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Antibody-Dependent Enhancement (ADE) and the role of complement system in disease pathogenesis

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ABSTRACT

Antibody-dependent enhancement (ADE) has been associated with severe disease outcomes in several viral infections, including respiratory infections. In vitro and in vivo studies showed that antibody-response to SARS-CoV and MERS-CoV could exacerbate infection via ADE. Recently in SARS-CoV-2, the in vitro studies and structural analysis shows a risk of disease severity via ADE. This phenomenon is partially attributed to non-neutralizing antibodies or antibodies at sub-neutralizing levels. These antibodies result in antigen-antibody complex deposition and propagation of a chronic inflammatory process that destroys affected tissues. Further, antigen-antibody complexes may enhance the internalization of the virus into cells through the Fc gamma receptor (FcyR) and lead to further virus replication. Thus, ADE occur via two mechanisms; 1. Antibody mediated replication and 2. Enhanced immune activation. Antibody-mediated effector functions are mainly driven by complement activation, and the first complement in the cascade is complement 1q (C1q) which binds to the virus-antibody complex. Reports say that deficiency in circulating plasma levels of C1q, an independent predictor of mortality in high-risk patients, including diabetes, is associated with severe viral infections. Complement mediated ADE is reported in several viral infections such as dengue, West Nile virus, measles, RSV, Human immunodeficiency virus (HIV), and Ebola virus. This review discusses ADE in viral infections and the in vitro evidence of ADE in coronaviruses. We outline the mechanisms of ADE, emphasizing the role of complements, especially C1q in the outcome of the enhanced disease.

1. Introduction

Antibodies induced by infection and vaccination can be a double-edged sword, as they play a vital role in protection, however in certain cases can enhance the illness. Such differential effects of antibody response depend on many factors, including the targeted epitope on the virus, cross-reactivity with host proteins, glycosylation pattern of the antibody-Fc fragment, host complement system, and others (Borsos and Rapp, 1965; Shim, 2011). In part, the virus may utilize the non-neutralizing antibodies bound to viral surface proteins for a more efficient entry into target cells and thus, elevates the viral infection (Hohdatsu et al., 1991). This phenomenon of increased viral infectivity by sub-neutralizing concentrations of antibodies or by non-neutralizing antibodies is termed antibody-dependent enhancement (ADE). The interrelation of prior available antibodies with the increased severity of disease progression has been perceived in many respiratory viruses, including RSV, measles (Kim et al., 1969; Nader et al., 1968), and other viruses including Flaviviruses (Peiris and Porterfield, 1982), Human immune deficiency virus (HIV) (Robinson et al., 1988a), and Ebola virus (EBOV) (Takada et al., 2003, 2001). In vitro studies also showed evidence of ADE in SARS, MERS, and COVID-19 (Jankov et al., 2006; Osioyewu et al., 1994; Wan et al., 2020; Wu et al., 2020; Yip et al., 2016). However, in respiratory infections, the non-neutralizing antibodies might lead to an immune complex formation that could be deposited in...
the lung or other tissues, causing complement deposition, enhanced inflammation and immunopathology (Nader et al., 1968; Graham, 2016a). This review will focus on the role of complements in ADE.

2. Different ADE mechanisms

ADE can occur via two different mechanisms: antibody-mediated replication and enhanced immune activation (Fig. 1). The antibody-mediated replication is mainly observed in viruses that infect immune cells, including Dengue and HIV (Dejnirattisai et al., 2010; Gorlani and Forthal, 2013), where the virus enters the cell via Fc-FcR (Fc on the antibody and FcR on cells) and further replicates inside the cells. This is otherwise called extrinsic ADE. Extrinsic ADE occurs when the virus, in the presence of sub-neutralizing levels or non-neutralizing antibodies, infects FcR expressing cells, including macrophages or monocytes (Dejnirattisai et al., 2010). The FcR is a receptor expressed predominantly on the surface of immune cells and possesses a vital role in the immune system’s protective functions. The FcR interacts with the Fc portion of the antibody when the Fab portions bind to the antigen surface resulting in virus-immune complex entry into cells (Mancardi and Daieron, 2014). There are three main classes of FcRs; Fc gamma receptor (FcγR), Fc alpha receptor (FcαR), and Fc epsilon receptor (FcεR). The FcR involved in the ADE is FcγR (Mancardi and Daieron, 2014).

On the other hand, the enhanced immune activation involves the formation of antigen-antibody-complement formation and deposition in certain tissues, particularly, respiratory system. This type of ADE mechanism is observed in non-macrophage tropic viruses, primarily respiratory viruses, including RSV and measles (Kim et al., 1969; Nader et al., 1968; Graham, 2016b). Though this mechanism occurs due to the non-neutralizing antibodies, the disease enhancement is mediated via excess secretion of pro-inflammatory cytokines and complement deposition in the tissues. This is otherwise called intrinsic ADE (Nader et al., 1968; Polack et al., 2002a). The complement cascade is composed of more than 50 small plasma proteins and glycoproteins synthesized primarily by liver and also by tissue macrophages and monocytes. These proteins and glycoproteins function as a cascade to help immune system eliminate the virus by inducing series of inflammatory responses (Byrne and Talarico, 2021; Dunkelberger and Song, 2010). Complement activity is consumed or activated by antigen-antibody complex. The first complement to get activated is C1q and further C3 to C9 complements get activated, and convertases are formed to release the final membrane attack complex (MAC). The MAC attacks and destroys the infected cell along with the virus-antibody complex (Dunkelberger and Song, 2010). Complement activation has been reported to be associated with disease severity in dengue (Churdboonchart et al., 1983) and HIV (Füst et al., 1994) infections. Moreover, complement activation and deposition are reported in respiratory infections including RSV-induced infection after formalin-inactivated RSV (FI-RSV) vaccination followed by RSV challenge in mice (Melendi et al., 2007). The infected mice weeks after vaccination were reported to enhance complement activation compared to infected mice without any vaccination. Recent studies have observed enhanced complement activation and deposition in patients with a severe infection in COVID-19 patients (de Nooijer et al., 2021; Gao et al., 2020a; Zinellu and Mangoni, 2021). For coronavirus, a non-macrophage tropic virus, the ADE mechanism would involve the intrinsic (complement-mediated) mechanism by activating complement and cytokine pathways leading to obstruction in the airway tissues.

A recent study reports the association of elevated serum C3a with the disease severity and mortality in severe COVID-19 patients (Henry et al., 2021a, 2020). More studies are required to understand the role of complements in disease enhancement and the exact mechanism in ADE. This would spread light to more efficient vaccine development strategies.

3. Epidemiological and experimental evidences for ADE

The first report on ADE was made in 1964 by Hawkes et al (Hawkes, 1964). The study assessed arbovirus neutralization using antiviral antibodies against four different flavivirus strains. They observed that virus-specific antibodies, especially immunoglobulin (Ig) G, at sub-neutralizing concentrations, elevated viral titers in chick embryonic cells (Hawkes, 1964; Hawkes and Lafferty, 1967). However, at high titers of antibodies, the infection was prevented. Thenceforth, ADE has been identified in many virus-cell systems, predominantly for the Flaviviridae family. However, dengue viruses (DENV) are the most studied, been identified in many virus-cell systems, predominantly for the Flaviviridae family. However, dengue viruses (DENV) are the most studied, including four serotypes (Halstead et al., 1970; WHO, 2021). The emergence of severe or fatal dengue shock syndrome (DSS) and dengue hemorrhagic fever (DHF) during the 1960 s in Thailand was investigated by Halstead et al. and associates. Experimental studies have shown that individuals who have a pre-existing dengue immunity are more likely to develop severe dengue shock syndrome (DSS) and dengue hemorrhagic fever (DHF) (Halstead et al., 1970, 1967). A long-term study (2004–2016) conducted among children between 2 and 14 years...
observed a significant relationship between dengue disease severity and pre-existing antibodies to dengue serotypes. The results showed a 7.64-fold higher hazard of DHS or DSS in children with antibodies from previous infection (Katzelnick et al., 2017). Similarly, mice that received West Nile Virus (WNV) and DENV antisera from infected blood donors demonstrated IgG-mediated ADE when challenged with zika virus (ZIKAV) (Bardina et al., 2017). The mice that received DENV positive plasma exhibited a 21.4% survival rate only, while those that received control plasma showed a 93.3% survival rate. In the same study, the in vitro analysis using K562 cells, DENV, and WNV antisera enhanced the infection of K562 cells when treated with ZIKAV. However, the infection was reduced when treated with IgG-depleted sera (Bardina et al., 2017).

