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Analysis of potential Antibody Dependent Enhancement (ADE) in SARS-CoV-2 infections

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Abstract

Antibody Dependent Enhancement (ADE) is a phenomenon in which viral particles bind to virus-specific antibodies, enhancing their uptake by host cells and resulting in increased viral replication and infection. First discovered while studying dengue virus (DENV) in primates, ADE can be explained by two general mechanisms, depending on the type of virus involved. In DENV infection, ADE manifests as largely different reactivity upon secondary infection with a cross-reactive serotype and this information provides a large implication to the authorization of DENGVAXIA vaccine use. Looking into the need of vaccines considering the current SARS-CoV-2 pandemic, authors are providing a review of current knowledge correlating SARS-CoV-2 and ADE phenomenon. Using SARS-CoV-1 as a model, we can hypothesize the possible ADE pathway in SAR- CoV-2. The S protein binds to the ACE2 receptor on the host cell and triggers different immune responses depending on the titer of antibodies present. Despite in vitro studies suggesting some cross-reactivity of some antibodies generated in response to other coronaviruses to SARS-CoV-2, ADE does not appear to be a major concern in vivo. Similarly with vaccines, although there may be variation in efficacy to respective strains of SARS-CoV-2 there does not appear to be serious concern for ADE due to vaccination.

Introduction

In light of the SARS-CoV-2 pandemic and the emergence of vaccines, many researchers have given attention to the possible phenomenon of antibody dependent enhancement (ADE) in SARS-CoV-2 infections. Vaccines work to introduce neutralizing antibodies to the immune system but on some viral occasions, these antibodies could increase the ability of the virus to

enter cells resulting in a worsened clinical phenotype through the mechanism of ADE. Considering the implications of ADE in dengue virus that resulted in its special use vaccine DENGVAXIA, an analysis of ADE in SARS-CoV-2 infections and its respective vaccines are appropriate. In this paper, the authors give an overview of current knowledge of ADE with the directionality of possible ADE in SARS-CoV-2 infections.

Antibody Dependent Enhancement: knowns and unknowns

Definition and overview of ADE

The notion of Antibody Dependent Enhancement (ADE) was first suspected while studying dengue virus (DENV) in Rhesus macaques. Through in vivo studies on primates, an association was found between antibody response and higher dengue viremia, which served as evidence for ADE. However, the exact mechanism behind this phenomenon was not articulated until several years later, and still remains poorly understood in dengue, even less so with respect to other pathogens. A common consensus among several studies has been that the level of pre-existing anti-DENV antibodies has a direct association with the severity of secondary dengue disease in humans.[1] But this is not unique to dengue, as ADE has appeared to play a role in further exacerbating infection in Respiratory Syncytial Virus (RSV) and Severe acute respiratory syndrome (SARS), elicited via the FCγR pathway.[2] The FCγ receptors are surface receptors on immune cells that identify the Fc portion of Immunoglobulin G (IgG), allowing for the internalization of virus-bound IgG via phagocytosis by macrophages and therefore resulting in productive infection.[3]

With large-scale efforts being made to contain the effects of the ongoing COVID-19 pandemic by vaccinating the general population against the SARS-CoV-2 virus, there has been an increased focus on immunity and antibodies and their role in protecting from infection. This has also led to a greater focus on the possibility of enhanced infection due to our body's protective antibodies, a phenomenon known as ADE. The possibility of ADE was suspected in SARS-CoV-2 when it was discovered that higher antibody titers against SARS-CoV-2 were associated with a more severe, or enhanced infection. Despite this recent surge in studies relating to the possible role of ADE in coronaviruses, there has been no conclusive evidence in support of the same. A recent study by Gao et al, for instance, found that no ADE of infection was observed for vaccinated macaques—that is, no evidence for IgG-mediated pathology or increased susceptibility to vaccine-associated enhanced respiratory disease (VAERD) was found despite the observation of protective IgG responses.[4] Additionally, given that viruses from the same group share cross reactivity, it is not uncommon to have cross reactive antibody responses, which could falsely indicate enhanced infection due to ADE.[5] However, there is much that is still unknown to us and that we must fully understand before we can attribute any observed enhanced infection in SARS-CoV-2 to ADE. For instance, in RSV, increased infection may occur due to vaccine hypersensitivity reactions caused by a skewed Th2 T cell immune response, not ADE, and we must be aware of the existence of similar patterns in SARS-CoV-2.[6]

General mechanism and diversity of ADE

The most general immune response mechanism involves response stimulation via Immunoglobulin G (IgG) antibodies. IgG antibodies are produced both actively and passively. They are actively produced in response to antigenic challenges, and can be passively administered as a form of immunotherapy to provide immunity against forgein antigens. Their protective activity is mediated through their bifunctional nature: a variable Fab domain mediates antigen‑binding specificity, whereas the constant Fc domain engages Fcγ receptors (FcγRs) expressed on the surface of leukocytes to mediate effector functions.

There are two general mechanisms that are thought to explain the working of ADE, depending on the type of virus. The first involves antibody-mediated viral uptake via FCγ receptor (FcγRIIa), and has been extensively studied in macrophage-tropic viruses such as dengue, while the second mechanism involves the formation of an immune complex and is seen mainly in non-macrophage-tropic respiratory viruses.

