodies. In this Viewpoint, we discuss the data underlying our concerns, and suggest strategies for assessing this risk, vaccine development and deployment.

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Epidemiology of COVID-19 infection

Initial data regarding the epidemiology of COVID-19 shows that individuals ≤ 19 years of age accounted for $<3\%$ of all confirmed cases[4, 5]. Multiple reports have also indicated that individuals ≤ 19 years old have milder symptoms, a lower hospitalization risk, and lower case fatality rates[2, 3, 4]. However, recent work by the Shenzhen Center for Disease Control, following 1286 close contacts of 391 index cases over a 28 day period, demonstrated that COVID-19 infection rates among close contacts ≤ 19 years old were equivalent to those found in older cohorts[3]. Importantly, this younger cohort was often asymptomatic ($<50\%$ presenting with fever) and had less severe infection even when symptomatic. Similar patterns have been observed for the SARS[7] and MERS[8] coronaviruses, with a low incidence of symptomatic infection and fewer severe cases. Indeed, the inverse relationship of age and asymptomatic coronavirus infection with less pathogenic human strains (e.g. 229E, NL63, OC43) has been known for over a decade[9].

Anti-CoV IgG levels in children and adults

An important difference between children and adults is the presence of IgG antibodies directed at common circulating human coronavirus strains. Children lack anti-CoV IgG prior to 6 years of age, but then begin to develop antibodies against the common circulating strains in humans (229E, NL63, OC43, HKU1). The presence of such anti-CoV IgG increases with age, with several studies demonstrating high titers in people >6 years old[10]. There is also some evidence of cross-strain binding for anti-CoV IgG directed against the common strains circulating in humans[11]. Importantly, the anti-CoV IgG repertoire in children may consist of predominately low affinity IgG, which will mature to high affinity anti-CoV IgG only after repeated infections.

Antibody dependent enhancement in CoV infections

Coronaviruses make use of antibody-dependent enhancement (ADE) of infection as an alternate mode of viral fusion with target cells[12, 13]. The coronavirus S (spike) protein contains a ligand binding domain, the angiotensin converting enzyme 2 (ACE2) protein for COVID-19 and SARS[14, 15], and dipeptidyl peptidase 4 (DDP4/CD26) for MERS[16]. Antibodies targeting the receptor binding sites can prevent S-protein:ligand binding and potentially viral fusion[17, 18, 19]. However, anti-S protein IgG complexed with virus will cause uptake of the virus-IgG complex via the Fc family of receptors[20], and subsequent viral fusion with macrophages, B cells, monocytes (Fc γ family), and vascular endothelium via the neonatal Fc receptor (nFcR)[21, 12]. Binding of complement to antigen-antibody complexes formed by IgG1 and IgG3 may also be a mechanism for ADE. Normally a mechanism

for viral clearance and antigen presentation, phagocytosis now becomes another route for viral infection. This mechanism is well known for other viruses, including respiratory syncytial (RSV), HIV, and dengue virus (DENV)[20].

For SARS and COVID-19, the protein sequences responsible for ADE have been identified on the S protein (Figure 1) [22]. Importantly, sera from SARS patients contain a mixture of IgG antibodies that can both inhibit CoV infection and cause ADE[23]. Similarly, vaccination with recombinant S protein can elicit both inhibitory IgG antibodies in animal models, as well as antibodies responsible for ADE[22]. Even the presence of neutralizing antibodies can cause severe disease. Anti-S protein:CoV complexes have been linked to increased cytokine release (e.g. MCP-1 and IL-8) in lung macrophages, and cytokine storm syndrome[24].

Pre-existing anti-CoV IgG antibodies to common strains of CoV that infect humans, but also react with COVID-19 and induce ADE, could be another risk factor for severe disease[13]. This is a well described phenomenon for DENV, where antibodies against one strain are a risk factor for severe disease during infection with another DENV strain[25], and anti-DENV IgG appears to potentiate Zika virus infection via ADE[26]. While high affinity anti-CoV IgG may be most effective in neutralizing CoV during infection[27], it may also increase risk for ADE. This is not a hypothetical concern. Clinical trial results for vaccines against DENV and RSV halted the development of these vaccines when they potentiated the severity of infection[28, 29]. ADE has thus been a major barrier to development of HIV, Zika Virus, and DENV vaccines[30, 31, 32].

Can lack of high affinity anti-CoV IgG explain less severe infections in children?

These findings suggest at least three mechanisms that may contribute to decreased severity of COVID-19 in subjects ≤ 20 years of age. At the time of infection, the absence or lower concentrations of high affinity, cross-reactive, anti-CoV antibodies capable of inducing ADE may contribute to lower viral loads and more rapid clearance of CoV. This may include a shift to CD8 T cell and NK cell mediated viral clearance. Secondly, the development of high-affinity, class switched IgG antibodies generally requires multiple rounds of antigenic exposure (e.g. priming and boosting). Even those antibodies that emerge during the immune response after the first exposure to will initially be of lower affinity. Finally, ADE has been linked to the development of cytokine storm syndrome, which occurs in the most severe cases of MERS, SARS and COVID-19 infection[6, 23]. Thus, absence of high affinity anti-CoV IgG could potentially mitigate infection severity, and potentially explain the milder disease in children and younger adults. Further work will need to be done to assess this hypothesis.