Several experimental studies reported that the FcR on the immune cells mediates ADE (Porterfield, 1986; Sondermann et al., 2001). The interaction of the FcR with the Fc on the antibody is observed in experimental studies using sera from secondary infection (Adey. Halstead, 1977). Sera from mice immunized with Zaire Ebola Virus (ZEOBV) glycoprotein was found to induce enhanced infectivity of VSV pseudotyped Ebola virus glycoprotein (EBOV GP) via FcR-mediated mechanism in human kidney cells (293 cell line) (Takada et al., 2001). Furthermore, in convalescent human plasma from EBOV-infected individuals, VSV pseudotyped Ebola virus glycoprotein (EBOV GP) showed enhanced internalization into 293 cells (Takada and Kawakita, 2003). In 2020, C1q mediated mechanism of ADE was reported in EBOV infection (Furuyama et al., 2020). The study reported that the mechanism of disease pathogenesis in EBOV is mediated via the cross-linking of C1q receptor on the immune cell surface with virus-antibody complex. ADE was also reported in HIV infections via FcR and complement-mediated mechanisms (Robinson et al., 1987). Human monoclonal antibodies directed to the transmembrane glycoprotein 41, such as V10–9, N2–4, and 120–16, increased HIV infectivity in vitro in MT2 (human cord leukocyte) cells. According to Robinson et al., T cells that express complement receptor 2 (CR2) are more likely to enhance HIV infectivity, implying that CR2 and CD4 play a vital role in complement-mediated disease enhancement (Robinson et al., 1988b). Mitchell et al. also reported the role of CR2 in ADE during HIV infection. They reported that murine monoclonal antibodies to CR2 and CD4 reduced HIV infection in vitro (Robinson et al., 1990). Similarly, another study reported the role of C1q in enhancing the neutralization of WNV by humanized IgG1 and IgG3 (Mehlhop et al., 2009a). ADE was also reported with other viral families. In the 1980 s, sub-neutralizing antibody titers against Sindbis viruses and Semliki Forest were reported to enhance the infection of macrophages in vitro experiments (Chanas et al., 1982). Enhanced infection of macrophages with Ross Rovers virus (RRV) was also shown to be FcR mediated in the presence of diluted antisera from infected patients (Linn et al., 1996). ADE was reported in animals also. FcR mediated ADE mechanism is reported in cats immunized against feline infectious peritonitis virus (FIPV). Cats were passively induced with FIPV serum antibodies. These cats had poor survival rates when infected with FIPV compared to the control ones. The study demonstrated ADE by entering a non-neutralizing antibody-virus complex into the peritoneal and alveolar macrophages leading to enhanced infection and disease outcomes (Weiss and Scott, 1981). The study also reported a faster decline of antibody titers in FIPV infected kittens when compared to non-infected controls. The mechanism of this internalization of the virus was later studied in vitro ADE assay via FcRRIIA on the immune cells (Takano et al., 2008). A recent study reported the role of C1q in ADE during Ebola virus infection via FcR independent mechanism in human kidney cells (Furuyama et al., 2020). The study suggested that virus-antibody-C1q complex can enter the cells via C1q receptor leading to enhanced replication. Another study in 2012 reported the role of Mannose binding lectin (MBL) in dengue virus infection (Shresta, 2012). The study reported an association between depressed level or activity of MBL protein with disease severity in dengue infection. Extensive complement mediated ADE was reported by HIV-1 strain in presence of non-neutralizing autologous antibodies was reported in the year 2011 by Nel et al. They observed enhanced infection of HIV in T cells expressing CR2 (Willey et al., 2011).

These shreds of evidence indicate that non-neutralizing antibodies facilitate immune cell infection via FcR-mediated virus entry. However, in non-macrophage tropic viruses, including respiratory viruses, the disease enhancement mediated by pre-existing antibodies from infection and vaccination is reported in many viruses.

3.1. ADE in respiratory viral infection

ADE can result in Enhanced respiratory disease (ERD), leading to acute lung injury and severe clinical symptoms (Acosta et al., 2015). The characteristics of ERD include monocytic infiltration and surfeit of eosinophils in the respiratory tract (Polack, 2007). This phenomenon can occur due to homotypic or heterotypic infection by a different serotype after natural infection, vaccination, or by the transfer of passive maternal immunity (Su et al., 2021). ADE in respiratory infections has been mostly reported to be vaccine-associated disease enhancement (VADE) and the mechanism involved is intrinsic in most of the respiratory viruses.

3.1.1. Respiratory syncytial virus

The first study report on ADE in respiratory infection was in 1969, following RSV vaccination trial. Formalin-inactivated RSV (FI-RSV) vaccination resulted in a higher incidence of increased hospitalization due to severe illness in children (80%) when compared to non-immunized (5%) (Kim et al., 1969). Since then, many in vitro studies have shown increased viral infectivity of different cell lines in the presence of vaccinated sera. In vitro enhancement of RSV infection in macrophages (U937 cells) was reported by Gimenez et al. when treated with the cell lines with the virus in the presence of diluted human serum samples from RSV infected children (Gimenez et al., 1989). Another study reported the enhanced infection of macrophages when monoclonal antibodies are directed to F (Fusion glycoprotein) and G (attachment glycoprotein) surface glycoproteins on RSV, which are the targets for neutralizing antibodies (Ananaba and Anderson, 1991; Krilov et al., 1989a). However, this virus uptake was much lesser when either Fc on the antibody or FcR on the cells were blocked (Krilov et al., 1989a). Further, Gimenez et al., 1996, reported their observation of neutralizing and enhancing abilities of the RSV antibodies with the help of four monoclonal antibodies (MABs) against G and F glycoproteins (Gimenez et al., 1996a). All four MABs showed significant ADE in U937 (human macrophage) cell line. They observed the same when a mixture of two MABs (against G and F) was used, which indicates that these antibody responses are synergic. Another two studies reported that immunization of mice with FI-RSV elicited a T-helper cell type 2 (Th2) dominant response, which enhances ADE (Moghadam et al., 2006; Castilow et al., 2007). In challenged mice with RSV, the enhanced disease along with lung inflammation and injury was found to be associated with pulmonary eosinophilia. This is linked to the Th-2 cytokine response by CD4 T cells (Castilow et al., 2007). Such Th-2 response was recorded for both F glycoprotein subunit and inactivated virus, resulting in the formation of immune complexes in the lungs of infected mice (Ananaba and Anderson, 1991). Gomez et al. reported the infection of lung dendritic cells via antibody-mediated virus (RSV) uptake that affected the normal T-cell activation (Gomez et al., 2016). In a set of in vitro experiments, Wicht et al. demonstrated an increased RSV infection in human monocyes (THP-1) in the presence of sub-neutralizing concentrations of RSV antibodies (van Erp et al., 2019).

Similarly, antibodies produced against the FI-RSV vaccine caused ADE in cotton rats (van Erp et al., 2017). Van et al. and associates also reported in their findings that human maternal antibodies also enhance viral infection in FcR-bearing human cell lines in vitro experiments as the antibodies were less neutralizing. Hence the association between reduced virus neutralization and ADE is clear in severe RSV infection (van Erp et al., 2017). On the other hand, the enhanced pathology by
weakly neutralizing antibodies through forming immune complexes and complement activation and deposition were observed in two children who died of enhanced RSV infection (Polack et al., 2002a). The formation of immune complexes and complements was demonstrated in mice experiments by immunizing the mice with Fl-RSV and then challenging them with RSV (Polack et al., 2002a). In 2003, Simos et al. studied ADE in Bonnet monkeys. They observed increased RSV infection in Fl-RSV immunized monkeys when compared to non-immunized counterparts (Ponnuraj et al., 2003). However, the clinical effects of ADE in RSV infection are still not thoroughly described.