In the first mechanism, as suggested by Figure 1, non-neutralizing antibodies bind to the viral surface and direct the virions to the macrophages, where the virions are internalized via FcγRIIa-mediated endocytosis. This results in the macrophages becoming infected and can lead to enhanced viral replication and more severe disease.

There are three types of human FCγ receptors—FCγ R I, II, and III. It is widely accepted that FCγRI and II are present on human macrophages, and that certain antibody types, such as IgG, bind well to these receptors. However, the virus molecules alone do not always have viral receptors on the target macrophage cells. Through the complexing of monoclonal antibodies (mAbs) with viral molecules, the mAbs-virus complex attaches itself to the macrophage through

the Fcγ receptors present on the macrophage. With the attachment of this complex, the virus can enter the macrophage via phagocytosis, resulting in increased replication and infection.[7] For instance, several studies regarding FIPV in cats demonstrated that virus replication was distinctly better in the peritoneal and alveolar macrophages recovered from the antibody-positive cats than those from the antibody-negative cats, and this enhancement could be suppressed by blockade of the Fc receptor region of the antibody.[8]

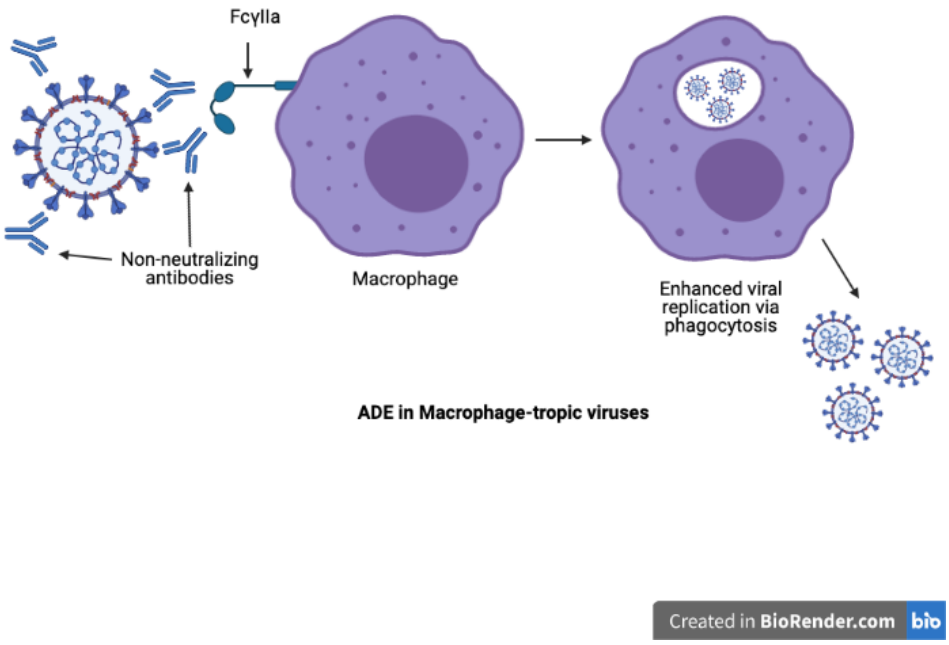

Figure 1: ADE in Macrophage-tropic viruses

In the second mechanism, which is seen in RSV and measles, non-neutralizing antibodies form an immune complex with viral antigens. These complexes form deposits inside airway tissues, causing the secretion of cytokines and the activation of complement pathways, leading to

inflammation and obstruction of airway tissues, as well as acute respiratory distress syndrome in severe cases, as seen in figure 2.[9]

The complement system works alongside pattern recognition receptors to stimulate host defense systems in advance of activation of the adaptive immune response. Studies have shown that complement-deficient mice have reduced neutrophilia in their lungs and reduced systemic inflammation (along with lower levels of inflammatory cytokines/chemokines in the lung and periphery), consistent with the observation that SARS-CoV pathogenesis follows the immune complex-complement pathway mechanism. The anaphylatoxins produced by this pathway are well-known for causing mast cell degranulation, initiating a cytokine storm, promoting vascular permeability, and contributing to acute lung injury.[10]