Figure 1. ADE associated CoV spike protein sequences.. The antibody-dependent enhancement (ADE) associated spike glycoprotein peptide sequences S₅₇₉₋₆₀₃ from SARS[22] are also conserved in two COVID-19 strains (isolates in bold) and the closely related bat coronavirus strain (RaTG). There is less sequence homology in the same S protein region of MERS or the common human coronavirus strains (HKU1, OC43, 229E). Sequence homologies analyzed with the Clustal Omega method using Unipro UGENE v33.0 software.

Implications for convalescent serum and monoclonal antibody therapy

The potential for ADE has implications for the use of convalescent sera or targeted monoclonal antibody therapies for treatment of COVID-19, which are being discussed as potential therapies and for clinical trials[33]. The hypothesis is that such sera or antibodies may improve infection morbidity and mortality, if given early enough in the course of COVID-19 illness. This is based on reports of the presence of neutralizing antibodies in the sera of individuals who have recovered from COVID-19 infection[34]. As noted above, such sera may also contain antibodies that induce antibody-dependent enhancement of COVID-19 infection. Neutralizing antibodies in sera have also been associated with worse a worse prognosis in some individuals with SARS[35]. Similar phenomena could also occur with targeted monoclonal antibodies[30, 36, 37]. This suggest that any wide-spread use of convalescent sera or monoclonal antibodies should take this possibility into account, and perhaps include screening for ADE *in vitro* in therapeutic preparations.

Implications for vaccine development

The above data suggest that development of a COVID-19 vaccine will require careful design and testing to assure both efficacy and safety. There are several vaccine types currently being pursued for COVID-19 including: mRNA, DNA, recombinant protein, virus-like particle, and live-attenuated or killed virus. With the potential exception of live, attenuated virus vaccines, the general goal is to induce adaptive immune response resulting in high affinity IgG against S or N viral capsid proteins. However, unless care is taken to modify the protein sequences to remove

or inactivate regions highly associated with ADE, if this is even possible, we may produce vaccines that enhance, rather than protect against, severe COVID-19 infection. This could be particularly problematic in children, with their reduced risk of severe infection.

Given these issues, we suggest several considerations for vaccine development (Table 1). First, the immunodominance of various antibody subsets should be assessed carefully for all COVID-19 vaccines. This should include identifying mapping epitope targets to COVID-19 protein sites, especially those known to induce ADE. Next, clinical trials will need to be designed to specifically look for ADE in vaccine recipients who are subsequently infected. This should include pre- and post- vaccination measurement of anti-CoV-19 reactive IgG. Additional *in vitro* and *in vivo* testing of vaccine recipient sera for ability to induce ADE should be performed. Given the incidence of milder disease in younger individuals, and the potential for increasing the risk of ADE in vaccinated children subsequently infected with COVID-19, initial clinical trials should consider carefully whether to include children.

Importantly, long term follow-up of vaccinated cohorts will be essential to assess both vaccine efficacy and increased risk of ADE. This should include monitoring of the broad anti-CoV IgG repertoire to further aid in the design of future coronavirus vaccines. We will likely need to track the proportion of protective versus ADE inducing antibodies generated by each vaccine. This may vary by due to a variety of factors, including HLA type, vaccine adjuvant, vaccine protein glycosylation, and prior exposure to other CoV strains. Multiplex methods developed for influenza can be quickly adapted to this use[38]. This may lead to further modifications vaccine antigens to alter or eliminate sequences associated with ADE. This may become even

more critical as the current human coronavirus mutates or becomes seasonal.

Table 1. Considerations for COVID-19 vaccine development

Screen for pre-vaccine anti-COVID-19 IgG
Identify immunodominant epitope targets
Test post-vaccine sera for ADE potential
Restrict initial vaccine trials to adults
Long-term follow-up of vaccinated subjects
Multiplex measurement of CoV IgG cross-reactivity

Similar issues may emerge with use of convalescent sera to treat COVID-19 infection, especially with hyper-immune serum vaccinated individuals, and with targeted monoclonal antibodies. It may be prudent to assess for ADE inducing antibodies in convalescent sera and therapeutic monoclonal antibodies prepared for clinical administration. Given the paucity of data on this issue, further work will be needed to rigorously determine whether this is indeed a significant issue.

The current urgency for COVID-19 therapies has brought together the scientific community to find treatments. In our rush to develop vaccines and antibody-based therapies, we should be mindful of what we have learned about ADE from SARS, HIV, and dengue virus research. A rapid and careful approach to vaccine and therapeutic

Contributors

JW and MZ conceived and wrote the manuscript, JW created the figure and performed the sequence analysis.

Disclosure

The authors have no competing interests to declare.

Revision History

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- Revision 2 (March 21, 2020) - Added more recent US data and references specific to COVID-19 induced ADE mechanism. Added discussion of targeted monoclonal antibody therapies and ADE.

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