3.1.2. Influenza virus

The first observation of enhanced virus replication concerning influenza infection was studied in a rat model in 1980. The study aimed to evaluate the response elicited after immunization using a subunit vaccine against heterologous challenges with different subtypes of Influenza A virus (Askonas and Webster, 1980). Another study showed enhanced infection of macrophage-like cell lines (P388D1) by Influenza A subtype H1NWS virus in two other antigenic drift strains. Enhanced infection was observed in the presence of cross-reactive antibodies against Influenza A viruses through FcγR-dependent mechanisms (Ochiai et al., 1990). Another study reported that pigs immunized with two different inactivated swine influenza viruses (H1N1 and H1N2) vaccines exhibited protection against homologous infection however, increased infectivity was seen in a heterologous infection module (Tamura et al., 1991). The study demonstrated antibodies produced against hemagglutinin (HA) and neuraminidase (NA) influenza virus-promoted virus uptake by antigen-presenting cells (APCs). The enhanced infection was FC-mediated but not Fab-mediated. A significant increase in viral infection was observed in the presence of sub-neutralizing concentrations of antibodies to the homologous virus (Tamura et al., 1991). Another study reported the role of cross-reactive anti H1N1 HA antibodies that were associated with the enhanced disease. This study characterized sera from pigs immunized with whole inactivated H1N1 vaccine, and the titers of neutralizing antibodies against the H1N1 virus were high. However, Fc mediated disease enhancement was observed in pigs when challenged with the H1N1 virus (Kurakna et al., 2013). Another recent study reported the ADE of influenza virus in a heterologous challenge. Infection caused enhanced lung infection in piglets when treated using maternally derived antibodies from patient’s sera (Rajao et al., 2016). A recent study in mice reported ADE during H3N2 infection in mouse model. Mice receiving lowest doses of monoclonal antibodies (MAb 78/2) against H3N2 strain experienced lung homogenate analysis. The results showed high levels of proinflammatory cytokines as well as several Th2 cytokines (Winasaki et al., 2019a). Different studies comparing the effect of influenza virus uptake in the presence of sera from vaccinated or naturally infected individuals indicated that antibodies from both natural infection and attenuated vaccine enhanced the uptake of different strains in the heterologous challenge. This denotes the Fc-FcR mediated cellular entry and complement mechanism of ADE in the influenza virus.

3.1.3. In vitro evidences of ADE in coronavirus

Coronaviruses belong to a large family, Coronaviridae, that infects a wide range of species and causes various diseases (Masters, 2006). Seven different strains of human coronaviruses have been identified: four viruses that cause the common cold (229E, NL63, OC43, and HKU1) and three viruses of SARS-CoV, MERS-CoV (MERS-CoV-2) that cause respiratory infections (Woo et al., 2010). Coronaviruses possess spikes on their surface that facilitate their attachment and entry into host cells (Huang et al., 2020). In both SARS-CoV and SARS-CoV-2, the spike proteins that have two subunits (S1 and S2) mediates virus entry by attaching to the receptor, angiotensin-converting enzyme 2 (ACE2), with the help of hydrolyzing transmembrane protease, serine 2 (TMPRSS2) present on the host cell membrane (Huang et al., 2020). The binding affinity of SARS-CoV-2 RBD to its receptor is 10–20 times higher than that of SARS-CoV (Wrapp et al., 2020; Lan et al., 2020).

Hypothetically, pre-existing antibodies formed due to prior SARS-CoV or MERS-CoV and other human coronaviruses infection may recognize the S protein. However, if it does not neutralize the virus, it would result in enhanced illness. In vitro studies conducted to test this hypothesis using sera from immunized mice demonstrated ADE at sub-neutralizing concentrations of antibodies (Jaume et al., 2011a). When SARS-CoV pseudotyped lentiviral particles (PP) were used to infect different cell lines expressing FcγRII but not ACE2 in the presence of sera from mice vaccinated with inactivated SARS-CoV resulted in the increased uptake of PP into FcγRII expressing cell lines (Jaume et al., 2011b). This indicates ADE in SARS-CoV via FcγR mediated mechanism. Also, diluted murine anti-spike antisera enhanced the entry and replication of SARS-CoV in human HLC-Z promyelocytic cell lines that express ACE2 in lower levels and FcγRII in higher levels (Wang et al., 2014). The infectivity assay results showed that ADE in SARS-CoV primarily occurs in the presence of diluted antisera, which refers to the sub-neutralizing concentrations of antibodies as the cause of ADE. In contrast, higher concentrations of antiserum neutralized SARS-CoV in mice (Wang et al., 2014). Enhanced infection was also observed in B cell lines in vitro experiments by SARS-CoV vaccine induced antibodies (Kam et al., 2007). Many other in vitro studies demonstrated that FcγR-expressing phagocytes showed enhanced SARS-CoV and MERS-CoV virions uptake when treated in the presence of diluted infected human antisera (Yip et al., 2016, 2014a; Jaume et al., 2011b; Cheung et al., 2005). Vaccination with recombinant full-length spike protein of SARS-CoV provided a protective immunity in macaques. However, an enhanced viral infection of human B lymphocytes was observed by ACE2-independent and FcγRII-dependent pathways, suggesting ADE in SARS-CoV (Wang et al., 2016a). Neutralizing monoclonal antibody (mAb) against the MERS-CoV RBD enhanced virus uptake into macrophages and other cell lines transfected with FcγRIIa (Wan et al., 2020). For both MERS-CoV and SARS-CoV, a low antibody concentration facilitated ADE, while the higher concentration neutralized the virus (Wan et al., 2020; Jaume et al., 2011c). In SARS-CoV vaccine studies in animal models, vaccinated animals with SARS-CoV demonstrated ERD or increased immunopathology (Deming et al., 2006; Tseng et al., 2012). Ralph Baric et al. observed that Venezuelan equine encephalitis virus replicon particles (VRP) expressed with SARS-CoV N glycoprotein enhanced the infection in mice after homologous and heterologous challenge. Mice demonstrated eosinophilic infiltrates in the lungs starting from day four and persisted till two weeks (Deming et al., 2006). In another study, Robert Couch et al. evaluated four candidate vaccines against SARS-CoV, including recombinant DNA vaccine, virus-like particle (VLP) vaccine, inactivated whole virus vaccine, and spike S protein vaccine. Their findings concluded that Th2 response of immunopathology was observed in mice given with inactivated vaccine (Tseng et al., 2012). There are only few studies in ADE of MERS, however, in 2018 Prescott et al. reported that antibodies against inactivated MERS virus resulted in hypersensitive lung pathology in rhesus macaques. A vaccine mediated increase in the production of eosinophil granulocytes resulted in interleukin (IL-5 and IL-13) secretion was observed in vaccinated macaques when compared to non-vaccinated (Prescott et al., 2018). Wan et al., in 2020 demonstrated that the MERS monoclonal antibodies at lower concentrations could exacerbate ADE. The viral entry to the cells expressing FcγR was much higher when compared to DPP4 expressing cells, which are the viral entry receptors (Wan et al., 2016).