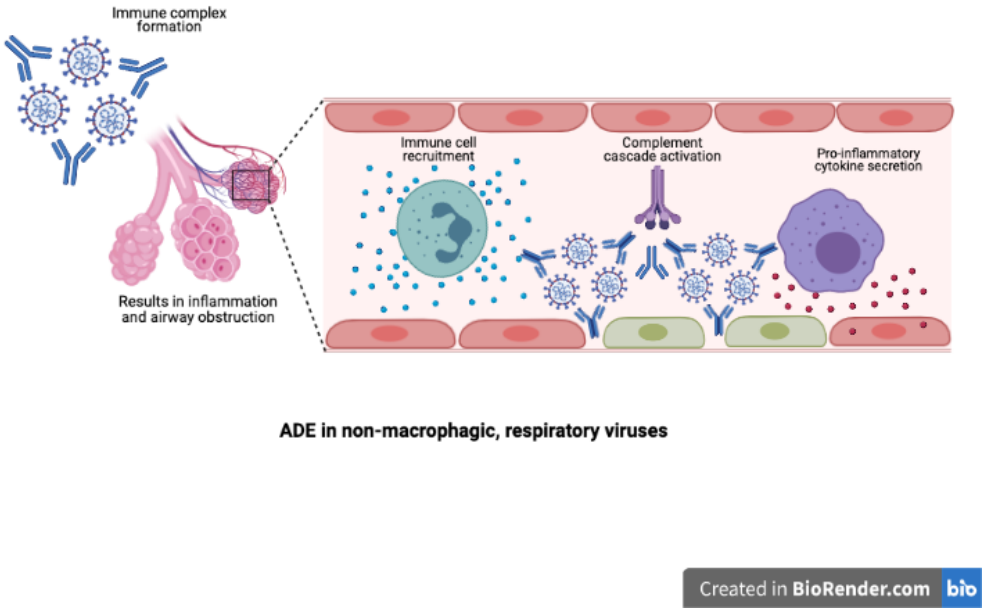

Figure 2: ADE in non-macrophagic, respiratory viruses

However, several studies have shown that SARS-CoV infected macrophages did not support the productive infection and replication of the virus [11] ,which would suggest that ADE in SARS-CoV-2 is likely to follow the second, immune complex formation mechanism described above.

Public health implications of ADE

The role of ADE in exacerbating infection has become increasingly important in public health settings, especially in regards to disease control by developing antibody-based vaccines and therapeutics.

With the race for developing effective vaccines against SARS-CoV-2 taking over the world by storm, it has become increasingly important to develop approaches with minimum or no risk for ADE. For instance, vaccines with a high theoretical risk of inducing pathologic ADE or ERD (enhanced respiratory disease) include inactivated viral vaccines, which may contain non-neutralizing antigen targets and/or the S protein in non-neutralizing conformations, providing a multitude of non-protective targets for antibodies that could drive additional inflammation via the mechanisms observed for other respiratory pathogens.[9]

Studying ADE through dengue virus

Dengue fever, which is also known as breakbone fever, is a serious condition borne out of mosquito-transmitted viruses. In many tropical areas of the world, dengue is a common occurrence, but studies have indicated that dengue operates via antibody-dependent

enhancement. This means that initial infection of the dengue virus does not provide the body with immunological memory for all serotypes of dengue. In fact, secondary exposure to the DENV virus may result in an elevated, and often deadly, viral infection.

Dengue fever arises from infection by one of four DENV virus serotypes, and is often transmitted through mosquitoes. These RNA viruses, like many parasitic viruses, have an envelope surrounding them as well as specific membrane proteins that facilitate the process of penetrating a host cell. The virus will then multiply within the host cell using the cell's machinery (primarily ribosomes) to duplicate itself many times over. Later, once assembled, the viruses are shipped out of the cell via the Golgi apparatus, which places the viruses in vesicles which are secreted from the cell via exocytosis.[12]

DENV enters a host cell through receptor-mediated endocytosis. As of now, it is still unknown which specific receptor protein facilities its entry, but current science indicated that the most likely candidate receptors are various glycoproteins, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), or a mannose receptor.[13] CLEC5A is a C-type lectin-like proinflammatory receptor that is said to propagate entry into macrophages for DENV, and a secondary viral E protein helps to increase binding to the host call via glycoprotein qualities.

Upon entry, the pathogen recognition receptors recognize the viral entry, in conjugation with MDA5 and RIG-I, all of which launch a response by secreting cytokines and other inflammatories into the bloodstream such as IL-12, IL-8, IFN-γ, and IFN-α.[14] DENV affects a variety of T-cells, including CD4+ and CD8+ cells, and CD4+ T-cells produce IFNγ, TNFα, TNFβ, interleukin (IL)-2, and CC-chemokine ligand 4 in response to infection of DENV to host cells.[15]

Dengue vaccines are being viewed with careful consideration in countries with high dengue-susceptible populations. Studies have observed that the threat of ADE from DENGVAXIA cannot be entirely mitigated at this time. Specifically in areas of extremely high to moderate dengue prevalence, vaccination has led to positive outcomes (i.e. a stark decline in reported dengue cases). However, surprisingly in areas of lower dengue prevalence, vaccination has led to a sizable increase in hospitalizations due to dengue. This is largely due to the nature of ADE in dengue, which will be discussed in greater depth in the following paragraph. These implications regarding the high risk nature of DENGVAXIA have led to a transition from the vaccine being public-use to special-use in order to minimize the effects of ADE.[14]

It is known to us that dengue's reactivity is largely different upon secondary infection with a cross-reactive serotype. This is because primary infection leads to the production of memory T-cells that are both serotype-specific and serotype-cross reactive. When a different serotype later attacks the host cell, all of the memory cells are reactivated to defend against the new pathogen. However, due to the difference in antigen sequences within various serotypes the T-cell response is limited by comparison due to low affinity of cross-reactive serotypes.[16] Due to this reduced T-cell response, the entire immunological sequence of events is affected, causing cross-reactivity in memory T-cells. Additionally, in a normal response, a wide array of cytokines are produced, and lysis of the cell occurs. In secondary response, the production of cytokines dwindles due to muted T-cell response. Overall, the secondary immune response is much more limited than the primary response, suggesting that high death rates from secondary DENV infection are caused by lack of immune response rather than stronger serotypes of DENV. [12]