Interestingly, a recent study based on cellular and structural biology analysis using sera from COVID-19 recovered patients suggest that some RBD-specific antibodies like 7F3 have dual nature: neutralizing pseudovirus and exhibiting ADE in Raji B cells (Wu et al., 2020). This was dependent on the concentration of antibodies and the receptor expression on the cells. It was an IgG-mediated enhancement of infection that is similar to SARS-CoV and MERS-CoV. However, the study reported that optimal antibody concentration blocks the virus entry through the
interception of RBD-ACE2 receptor binding. In contrast the sub-neutralizing antibody concentration promoted pseudovirus internalization into the cells expressing FcγR of antibody (Wu et al., 2020). The fact these antibodies to promote the phagocytic uptake of the virus is foreseen; however, the infection of FcγR expressing macrophages is abortive in case of SARS-CoV (Chen et al., 2021) unlike MERS-CoV (Zhou et al., 2014). Similarly, in SARS-CoV-2, ADE as the cause of disease severity has been actively investigated by many researchers. However, reports suggest that in severe COVID-19 patients, antibody titer is high, corresponding to disease severity and mortality (Lau et al., 2021). The reports from the structural analysis suggest that SARS-CoV-2 may escape from the neutralizing antibodies as the number of neutralizing epitopes on the virus is low compared to other RNA viruses (Bachmann et al., 2021). While other RNA viruses including SARS-CoV offer 20 or more repetitive antigenic epitopes that are rigid and induce effective B cell responses, the SARS-CoV-2 offer a smaller number of epitopes and are widely spaced on the S protein and hence the antibodies against them may offer only a short- life. Over activation of complement cascade have been reported in different clinical studies to result in the deposition of immune complexes in the airway tissues, leading to inflammatory lung injury in COVID-19 severe ICU patients. Studies reports that higher levels of complements are associated with disease severity in ICU patients (de Novoier et al., 2021; Zinelu and Mangoni, 2021; Henry et al., 2021b). This indicates that if ADE exists in SARS-CoV-2 it is more likely to be complement-mediated; however, a definite role of ADE in COVID-19 is not yet reported.

3.1.4. Clinical evidence of ADE of viral vaccines

Like a natural infection, vaccination with the live attenuated virus has been shown to induce cross-reactive non-neutralizing antibodies, resulting in increased disease severity (Guzman et al., 2013). ADE after vaccination has been reported in RSV, measles and dengue vaccines (Polack et al., 2002a; Delgado et al., 2009; Borges et al., 2019). In RSV, the incidence of enhanced infection after vaccination came up after the 1960 RSV vaccination, when infants above six months of age were frequently developing RSV infection. Kim et al. in 1969 reported on vaccine-induced ADE in RSV (Kim et al., 1969). For children administrated with formalin-inactivated RSV vaccine (FIRSV vaccine), 80% were hospitalized during RSV season. This study also reported an increased infection rate among children who had maternal antibodies when re-infected with RSV (Kim et al., 1969). The in vitro experiments to evaluate ADE after RSV vaccination demonstrated an enhanced RSV internalization in the presence of RSV-specific monoclonal antibodies into monocyte (U937, THP-1) and macrophage-like cell lines (Osiowy et al., 1994; Krilov et al., 1989b). This indicates that the serum antibodies against RSV are not always protective, however, may enhance the infection when exposed to different strains of RSV.

Similarly, severe illness was reported in children infected with the measles virus after being immunized with the measles virus vaccine (Vader et al., 1968; Polack, 2007). This was first reported after introducing formalin-inactivated measles virus (FIMV) vaccines to Europe and the United States in the 1960 s. Upon subsequent exposure to wild-type measles viruses, around 16% of children were severely infected with atypical measles (Carter et al., 1962; Fulginitti et al., 1967; Rauh and Schmidt, 1965). These children developed atypical pneumonia, high fever, and unusual rashes on their skin. As a result, the vaccine was withdrawn in 1967 (Fulginitti et al., 1967; Philadelphia, 2002). A model for the pathogenesis of this atypical infection was proposed by Russell et al. in 2006, which suggests that the H-specific IgG promotes the infection of monocytes and macrophages bearing FcγRII on their surface (Jankov et al., 2006). This relates the atypical measles (ADE) developed in FMV vaccinated children to the vaccine-generated antibodies that are non-neutralizing. However, there are only a few studies on this, and more investigation is required to explain the pathology and mechanism.

The first approved vaccine against Dengue virus, CYT-TDV, is an attenuated tetravalent vaccine composed of yellow fever 17D chimeras and four DENV serotypes. After completion of phase III clinical trials, the vaccine was approved in 2018. However, it was found to cause infection in children continuously over 4–5 years from 18 months after vaccination in 2016 (Halstead, 2018). Studies on the risk-benefit ratio in a seronegative population reported that though the efficacy of the CYT-TDV vaccine is high in terms of antibody titer, however, the serostatus of the patients determines the disease outcome after infection. In case of seronegative vaccine recipients, there was a higher risk of hospitalization compared to seropositive vaccine recipients (Sridhar et al., 2018). A recent study in immune primed rhesus macaques reported that the tetravalent DENV vaccine-induced low titer of neutralizing antibody response and hence the serum antibodies demonstrated higher ADE in BHK cells (McCracken et al., 2021).

These evidences indicate that there is a risk of ADE associated with vaccines (especially inactivated vaccines), when patients are re-infected with the same strain or different strains. Hence it is one of the most crucial aspects to be considered while designing vaccines.

Table 1 summarises the different studies indicative of ADE due to viral infections or vaccination.

4. Mechanisms of ADE in viral infections

ADE in viral infections has two presumed mechanisms (Fig. 1): i) increased virus uptake into phagocytic cells via FcγR, resulting in increased infection and replication (extrinsic ADE), or ii) formation of immune complexes (virus-antibody complex), which may lead to complement activation and deposition causing virus-tolerant states leading to increased inflammation (intrinsic ADE) (Lee et al., 2020). This creates airway congestion in respiratory infections and hence leading to ERD (Winarski et al., 2019b). In both ADE mechanisms, FcγR on the surface of the immune cells is the key receptor that promotes enhanced infection. FcγR is the receptor for the Fc portion of immunoglobulin G (IgG) on cells, including macrophages, eosinophils, neutrophils, dendritic cells, B cells, and mast cells. There are different types of FcγR expressed on immune cells that are studied to be associated with ADE in different viral infections and viral vaccines (Pinicetc et al., 2014) (Table 2).

ADE mediated by the entry of virus via FcγR uses two different mechanisms by various viruses. The Extrinsic and Intrinsic mechanisms.

4.1. Extrinsic ADE; DENV as an example

The intrinsic ADE seems to have a greater contribution to enhanced illness in flaviviruses infections (DENV, WNV, and Zika virus) as compared to extrinsic ADE. In the intrinsic ADE, augmented virus replication is related to the inhibition of type1 interferon and activation of interleukin-10 biosynthesis, which favors Th2 type immune response. This mechanism is best described using DENV virus. It has also been reported in other viruses, including HIV (Weiss and Scott, 1981; Halstead and O’Rourke, 1977b). DENV has four serotypes (1–4). Infection with one serotype induces protection from future infection with the same serotype. However, subsequent infection with another serotype (heterologous) results in enhanced disease by pre-existing cross-reactive but non-neutralizing antibodies (Dejnirattisai et al., 2010). When non-neutralizing antibodies bind to a virus without preventing or clearing the infection, an extrinsic ADE mechanism may occur. These antibodies bind to the virus’s surface glycoproteins, making them more prone to be engulfed by phagocytic cells (macrophages, monocytes, or DC) through Fc-FcR, specifically FcγR (Narayan and Tripathi, 2020). Once the immunocomplex ligation with the FcγR, the complex enters the endosome of the effecter cell. When the affinity for the FcγR to IgG is low, the complex is released inside the cell. This dissociation of immunocomplex from FcγR switches the antiviral innate immune mechanism to an immune-suppressive one. In DENV infection, ADE was more linked to mature DCs than immature DCs, knowing that mature DCs expresses more FcγRIIa but not FcγRIIb (Guilliams et al., 2014). It is also found
activates the cytokine suppressor gene (cytokine signaling genes),
2010). The first mechanism is cytokine-mediated. The immunocomplex
(inhibition of type 1 interferon) takes place in two ways (Ubol et al.,
Mohamad Zamberi et al., 2015). The immune suppressive pathway
mechanism is mediated by negative regulators that help virus replica-
ables heightened virus production (Taylor et al., 2015). The second
mechanism is mediated by negative regulators that help virus replica-
Expression on human effector
cells
Type of antibody
Ligand
Affinity for
Ligand
Reference
Phagocytosis, Respiratory burst activation, induction of microbe killing, cell activation
IgG1 and IgG3
No data available
High
(Gavin et al., 1998)
Phagocytosis, ROI production
Degranulation, Phagocytosis
IgG
DENV, SARS-CoV-2
Low
(Yip et al., 2014a; Halstead and O’Rourke, 1977a)
Degranulation, platelet activation
IgG
DENV, WNV, SARS-CoV-2
Low
(Wu et al., 2020; Yip et al., 2014b; Boonnak et al., 2013)
Initiate Antibody-dependent cell-mediated cytol
EG
Ebola virus
Low
(Mellor et al., 2013; Kuzmina et al., 2018)
Phagocytosis, cytokine and chemokine
production
IgG
No data available
Low
(Mellor et al., 2013)

that FcγRIIa has low affinity to its ligand on IgG (Boonnak et al., 2013; Mohamad Zamberi et al., 2015). The immune suppressive pathway
(inhibition of type 1 interferon) takes place in two ways (Ubol et al.,
Mohamad Zamberi et al., 2015). The first mechanism is cytokine-mediated. The immunocomplex
upregulates the production of TNF, IL-6, and IL-10. High levels of IL-10
activates the cytokine suppressor gene (cytokine signaling genes),
further suppress the expression of type 1 interferons, which in turn en-
ables heightened virus production (Taylor et al., 2015). The second
mechanism is mediated by negative regulators that help virus replica-
tion. The two reported negative regulators are autophagy-related pro-
teins (ATG 5 and 12) and dihydroxyacetone kinase. When these two are
activated, they further deactivate the signal cascade, which supresses
type 1 interferon production. Thus interferon-related antiviral responses
are affected, and the virus replicates inside the cells resulting in
enhanced infection (Ubol et al., 2010).

4.2. Intrinsic ADE; RSV as an example

Intrinsic ADE mechanisms are best studied in respiratory viruses. Respiratory disease enhancement and immunopathology result from
increased Fc-mediated antibody-effector functions. The formation of
virus-antibody complexes that activate immune cascades and lead to
noticeable lung pathology (Winarski et al., 2019b). The activation of immune cells (monocytes, macrophages, dendritic cells, neutrophils, and natural killer cells) by Fc-mediated response by non-neutralizing antibodies can cause dysregulated activation of the immune system (Winarski et al., 2019b; Ye et al., 2017). This ADE mechanism has been extensively investigated in vitro and in vitro via disease manifestations, immunopathology, and presence of inflammatory markers. Non-macrophage-tropic viruses including measles and respiratory syncytial virus (RSV) are clear examples of ADE triggered by increased immune activation. This leads to cytokine and complement pathway activation, which contributes to inflammation, triggering acute respiratory distress syndrome (Polack et al., 2002a, 2003). To further understand the mechanism of non-neutralizing immune complexes to cause enhanced disease, a handful of in vitro studies were performed. A study reported the role of complements, specifically complement 3 (C3), activated by non-neutralizing immune complex (Polack et al., 2002b).

The immune-complex formation and interaction with Clq further cleaves C3 into C3a and C3b. C3a is an anaphylatoxin, and C3b is an opsonin. C3b activates down the line complements and gets deposited in the airway tissues (Prohaska et al., 2004). The recruitment of immune cells results in the release of pro-inflammatory cytokines leading to increasing lung pathology (Polack et al., 2003). The released complements and non-neutralized virus-IgG complex gets deposited in the airway tissues causing obstruction leading to acute respiratory distress syndrome in severe infections (Polack et al., 2002a). Many studies reported the role of complement deposition along with immunocomplex associated with severe disease outcome in various respiratory virus infections.

A study observed colocalization of IgG and C3 in RSV vaccinated mice’s alveolar regions, which demonstrated ERD, unlike control mice. In this study Kim et al. in 1976 reported that C3 activates other complements to form complement 5b-9 (CSb-9) or activates complements like C3a, C4a, and C5a (anaphylatoxins), which can injure lung tissues. This leads to mucus secretion and congestion of bronchi, and this initiates recruitment of inflammatory cells and disease enhancement (Kim et al., 1976). Another study using six monoclonal antibodies specific to RSV G and F glycoproteins, observed that the immunocomplex initiates a type 2 helper cell (Th2) response that upregulates TNF-α, IL-4, IL-13 and IL-5 expression and down regulates cytotoxic T lymphocytes (Gimenez et al., 1996b). This further leads to dysregulation of the immune system, resulting in non-clearance of virus-infected host cells and enhanced disease. Over activation of the complement followed by the inflammatory lung injury was also observed in SARS and COVID-19 (Wang et al., 2020; Gui, 2020). From the available reports, SARS-CoV-2 is not known to infect macrophages (Narayan and Tripathi, 2020). Thus, the possible mechanism of ADE in SARS-CoV-2 pathology has been explained by the formation of immune complexes that promote excessive immune cascade activation in lung cells (Wu et al., 2020). Clinical evidences reports that excessive complement activation in COVID-19 severe cases and ICU admissions are associated with respiratory failure (Holter et al., 2020a; Chouaki Benmansour et al., 2021; Cugno et al., 2020). Recent studies report that the immune complex may activate C3 that further activates C5a and its convertase. These complements promote the recruitment of macrophages/monocytes and neutrophils. These activated cells secrete pro-inflammatory cytokines TNF-α and IL-6 that contributes to the cytokine storm (Chouaki Benmansour et al., 2021) resulting in disease severity.

5. Evidence on the role of complements in ERD of respiratory viral infections

The activation of complements associated with ERD of respiratory viral infections was studied in influenza, RSV, SARS-CoV, and MERS (Garcia et al., 2013). In all the three pathways including classical pathway, lectin pathway and alternative pathway, the central component of the complement cascade is complement 3 (C3). The classical pathway (CP) gets activated when Clq-virus-antibody complex activates C3. C3 gets cleaved to form C3 convertase that further activates other components of the cascade. However, the lectin pathway (LP) gets activated by the activation of Mannose-binding Lectin (MBL) and ficolin complex together named as MBL-associated serine proteases (MBLSPs), when they recognize the carbohydrate patterns on the surface of antigens. MBLSPs cleaves C4 and C2 to form the C4b2aC3 convertase. In the third pathway, the alternative pathway (AP) the complement cascade is activated by hydrolysis of C3. This pathway also serves as an alternative way to cleave C3 to its products. Both in CP and LP the convertases cleave C3 to C3a and C3b, C3a is an anaphylatoxin and C3b further activates C5 convertases to cleave C5 to C5a and C5b. C5a is an anaphylatoxin and C5b further activates the complements in the cascade. Both these C3a and C5a anaphylatoxins are involved in chemotaxis and cellular effector functions of innate and adaptive immune response (Stoermer and Morrison, 2011). Experiments in mice to study the role of complement 5a (C5a) in ADE revealed that the deficiency of the C5a receptor in mice showed reduced clinical symptoms of influenza infection. They also observed similar effects after blocking the C5aR using specific antibodies (Song et al., 2018). The excess activation of C3a and its role in disease severity were also studied in mice during H5N1 infection (Sun et al., 2013; O’Brien et al., 2011). The study observed a significant reduction in the infiltration of neutrophils and eosinophils in the lungs and reduced viral replication using a C3a receptor (C3aR) antagonist. In SARS CoV infection, a study in mice injected with mouse-adapted SARS-CoV (SARS-CoV MA 15) observed a significant increase in complements, including C3a and C4b, in mice infected with a lethal dose of SARS-CoV (Gralinski et al., 2018). However, silencing of C3 resulted in reduced production of IL-1, IL-6, and TNF-α which are found to be elevated in patients with severe respiratory diseases. The study presents complements as a prime component of disease severity in SARS-CoV infection (Gralinski et al., 2018). A similar investigation was also done in MERS after observing increased levels of C5a in infected patients (Jiang et al., 2018). MERS-CoV infection upregulated the expression of C3a and its receptor (C3aR) in monocytes (THP-1) and macrophages (differentiated THP-1 macrophages) (Jiang et al., 2019). Similarly, in SARS-CoV-2, patients admitted to ICU reported high levels of C5a, C3bc, C3bBbP, C4d, and MAC (Jostet et al., 2013). A handful of studies reported the activation of complements via the lectin pathway (Holter et al., 2020b; Gao et al., 2020b; Malaguis et al., 2021). These reports highlight the fact that the ERD in respiratory infections via ADE is complement-mediated though the non-neutralizing antibodies facilitate this phenomenon via FcγR entry in to immune cells.