DENV uptake is commonly enhanced by antibody–virus complexes attached to Fc-gamma receptors(FcγR). Host cells that are infected are thought to produce TNFα and NO,

which increase permeability and allow for infectious elements to enter, but so far this hypothesis has not been fully proven.[17]

One major issue with the four different serotypes of DENV is the nature of ADE associated with them. Antibodies that have been developed against various serotypes are not all-encompassing, meaning that they do not neutralize all serotypes. Because of this, if an individual is reinfected with a different serotype of dengue, viral entry is extremely dangerous, and can result in dengue shock syndrome or hemorrhagic fevers. This is because viral entry into a host cell is cell-mediated, and thus, enhanced, when the above conditions hold. Viral entry during a second infection, unlike in a primary infection, is similar to phagocytosis. Type-1 interferons, which would usually be activated, are avoided by the secondary infection because DENV can use the inhibitor, receptor -B1 to inhibit this immune response. FcγR signaling is also inhibited here. By evading the body's immune response, DENV is able to successfully enter the body and reproduce extensively more than in a primary infection, signalling the truly devastating nature of ADE. [14] Enhancement of dengue via ADE has brought about questions regarding ADE in SARS-coV-2. Due to the expedient production of the SARS-coV-2 vaccines, it is important to continue to study its long-term effects.

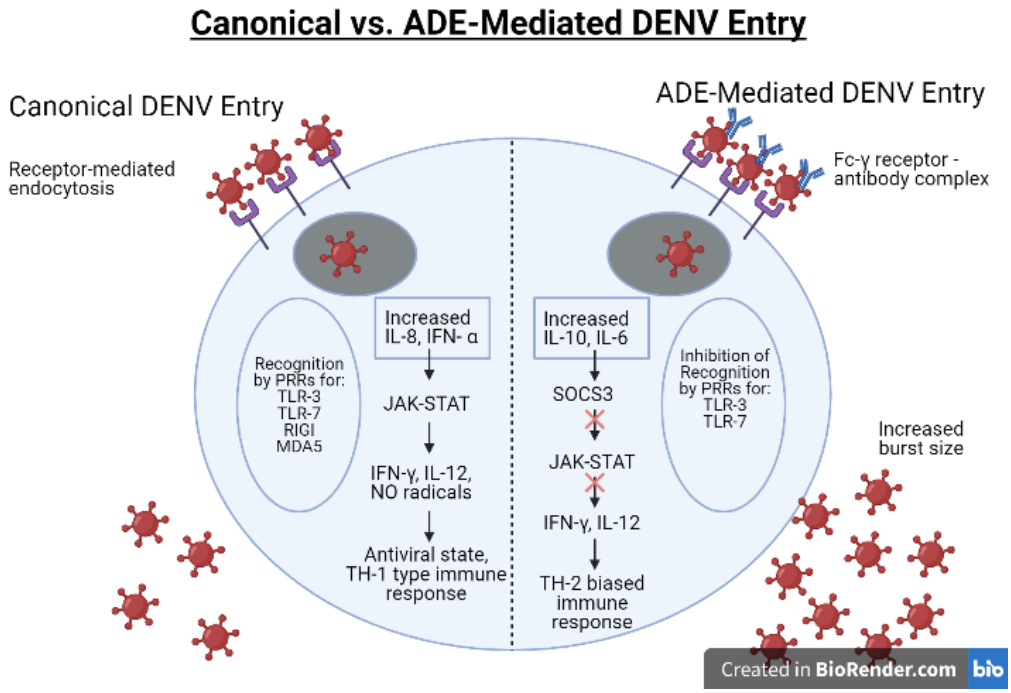

Figure 3: Canonical vs ADE-mediated DENV Entry

ADE in SARS-CoV-2

Introducing SARS-CoV-2 viral entry and possible ADE mechanism

SARS-CoV-2 uses a mechanism similar to SARS-CoV-1 for host cell entry via the angiotensin-converting enzyme 2 (ACE2) receptor.[18] The coronavirus's spike (S) protein facilitates entry into the host cell. The S protein contains the S1 subunit that contains a receptor binding domain (RBD) and the S2 subunit, which mediates membrane fusion to allow the virus to enter the host cell.[19]

The presence of ADE in SARS-CoV-2 is currently relatively unknown and under

investigation. While no established mechanism of ADE has been identified, studies have found that among COVID-19 patients, higher antibody titers against SARS-CoV-2 are associated with a more severe disease.[20] There also exists the potential concern that ADE in SARS-CoV-2 is further exacerbated by pre-existing antibodies against other coronaviruses, such as SARS-CoV-1.[21] The geographic discrepancies in severity of symptoms of COVID-19, where areas previously exposed to SARS-CoV-1 experienced worse outcomes and symptoms, indicate the possible role of ADE in causing more severe symptoms among patients.

A potential mechanism of ADE in SARS-CoV-2 is outlined in the figure below (Figure 4). In the first infection, S proteins bind to the ACE2 receptor of a host cell. Neutralizing antibodies (NAb) are produced as a result of the body's immune response. If the body receives the same strain of SARS-CoV-2 in a second infection and the NAb titer is high enough, the body's immune system immediately destroys the virus. Low NAb titers, however, mediate ADE and lead to further virus production via IgG induced stimulation. In the event that the second infection is with an RBD mutated strain, already existing antibodies can bind to the mutated RBD with reduced affinity, undergo internalization via the ACE2 receptor, and increase viral production through ADE. If there are sufficient antibodies that can block the mutated RBD virus, it can bind to the Fcγ receptors II (FcγRII), which facilitates infection of SARS-CoV-2 in immune cells and results in an ADE response.

The specifics of this mechanism and the exact role of antibodies are still up for debate by scientists; one study indicates that symptomatic patients had higher antibody titers yet cleared the virus from the upper respiratory tract more quickly.[19] However, there is a general consensus of the use of the ACE2 receptor for viral entry and the presence of higher antibody titers leading to an increased severity of infection, suggesting the presence of ADE in the SARS-CoV-2

infections.

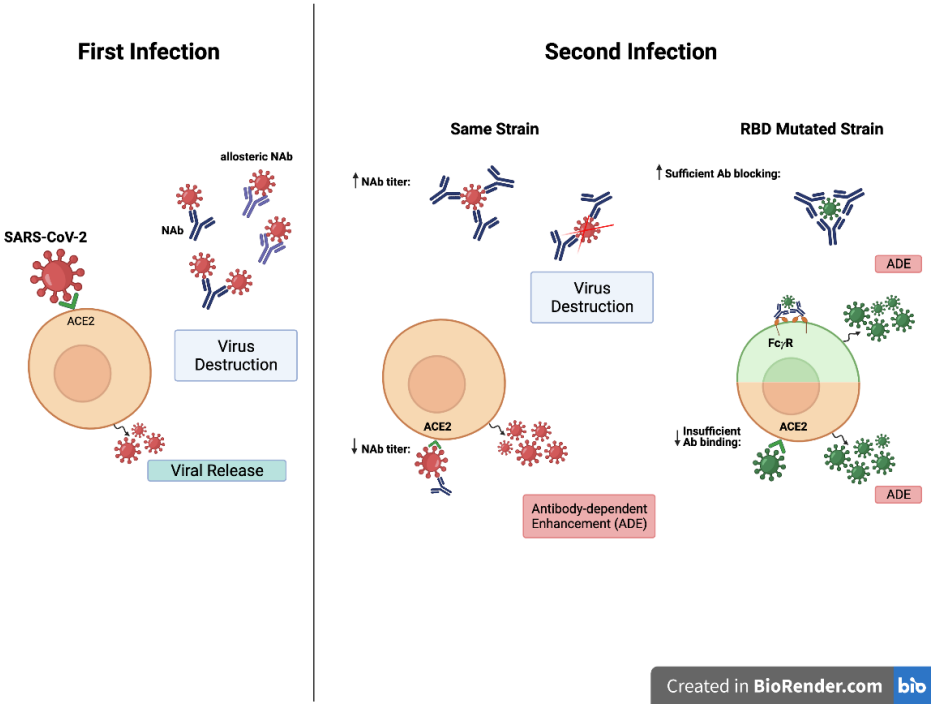

Figure 4: Potential Mechanism of ADE in SARS-CoV-2[22]

SARS CoV-2 ADE mechanism based on other infections

A variety of other respiratory illnesses, including SARS-CoV-1, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), and dengue virus (DENV) have helped to better understand the ADE mechanism for SARS-CoV-2. The SARS-CoV-2 whole genome sequence shares 79% sequence identity with SARS-CoV-1.[23] Both these coronaviruses have spike (S) proteins that bind to the RBD of ACE2 on the host cell surface which facilitates viral entry. Data from past SARS-CoV-1 outbreaks demonstrate that infection and severity are positively correlated with RBD/ACE2 binding affinity.[24, 25] Li et al. analyzed the S proteins derived from the 2002-2003 outbreak and compared to those derived from the less severe 2003-2004

outbreak.[25] The RBDs of the S proteins from the 2002-2003 outbreak, in which more severe symptoms were observed, bound to the human ACE2 most efficiently, indicating this high binding affinity is associated with a higher severity of infection. This information is in line with what was previously mentioned about SARS-CoV-2's viral entry: the RBD of the S protein on SARS-CoV-2 relies on the ACE2 receptor of host cells for viral entry, so a higher affinity leads to more efficient viral entry which leads to a higher viral load and thus more severe symptoms from the infection.