5.1. Role of Clq in ADE of viral infections

The complement system consists of approximately 50 proteins that function via three different pathways: i) lectin pathway (LP), ii) classical pathway (CP), and iii) alternative pathway (AP) (Dunkelberger and Song, 2010). The antibody-attached virus activates the complement proteins to form the membrane attack complex (MAC), which destroys the infected cell (Duensing and Watson, 2018). This is known as complement-mediated cytotoxicity. The first complement in the system to get activated is complement 1q (Clq), which has two subunits C1r and C1s, mainly produced by macrophages, monocytes, and immature dendritic cells. In contrast, the liver produces a majority of the other complement proteins (Merle et al., 2015). Clq is required for the activation of normal IgG responses (Mehlhop and Diamond, 2006). In association with natural IgM or IgG antibodies, Clq activates the classical pathway during primary infection (Stoermer and Morrison, 2011). Clq binding to IgG depends on the clustering of IgG, and this clustering is driven by antigens. Antigens induce the formation of IgG hexamers, promoting multivalent Clq binding on the surface of the antigen-antibody complex, mediated by IgG Fc: Fc interactions, leading to activation of the complement system (Diebolder et al., 2014; Wang et al., 2016b). The primary Clq binding residues are revealed on a
Clq binds to virus-antibody complexes, facilitating viral capsule fusion to the cell membrane via the interaction of Clq and its receptor, forming a virus-antibody-Clq complex (von Kietzell et al., 2014). Further, this complex binds to the receptor for Clq on the host cell, triggering the intracellular signaling cascade and enhancing virus-receptor binding. By cleaving C2 and C4 from Cls, Clq can activate the binding of complement C3 and its receptors (Stoermer and Morrison, 2011). Likewise, the virus antibody complex can bind to complement receptors (Dustin, 2016). The mechanism of complement-mediated ADE is also reported in WNV and HIV (Mehlhop et al., 2007). During HIV infection, the Clq protein can bind directly to gp41, a glycoprotein on the outer membrane of HIV. Clq receptors are present on inflammatory monocytes and macrophages, B cells, neutrophils, fibroblasts, smooth muscle cells, endothelial cells, and other cell types. When these antibodies are at sub-neutralizing concentrations for example; the antiviral serum at an early stage may exhibit an increased human monocyte infection. This kind of enhanced infection was reported in HIV by Robinson and his team using MT-2 cells (Robinson et al., 1988b). A similar mechanism was also found in EBOV (von Kietzell et al., 2014). These evidences explain the role of normal levels of Clq in preventing ADE. (Fig. 3).

ADE of homologous and heterologous DENV infection was reported to be Clq-dependent using a mouse model (Yamanaka et al., 2008; Mehlhop et al., 2009b). Mehlhop et al. in 2007 demonstrated that Clq could inhibit ADE both in vitro and in vivo using flavivirus antiserum obtained by infecting mice with WNV (Mehlhop et al., 2007). His study reported that Clq can inhibit virus internalization in macrophages in vitro and also in Clq deficient mice when treated using purified Clq. However, it was also shown that ADE was not reduced in the presence of Clq- and C3-depleted serum in vitro, but at high levels of C3 and normal levels of Clq (by reconstituting complement factors to the deficient serum), the rate of ADE was reduced (Yamanaka et al., 2008). All these shreds of evidence indicate the ability of Clq to make changes in the conformation of IgG needed for the fusion to viral E proteins, which promotes the entry of the antibody-bound virus. When Clq is present, the IgG subtype is more determinant of protection (Mehlhop et al., 2007). In the absence of Clq, human IgG1 and IgG3 neutralized WNV better than IgG2 and IgG4. Mehlhop et al. found that the IgG2b class had no impact on the neutralization potential of humanized E16 antibodies in Clq deficient mice. It could be due to the affinity of this IgG subclass for the specific FcγR expressed. The different affinities of different FcR determine the competition between FcR and Clq for IgG subtypes due to overlapping FcR and Clq binding sites (Mehlhop et al., 2007). It was also found that Clq binding to virus surface doesn’t prevent cellular attachment, increasing the possibility that the attachment is mediated by FcR after binding Clq to the antigen-antibody complex (Mehlhop et al., 2009b). His findings concluded that Clq could increase the efficacy of antiviral antibodies by improving the stoichiometric conditions that are required for complete neutralization. In animal models the role of complements was investigated by few studies. These evidences describe the role of Clq or other complements in preventing ADE and moreover its affinity to IgG isotypes, which are vital information in the development of vaccines.

6. Conclusion

Viral infections are a significant public health concern, as exhibited by the current COVID-19 pandemic. With the absence of effective antivirals and vaccines against many viral infections, it is essential to understand the molecular details of disease prognosis. Regardless of massive vaccination in many countries, COVID-19 cases were in continuous increase. The inactivated SARS-CoV-2 vaccine (BIBP) showed less protective efficacy, reported in Bahrain (Khouri et al., 2021). Later the country offered a booster dose of the mRNA vaccine. From the earlier reports, vaccination with Fi-RSV, measles, and other viruses was shown to increase morbidity and mortality after infection due to ADE. Hence, ADE is considered a significant barrier in some viral vaccine development. Understanding the molecular mechanisms of ADE may pave the way towards developing safe vaccine approaches and therapeutic strategies. ADE is a phenomenon widely studied in vitro, however the transferability of its approaches to in vivo and human applications is widely necessary. Animal model studies on RSV and SARS-CoV vaccines indicated the elicitation of Th2-mediated enhanced lung pathology; however, Th1 response is not reported to be associated with such ERD. There is evidence that an adenovirus-based vaccine for MERS S1 fusion protein that could produce Th1 response; preventing vaccine-induced enhanced disease in mice (Hashem et al., 2019). Among all the vaccines against COVID-19 the Adenovirus type 5

![Fig. 2. Complement-mediated ADE: The non-neutralizing antibody forms hexamer and binds to the virus. Further, Clq binds to this complex and activates complement cascade. The recruitment of immune cells leads to the secretion of pro-inflammatory cytokines and the deposition of complements in the tissues, forming an obstruction.](image-url)
expressing spike protein of SARS CoV-2, is reported to be associated with Th1 cytokine, but not with inflammatory cytokine response in immunized BALF (Chung et al., 2022). In the preclinical animal studies, a modified vaccinia virus Ankara (MVA) expressing SARS CoV was reported to demonstrate enhanced hepatitis in ferrets (Weingartl et al., 2004). However, in contest of SARS CoV-2 the possibility of ADE is unclear though some experimental evidences support the same. Animal model studies are essential to understand the risk of enhanced infection at a sub-neutralizing concentration of antibodies against SARS CoV-2. It is very important to understand the need of routine vaccination against SARS CoV-2 considering the emergence of variants as well as the existence of other coronaviruses. This evidence demands the need for more studies using animal models during vaccine development to eliminate the possibility of ADE. The role of complements in homeostasis and innate immunity is pivotal, where antibodies can initiate protective mechanisms against viruses through complex pathways. Studies of ADE in respiratory infections clearly indicates that the ERD due to ADE is complement mediated. These findings indicate that complement activation may contribute to pathogen clearance and inflammation, highlighting complement’s dual function. Reports have suggested that patients with pre-existing conditions are more likely to suffer from severe diseases during COVID-19 infection. ADE can be a possible cause of ERD of SARS-CoV-2 in patients with different severities. Vaccines that promote humoral immune responses with predominant protective interactions may be developed considering the circumstances surrounding the interaction between the above-mentioned immune components and the resulting ADE limitation.

**Data availability**

There are no data to share.

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**References**

Acosta, P.L., Caballero, M.T., Polack, F.P., 2015. Brief history and characterization of enhanced respiratory syncytial virus disease. Clin. Vaccin. Immunol.: CVI 23 (3), 189–195.

Aranaba, G.A., Anderson, L.J., 1991. Antibody enhancement of respiratory syncytial virus stimulation of leukotriene production by a macrophagelike cell line. J. Virol. 65 (9), 5052–5060.

Askonas, B.A., Webster, R.G., 1980. Monoclonal antibodies to hemagglutinin and to H-2 inhibit the cross-reactive cytotoxic T cell populations induced by influenza. Eur. J. Immunol. 10 (2), 151–156.

Bachmann, M.F., et al., 2021. SARS-CoV-2 structural features may explain limited neutralizing-antibody responses. npj Vaccin. 6 (1), 2.

Bardina, S.V., et al., 2017. Enhancement of Zika virus pathogenesis by preexisting antivirus immunity. Science 356 (6334), 175–180.

Boonnak, K., et al., 2013. Human FcεRI cytoplasmic domains differentially influence antibody-mediated dengue virus infection. J. Immunol. (Baltim., Md.: 1950) 190 (11), 5659–5665.

Borges, M.B., et al., 2019. Detection of post-vaccination enhanced dengue virus infection in macaques: An improved model for early assessment of dengue vaccines. PLoS Pathog. 15 (4) e1007721-e1007721.

Borsos, T., Rapp, H.J., 1965. Complement Fixation on Cell Surfaces by 19S and 7S Antibodies. Science 150 (3695), 505–506.

Byrne, A.B., Talarico, L.B., 2021. Role of the complement system in antibody-dependent enhancement of flavivirus infections. Int. J. Infect. Dis. 103, 404–411.

Carter, C.H., et al., 1962. Serologic response of children to inactivated measles vaccine. JAMA 179 (11), 848–853.

Castilow, E.M., Olson, M.R., Varga, S.M., 2007. Understanding respiratory syncytial virus (RSV) vaccine-enhanced disease. Immunol. Res 39 (1–3), 225–239.

Chanas, A.C., et al., 1982. Monoclonal antibodies to Sindbis virus glycoprotein E1 can neutralize, enhance infectivity, and independently inhibit haemagglutination or haemolysis. J. Gen. Virol. 58 (Pt 1), 37–46.

Chen, R.E., et al., 2021. Resistance of SARS-CoV-2 variants to neutralization by monoclonal and serum-derived polyclonal antibodies. Nat. Med. 27 (4), 717–726.

Cheung, C.Y., et al., 2005. Cytokine responses in severe acute respiratory syndrome coronavirus-infected macrophages in vitro: possible relevance to pathogenesis. J. Virol. 79 (12), 7819–7826.

Chouraki Benmansour, N., Carvelli, J., Vivier, E., 2021. Complement cascade in severe forms of COVID-19: Recent advances in therapy. Eur. J. Immunol. 51 (7), 1652–1659.

Chung, N.-H., et al., 2022. Induction of Th1 and Th2 in the protection against SARS-CoV-2 through mucosal delivery of an adenovirus vaccine expressing an engineered spike protein. Vaccine 40 (4), 574–586.

Chudnovskich, V., Bhamarapravati, N., Putrakul, P., 1983. Crossed immunoelectrophoresis for the detection of split products of the third complement in Dengue hemorrhagic fever. Am. J. Trop. Med. Hyg. 32 (3), 569–576.

Cugno, M., et al., 2020. Complement activation in patients with COVID-19: A novel therapeutic target. J. Allergy Clin. Immunol. 146 (1), 215–217.

Dejnirattisai, W., et al., 2020. Cross-reacting antibodies enhance dengue virus infection in humans. Science 328 (5979), 745–748.

Delgado, M.F., et al., 2009. Lack of antibody affinity maturation due to poor Toll-like receptor stimulation leads to enhanced respiratory syncytial virus disease. Nat. Med. 15 (1), 34–41.

Deming, D., et al., 2006. Vaccine efficacy in senescent mice challenged with recombiant SARS-CoV bearing epidemic and zoonotic spike variants. PLoS Med 3 (12), e255.

Diebold, C.A., et al., 2014. Complement is activated by IgG hexamers assembled at the cell surface. Science 343 (6176), 1260–1263.

Dundas, A.B., Watson, S.R., 2018. Complement-dependent cytotoxicity assay. Cold Spring Harb. Protoc. 2018 (2).

Dunkelberger, J.R., Song, W.-C., 2010. Complement and its role in innate and adaptive immune responses. Cell Res. 20 (1), 34–50.

Dustin, M.L., 2016. Complement Receptors in Myeloid Cell Adhesion and Phagocytosis. Microbiol Spectr. 4, 6.

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**Fig. 3.** C1q response to antigen-antibody complex: C1q response to antigen-antibody complex: The antibody (IgG), when identifying a virus, forms a hexamer unit by clustering six IgGs. This activates C1q to combine with its activated subunits C1r and C1s to form the C1 complex. As one arm of IgG binds to another IgG, the second arm stretches towards C1 complex. This complex further activates C3 and other complements in the system leading to antigen clearance.
van Erp, E.A., et al., 2017. In Vitro Enhancement of Respiratory Syncytial Virus Infection by Maternal Antibodies Does Not Explain Disease Severity in Infants. J. Virol. 91 (9), e00581–e00525.
van Erp, E.A., et al., 2019. Fe-mediated antibody effector functions during respiratory syncytial virus infection and disease. Front. Immunol. 10, 548.
Fulginiti, V.A., et al., 1967. Altered reactivity to measles virus: atypical measles in children previously immunized with inactivated measles virus vaccines. JAMA 202 (12), 1075–1080.
Furuyama, W., et al., 2020. A complement component C1q-mediated mechanism of antibody-dependent enhancement of Ebola virus infection. PLOS Negl. Trop. Dis. 14 (9), e000602.
Füst, G., et al., 1994. Neutralizing and enhancing antibodies measured in complement-restored serum samples from HIV-1 infected individuals correlate with immunosuppression. AIDS Res. Hum. Retroviruses 10 (5), 605–609.
Gao, T., et al., 2020a. Highly pathogenic coronavirus N protein aggravates lung injury by MASp-2-mediated complement over-activation. MedRxiv.
Gao, T., et al., 2020b. Highly pathogenic coronavirus N protein aggravates lung injury by MASp-2-mediated complement over-activation. medRxiv. 2020.03.29.20041962.
Garcia, C.C., et al., 2013. Complement C5 activation during influenza A infection in mice contributes to neutrophil recruitment and lung injury. PLoS One 8 (5), e64443.
Gavin, A.L., Tan, P.S., Hogarth, P.M., 1998. Gain-of-function mutations in FcγRI of reovirus infected cells enhance virus spread and viral persistence in macrophages. Nat. Rev. Immunol. 14 (2), 94–108.
Guzman, M.G., Alvarez, M., Halstead, S.B., 2013. Secondary infection as a risk factor for dengue hemorrhagic fever/dengue shock syndrome: an historical perspective and role of antibody-dependent enhancement of infection. Arch. Virol. 158 (7), 1445–1459.
Halstead, S.B., O’rouke, E., 1977. Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. J. Exp. Med. 146 (1), 201–217.
Halstead, S.B., et al., 1967. Hemorrhagic fever in Thailand; recent knowledge regarding etiology. Jpn J. Med. Sci. 20 (Suppl. p), 407–411.
Halstead, S.B., et al., 1970. Observations related to pathogenesis of dengue hemorrhagic fever. I. Experience with classification of dengue viruses. Yale J. Biol. Med. 42 (5), 261–275.
Halstead, S.B., 2018. Safety issues from a Phase 3 clinical trial of a live attenuated chimeric yellow fever/tetravalent dengue vaccine. Hum. Vaccin Immunother. 14 (9), 2158–2162.
Halstead, S.B., O’Rourke, E.J., 1977a. Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. The. J. Exp. Med. 146 (1), 201–217.
Halstead, S.B., O’Rourke, E.J., 1977b. Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. J. Exp. Med. 146 (1), 201–217.
Haschem, A.M., et al., 2019. A Highly Immunogenic, Protective, and Safe Adenovirus-Based Vaccine Expressing Middle East Respiratory Syndrome Coronavirus S1-CMDM Fusion Protein in a Transgenic Human Dipeptidyl Peptidase 4 Mouse Model. J. Infect. Dis. 220 (10), 1558–1567.
Hawkes, R., 1964. Enhancement of the infectivity of arboviruses by specific antibodies produced in domestic fowls. Aust. J. Exp. Biol. Med. Sci. 42 (4), 465–482.
Hawkes, R.A., Lafferty, K.J., 1967. The enhancement of virus infectivity by antibody. Virology 33 (2), 250–261.
Henry, B.M., et al., 2020. Circulating plasmocytogen concentration at admission in patients with coronavirus disease 2019 (COVID-19). Seminars in Thrombosis and Hemostasis. Thieme Medical Publishers.etc.
Henry, B.M., et al., 2021a. Complement levels at admission as a reflection of coronavirus disease 2019 (COVID-19) severity state. J. Med. Virol. 93 (9), 5515–5522.
Henry, B.M., et al., 2021b. Complement level at admission as a reflection of coronavirus disease 2019 (COVID-19) severity state. J. Med. Virol. 93 (9), 5515–5522.
Hohdatsu, T., et al., 1991. A study on the mechanism of antibody-dependent enhancement of the infectious virion of dengue fever in feline macrophages by monoclonal antibodies. Arch. Virol. 120 (3–4), 207–217.
Holter, J.C., et al., 2020a. Systemic complement activation is associated with respiratory failure in COVID-19 hospitalized patients. Proc. Natl. Acad. Sci. 117 (40), 25018–25025.
Polack, F.P., et al., 2002b. A role for immune complexes in enhanced respiratory syncytial virus disease. J. Infect. Dis. 190 (2), 214–224.