The mechanism of MERS-CoV is also useful in understanding SARS-CoV-2. MERS-CoV consists of neutralizing antibodies forming a complex between the RBD of the S protein of MERS-CoV and the FcγRIIa on the host cell.[26] This complex functionally replicates the cell surface virus receptor which then promotes further virus entry into IgG Fc receptor-expressing cells. This facilitation is caused by conformational changes that make the S protein better equipped for proteolytic activation. Thus, neutralizing antibodies seem to facilitate virus entry rather than block it. Because MERS-CoV and SARS-CoV-2 are closely related since they share 50% of the whole genome sequence[23], we must keep this conclusion in mind when considering potential vaccines and neutralizing antibodies-based therapies to prevent ADE in SARS-CoV-2.

Studying DENV has also shown support for the theory of neutralizing antibodies facilitating ADE. Since DENV has four serotypes, exposure to one can produce poorly neutralizing antibodies that then cross-react with the other serotypes, promoting viral entry also through the FcγR and leading to severe dengue disease via ADE. In DENV-ADE, non-neutralizing antibodies bind DENV, forming a complex which is recognized and internalized by Fcγ receptor-bearing cells. This internalization leads to a higher number of cell infections

which allows for an increased viral load that causes enhanced illness. The key component to DENV over other infections is the ability for its antibodies to cross-react and prevent neutralization of the disease.[27]

Understanding the ADE mechanisms of SARS-CoV-1, MERS-CoV, and DENV have shown the role neutralizing antibodies from previous exposure to the virus play in facilitating ADE and increasing the severity of the disease upon subsequent infections. With this knowledge, it is certainly plausible that a similar mechanism occurs in SARS-CoV-2, a possibility that must be examined further and thoroughly for vaccines and therapies to combat COVID-19.

SARS-CoV-2 and Other Coronaviruses

SARS-CoV-2 utilizes the ACE2 receptor on epithelial cells to mediate viral entry into cells. It has been documented that this coronavirus is also capable of entering immune cells lacking the ACE2 receptor, likely a result of ADE.[28] Further, high concentrations of anti-spike antibodies protected from SARS-CoV-1. Whereas diluted anti-spike antibodies bolstered infectivity. This is not observed with antibodies against nucleoprotein indicating that anti-spike antibodies are significant to ADE in SARS-CoV.[29] The required concentration of antibody, their affinity for the epitope, and the location of the epitope all play a major role in ADE.[30] For instance given an epitope in a difficult to reach region, even at saturating concentrations of a high affinity antibody the stoichiometry of the antibodies may render them insignificant for

neutralization. Other times minimal concentrations of antibody for a readily available epitope may confer protection.

Antibodies generated in response to prior infection from SARS-CoV-1 or even endemic corona viruses could play a role in the ADE of SARS-CoV-2. As mentioned, the whole genome sequence similarity between SARS-CoV-2 and SARS-CoV-1 is 79%. The amino acid sequences of the spike proteins of SARS-CoV-1 and SARS-CoV-2 share 76% sequence similarity.[23] Thus, there is the possibility that antibodies specific for epitopes of SARS-CoV-1, such as 447-458, are not neutralizing in SARS-CoV-2 as there is only 72.7% sequence similarity. However given the low seroprevalence of SARS-CoV-1 in the population it is unlikely that this is a principal priming virus.[21] Further, there are likely significant differences in spike protein RBD as SARS-CoV-2 binds ACE2 with as much as 20 fold higher affinity than SARS-CoV-1.[31]

A study analyzing serologic cross-reactivity between common endemic coronaviruses and pandemic SARS-CoV-2 using an antigen microarray consisting of 67 antigens demonstrated high IgG seroreactivity to common human coronaviruses but low IgG reactivity to epidemic SARS-CoV-2 coronaviruses.[32] Interestingly, antibodies isolated from those who had SARS-CoV-1 have been demonstrated to be cross-neutralizing for SARS-CoV-2.[33, 34] However, It is unclear if such antibodies are produced as a result of infection with SARS-CoV-2. In terms of reexposure to SARS-CoV-2, a study in Rhesus Macaques displayed that previous primary exposure to identical strains of SARS-CoV-2 protects from reinfection.[27] A cohort of 1038 previously infected healthcare workers followed for about 7 months showed no reinfections.[35] Similarly a study of 12,000 healthcare workers showed protection from reinfection up to 31 weeks.[35] More work needs to be done to fully understand if other coronaviruses and potentially strains of SARS-CoV-2 are contributing to ADE in clinically severe cases. As of now, clinical

severity is associated with elders with pre-existing conditions and not those with previous coronavirus infection.[21, 36]

Convalescent Plasma

Convalescent plasma has been widely used in the treatment of SARS-CoV-2 under the assumption that it contains therapeutic antibodies that can be passively transferred to the plasma recipient. These antibodies are thought to help neutralize the virus and may also have an immunomodulatory effect on aspects such as inflammation. The efficacy of convalescent plasma therapy is deeply tied to the types and titers of a few very specific neutralizing antibodies present in sera consisting of around 10^{21} different types of antibodies.[37] Identification of sera containing neutralizing antibodies can be found via a neutralization test using pseudovirus and identification of sera with antibodies that may induce ADE can potentially be found with cell lines expressing Fcγ and complement receptors. [38, 39] This is necessary to avoid the risks of ADE caused by non-neutralizing antibodies against other epitopes. As a result, the process of selecting appropriate convalescent plasma can be difficult and is not streamlined.