O’Brian, K.T., et al., 2011. A protective role for complement C3 protein during pandemic 2009 H1N1 and H5N1 influenza A virus infection. PloS One 6 (3) e17377.

Ochiai, H., et al., 1990. Infection enhancement of influenza A H1 subtype viruses in macrophage-like P815D1 cells by cross-reactive antibodies. J. Med Virol. 30 (4), 258–265.

Oisozy, C., Horne, D., Anderson, R., 1994. Antibody-dependent enhancement of respiratory syncytial virus infection by sera from young infants. Clin. Diagn. Lab. Immunol. 1 (6), 670–677.

Peiris, J.S., Porterfield, J.S., 1982. Antibody-dependent plaque enhancement: its antigenic specificity in relation to Togaviridae. J. Gen. Virol. 58 (Pt 2), 291–296.

Prabuzaika, Z., et al., 2004. Complement-Mediated Antibody-Dependent Enhancement of Viral Infections. In: Szabó, Z. (Ed.), The Complement System: Novel Roles in Health and Disease. Springer US, Boston, MA. pp. 249–264.

Rauh, L.W., Schmidt, R., 1965. Measles immunization with killed virus vaccine: serum antibody titers and experience to exposure to measles epidemic. Am. J. Dis. Child. 109 (3), 232–237.

Song, N., et al., 2018. Csf receptor1 inhibition alleviates influenza virus-induced acute lung injury. Int Immunopharmacol. 59, 12–20.

Tamura, M., Webster, R.G., Ennis, F.A., 1991. Antibodies to HA and NA augment uptake of influenza A viruses into cells via Fc receptor entry. Virology 182 (1), 211–219.

Teng, C.T., et al., 2012. Immunization with SARS coronavirus vaccines leads to pulmonary immunopathology on challenge with the SARS virus. PLoS One 7 (4), e35421.

Wang, G., et al., 2016b. Molecular basis of assembly and activation of complement component C1 in complex with immunoglobulin G1 and antigen. Mol. Cell 63 (1), 135–145.

Wang, Q., et al., 2016a. Immunodominant SARS Coronavirus Epitopes in Humans Elicited both Enhancing and Neutralizing Effects on Infection in Non-human Primates. ACS Infect. Dis. 2 (5), 361–376.

Wang, S.F., et al., 2014. Antibody-dependent SARS coronavirus infection is mediated by antibodies against spike proteins. Biochem Biophys. Res Commun. Res 451 (2), 308–316.

Weingartl, H., et al., 2004. Immunization with modified vaccinia virus Ankara-based recombinant vaccine against severe acute respiratory syndrome is associated with enhanced hepatitis in ferrets. J. Virol. 78 (22), 12672–12676.

Weiss, R.C., Scott, F.W., 1981. Antibody-enhanced infection of disease in feline infectious peritonitis: comparisons with dengue hemorrhagic fever. Comp. Immunol. Microbiol. Infect. Dis. 4 (2), 175–189.

Wiley, S., et al., 2011. Extensive complement-dependent enhancement of HIV-1 by autologous non-neutralising antibodies at early stages of infection. Retrovirology 8 (1), 16.

Winarski, K.L., et al., 2019a. Antibody-dependent enhancement of influenza disease promoted by increase in hemagglutinin stem flexibility and virus fusion kinetics. Proc. Natl. Acad. Sci. 116 (30), 15194–15199.

Winarski, K.L., et al., 2019b. Antibody-dependent enhancement of influenza disease promoted by increase in hemagglutinin stem flexibility and virus fusion kinetics. Proc. Natl. Acad. Sci. USA 116 (30), 15194–15199.

Woo, P.C., et al., 2010. Coronavirus genomics and bioinformatics analysis. Viruses 2 (8), 1804–1820.

Wrap, D., et al., 2020. Cyto-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 367 (6483), 1260–1263.

Wu, F., et al., 2020. Antibody-dependent enhancement (ADE) of SARS-CoV-2 infection in recovered COVID-19 patients: studies based on cellular and structural biology analysis. medRxiv, 2020.10.08.20209114.

Yamanaka, A., Kosugi, S., Konishi, E., 2008. Infection-enhancing and-neutralizing activities of mouse monoclonal antibodies against dengue type 2 and 4 viruses are controlled by complement levels. J. Virol. 82 (2), 927–937.

Ye, Z.W., et al., 2017. Antibody-Dependent Cell-Mediated Cytotoxicity Epitopes on the Hemagglutinin Head Region of Pandemic H1N1 Influenza Virus Play Detrimental Roles in H1N1-Infected Mice. Front. Immunol. 8, 317.

Yip, M.S., et al., 2014a. Antibody-dependent enhancement of human macrophages by severe acute respiratory syndrome coronavirus. Virol. J. 11, 82.

Yip, M.S., et al., 2014b. Antibody-dependent infection of human macrophages by severe acute respiratory syndrome coronavirus. Virol. J. 11, 82–82.

Yip, M.S., et al., 2016. Antibody-dependent enhancement of SARS coronavirus infection and its role in the pathogenesis of SARS. Hong. Keng Med J 22 (3 Suppl 4), 25–31.

Zhou, J., et al., 2014. Active replication of Middle East respiratory syndrome coronavirus and aberrant inhibition of inflammatory cytokines and chemokines in human macrophages: implications for pathogenesis. J. Infect. Dis. 209 (9), 1331–1342.

Zinelu, A., Mangoni, A.A., 2021. Serum Complement Component C3 and C4 and COVID-19 Severity and Mortality: A Systematic Review and Meta-Analysis With Meta-Regression. Front. Immunol. 12, 2184.