A meta-analysis examined the effectiveness of convalescent plasma in SARS-CoV-2 treatment in 19 studies with 38,160 participants. The analysis showed that convalescent plasma therapy might have little improvement in clinical symptoms at 7 days but improved clinical symptoms at up to 15 and 30 days respectively, but it was not published whether treatment decreased mortality.[40] A systemic analysis conducted involving 18 clinical trials and 10,436 SARS-CoV-2 patients demonstrated that those treated with convalescent plasma had 51% reduced morbidity compared with patients receiving the standard treatments.[41] Despite this there

remains skepticism surrounding ideal titers, antibody testing assays, and possibility of ADE from convalescent plasma.[42] In patients with severe or life threatening COVID-19 convalescent plasma therapy in addition to standard treatment had no statistically significant improvement in outcomes compared with standard treatment alone within 28 days.[43]

Based on the hundreds of thousands given convalescent plasma without signs of antibody dependent enhancement, the threat of ADE as a result of CP in non-severe COVID-19 patients appears minimal. The statistical significance of improvements in clinical outcomes or all cause mortality of SARS-CoV-2 as a result of convalescent plasma therapy continues to lack evidence.[44]

Monoclonal Antibodies

The neutralizing antibodies within convalescent plasma, immunized animal models, and re-engineered SARS-CoV-1 antibodies inspire monoclonal antibody (mAB) production. mABs offer promise in that they can be engineered to limit some of the drawbacks seen with convalescent plasma by generating specific titers of specific neutralizing antibodies and modifying the Fc receptor to alter uptake into macrophages or the formation of immune complexes.

There is considerable variability in the neutralizing activity of recovered individuals.[45] A study that purified monoclonals from the sera of individuals with potent serum neutralization ability found that these individuals did not necessarily have potent neutralizing IgG antibodies. The authors offered the possible explanation that the difference is likely explained by non-IgG antibodies such as secretory IgA which plays an important role in mucosal protection in

respiratory viruses.[46] Robust IgA production is observed in individuals with SARS-CoV-2.[47] Monoclonal IgA antibodies have been demonstrated to be as effective as IgG antibodies via in vitro neutralization assays.[48]

In the prior study, 48 monoclonals were isolated from potently neutralizing sera. 11 of these 48 displayed ADE of pseudo-viral entry into Raji cells, a B cell line expressing FcγRII. No ADE was observed when the sera from which the antibodies were derived was used. This could possibly be explained by the enhancing antibody titers being too low in the sera. No Raji cells were infected in the absence of antibodies. When the Fc binding site was mutated or blocked with an anti-FcγRII antibody viral uptake was nullified, suggesting ADE through a Fcγ receptor-dependent mechanism. A similar result was observed with authentic SARS-CoV-2. Paralleling previous findings with SARS-CoV-1, it appears that SARS-CoV-2 entry into Raji cells yields no significant viral replication within such immune cells.[46, 2]

Specific monoclonal antibodies have been cited to reduce chances of hospitalion and death somewhere from 75-87% when administered soon after infection.[49] Phase 3 clinical trials demonstrate casirivimab (REGN10933) and imdevimab (REGN10987) combined into a cocktail reduced the chances of hospitalization or death by 70% when administered to non-hospitalized SARS-CoV-2 patients.[50] Administration of these mABs in outpatient settings resulted in adverse immune effects like hypersensitivity in 1% of patients compared with 2% in the placebo control.[51] It is less clear whether this form of passive immunization has a benefit in severely ill individuals and many trials are now excluding patients requiring oxygen or mechanical ventilation.[52] This could be because it appears that the immune system itself plays a more important role in severe pathology than the virus.[53]

Given the specificity of monoclonal antibodies there is the possibility that SARS-CoV-2 may incur a mutation that renders the virus resistant. Neutralizing mABs have been made into cocktails consisting of two or more monoclonal antibodies to combat mutational escape.[54] Further studies are warranted to determine the need to engineer the Fc regions of the antibody to remove their effector functions potentially limiting the possibility of ADE with the monoclonal antibody.

Vaccines

Concomitantly with monoclonal antibody development there has been a global effort toward a SARS-CoV-2 vaccine. ADE as a result of vaccines was seen in dengue with human children in the Philippines, RSV in the Bonnet monkey model, and multiple animal models of SARS-CoV-1.[55, 56, 57, 58] Throughout the development of SARS-CoV-2 vaccines, much consideration has been taken for the possibility of vaccine driven ADE.[59]

Animal models were used to specifically test for ADE in candidate vaccines during development. This was done by administering the vaccine and later exposing the animals to the virus and analyzing their immunopathology. Particularly examined was the skewing toward a Th2 immune response and eosinophilic pulmonary invasion, which could be indicative of ADE. Testing of frontrunning SARS-CoV-2 vaccines such as mRNA-1273 and others has demonstrated a Th1 skewed immune response and no lung pathologic infiltrates.[60, 61, 62, 63] The limitation here is the short duration of testing; if these vaccines may lead to ADE in the long term is not known.[64]

There are four types of vaccines currently being administered for SARS-CoV-2 throughout the world: RNA, adenovirus vector, inactivated virus, and protein subunit. In the

United States there is approved use of two RNA based vaccines and one adenovirus based vaccine.

RNA vaccines utilize mRNA in a lipid nanoparticle which can be absorbed into cells. The mRNA will be translated in the cells into immunogenic proteins. The two vaccines with approved emergency use authorizations (EUAs) in this category are BNT162b2 and mRNA-1273. Both vaccines contain nucleoside modified mRNA (modRNA) coding for regions of the spike protein. The RNA is encapsulated in lipid nanoparticles which permits absorption into cells. In phase III trials BNT162b2 was found to have an efficacy of 95% indicating that vaccine motivated ADE is not enhancing infection rates.[65] A case control study in Israel showed that the BNT162b2 vaccine reduced symptomatic SARS-CoV-2 cases by 94%. [66] The other EUA approved RNA vaccine, mRNA-1273, phase III clinical trial data demonstrates no indication of vaccine-induced enhanced respiratory disease in observing Th1/Th2 responses and 94% efficacy suggesting that ADE is unlikely.[64] However, long term outcomes of these vaccines remain unknown.

Adenovirus vaccines consist of a non-replicative adenovirus shell containing DNA coding for an antigenic protein. In the U.S. Ad26.COV2.S has an approved EUA, but there are others in use elsewhere. In phase 1-II interim analysis Ad26.COV2.S T cell responses were Th1 skewed reducing concern of vaccine associated enhanced respiratory disease. The vaccine induces neutralizing antibodies after one dose with an efficacy of 66% in preventing symptomatic infection, 85% in preventing severe infection, and 100% efficacy of preventing hospitalization or death caused by SARS-CoV-2 infection.[67, 66] This suggests that there is not likely ADE induced by Ad26.COV2.S vaccination.

Variants

Of concern are new variants of SARS-CoV-2 and how they might impact the effectiveness of current vaccines.The B.1.1.7 variant seems to be no more infectious against those vaccinated with mRNA-1273 or BNT162b2.[68, 69] But the NVX-CoV2373 protein subunit vaccine showed a decrease in efficacy from 96% to 86% against this variant and 60% against the B.1.351 variant.[70] Using cell lines and pseudovirus, a study showed The P.1 and B.1.351 variants are inhibited less efficiently in sera of individuals vaccinated with BNT162b2.[71] While this is important in the effectiveness of vaccines in the population to hinder the spread of virus, the potential for ADE due to variants needs to continue to be monitored. Although the antibodies generated by these vaccines may be less effective, there remains 100% efficacy to death which potentially illustrates the importance of T cells in immunity and the importance of priming them in vaccinations.

Conclusion

The phenomenon of antibody dependent enhancement can be explained via two general mechanisms, depending on the type of virus. The first mechanism, extensively studied in macrophage-tropic viruses such as DENV, involves antibody-mediated viral uptake via FCγ receptor (FcγRIIa), leading to increased viral replication and infection. The second mechanism, seen predominantly in non-macrophage-tropic respiratory viruses such as RSV, works via the

formation of an immune complex, and results in the secretion of cytokines and the activation of complement pathways, leading to inflammation and obstruction of airway tissues.

First studied extensively in dengue virus, ADE has been devastating for DENV-susceptible populations across the world due to cross-reactive antibody enhancement of viral infection. The mechanism by which ADE is propagated within dengue is limiting secondary immune response compared to primary response due to muted T-cell and low cytokine production that is caused by low-affinity of cross-reactive serotypes. The dengue vaccine, DENGVAXIA, has been shown to have some implications for ADE.

Due to the similarities between SARS-CoV-1 and SARS-CoV-2, the SARS-CoV-1 ADE infection pathway can be used to hypothesize the mechanism for SARS-CoV-2. SARS-CoV-2's spike proteins contain a receptor binding domain (RBD) which binds to the ACE2 receptors on host cells to mediate viral entry. Studies of COVID-19 patients have demonstrated that the antibody titers present in the body affect the severity of the disease. Higher antibody titers correlate with a higher severity of disease, and patients who had antibodies after recovering from SARS-CoV-1fared worse when infected with SARS-CoV-2. These findings suggest the role that antibodies play in disease outcome, indicating the potential of ADE in SARS-CoV-2.

While higher antibody titers are associated with more disease severity, whether these antibodies are a cause or an effect of such severity is less certain. In vitro experimentation demonstrates little productive intracellular replication of the virus. This could explain the absence of changes in disease severity despite the ability of the virus to have increased uptake due to an ADE-like

mechanism. Antibody-related treatments for SARS-CoV-2 infection such as convalescent plasma appear to be safe but its efficacy for actual improvement in outcomes remains uncertain. Monoclonal antibodies have been demonstrated to be effective and safe, especially when administered as a cocktail. Furthermore, ADE due to vaccinations seems unlikely but long term outcomes with continuing research are needed to be certain. Newly surfaced variants may affect vaccine efficacy but may not be a serious concern as it pertains directly to the mechanism of ADE described in this paper. As antibody titers wane, prolonged studies are needed to verify these data.

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