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Balance of Neutralizing Antibodies and Antibody Dependent Enhancement (ADE) in Dengue Virus (DENV) Infection: A Literature Review

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ABSTRACT

Dengue fever is a major public health concern worldwide, especially in regions with warm temperatures. Dengue Virus (DENV) from Flaviviridae, which has 4 serotypes DENV-1, DENV-2, DENV-3, and DENV-4) caused Dengue. DENV can cause three different types of dengue fever: Classical DF, DHF, and DSS. To fight off DENV infection, the body will produce an immunological response. Neutralizing antibodies are one of the immune responses that play a role in preventing viral infections. However, antibodies can also contribute to DENV infection severity through a process called Antibody Dependent Enhancement (ADE). The amount of antibodies bound to virus particles is related to viral neutralization and ADE. Neutralization occurs when antibody concentrations exceed the stoichiometric threshold and when antibody concentrations are higher than the minimal amount that is required for stable attachment of virions to cells. ADE occurs in the presence of antibodies below the stoichiometric threshold for neutralization and less than the minimal amount required for stable attachment of viral particles to cells. Antibody binding stoichiometry is determined by the affinity of the antibody and the number of epitopes that can bind to the virion. According to scholarly discourse, it is commonly held that the balance between neutralizing antibodies and ADE can be influenced by two contexts, namely the affinity and amount of antibodies.

INTRODUCTION

Dengue fever is a major public health concern worldwide, especially in regions with warm temperatures. According to data, dengue infects 400 million individuals annually across the entire world. Around 100 million become ill and 40,000 pass away due to severe dengue disease (Centre for Disease Control and Prevention, 2022).In 2022 there have been reported 4,110,465 cases and 4,099 deaths caused by dengue disease. The number of confirmed cases in Indonesia alone amounted to 125,888 cases and accounted for 1,082 deaths. Compared to 2021, when there were 73,518 cases of dengue with a fatality rate of 705 cases per year, the number of illnesses and deaths increased (European Centre for Disease Control and Prevention, 2022)

Dengue is an infection caused by the dengue virus (DENV), which is a member of the Flaviviridae family and has four different serotypes (DENV-1, DENV-2, DENV-3, DENV-4) that may cause dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS).

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DENV is transmitted by the bite of infected Aedes species mosquitoes (Ae. aegypti and Ae. albopictus) (Centre for Disease Control and Prevention, 2022.; Hasan et al., 2016; Langerak et al., 2019; World Health Organization, 2022.). DENV has a unique morphology where the glycoproteins in its mature virions lie flat on the surface and give the appearance of a smooth golf ball. DENV is a virus with RNA-positive single strain genetic material that is round with a diameter of about 50 nm. The DENV genome consists of three structural proteins that form virions, namely Capsid (C), Membrane (M), and Envelope (E), and seven non-structural proteins that are involved in viral replication, namely NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 (R. et al., 2018; Souza et al., 2022). Envelope (E) and pre-membrane (prM) are integral membrane proteins found in the envelope of DENV. The E protein plays a role in binding to cellular receptors, mediating viral fusion so that the virus enters the cell, and regulating the process of virus assembly and budding. E proteins are the primary target of neutralizing antibodies as well. The subunit of protein E is composed of three domains: domain I (EDI), domain II (EDII), and domain III (EDIII). At the end of domain II lies a viral fusion peptide that is hydrophobic and protected by domain III of the adjacent subunit. Domain III plays a role in binding to cellular receptors for virus entry. All three domains of protein E have epitopes that can be recognized by neutralizing antibodies (Dowd & Pierson, 2011; Souza et al., 2022).

In dengue infection antibodies play an important role. The body will produce an immune response as a form of protection against DENV infection. The immune response consists of innate and adaptive immune responses, with humoral and cellular immune responses creating the adaptive immune response. The humoral immune response can be in the form of neutralizing antibodies and non-neutralizing antibodies (Abbas A, Litchman A, 2019). Neutralizing antibodies can be as a specific defense against DENV that attack the body. Neutralizing antibodies not only bind to the virus but can also block infection. Neutralizing antibodies can block the interaction of the virus with receptors or can bind to the viral capsid by inhibiting the genome uncoating process (Payne, 2023). Neutralizing antibodies are generated after primary induction of DENV, these antibodies maintain immunological memory, are able to bind and neutralize homologous dengue serotypes and provide lifelong specific immunity (Sarker et al., 2023). However, in some cases, the presence of these antibodies can increase the severity of DENV infection. Where these antibodies are derived from non-neutralized antibodies. Antibody Dependent Enhancement (ADE) is a phenomenon in which antibodies produced in DENV infection can recognize and bind different serotypes of DENV from the primary infection but do not neutralize them so that the virus-specific antibodies will increase the entry of the virus into the host cell and its replication in the cell through sub-optimal antibody interactions with the virus and Fcγ receptors (Guzman et al., 2010; Lee et al., 2020). ADE has been shown to result in higher levels of viral internalization through an increase in the number of fusions per cell (Tirado & Yoon, 2003). According to research conducted by de Alwis R et al. that homotypic human serum (primary DENV-2) did not cause infection or death in mice exposed to sublethal doses of DENV-2, whereas passive transfer of equal amounts of heterotypic human serum (primary DENV-1, DENV-3 or DENV-4 antibody serum) caused significant ADE-induced disease and death (de Alwis et al., 2014).

The occurrence of antibody neutralizing and ADE can be initiated by epitopes. The following are epitopes that can play a role in antibody neutralizing and ADE, with serotype specificity, antibody source, antibody code, and epitope region. It can be seen in the table for epitopes that induce neutralization are generally in domain I and III regions (Table 1). Epitopes that induce ADE can target PrM and fusion loop epitope (FLE) epitopes (Xu et al., 2017). ADE can be affected by epitope-specific antibodies that are difficult to reach. Antibodies often target cross-reactive

epitopes and serotype-specific epitopes located in the interdomain or outside the E protein domain (Shukla et al., 2020).

Table 1. Specific epitopes for antibody neutralization and ADE

Induction	Serotype Specificity	Antibody Source	Antibody Code	Epitope Regions
Neutralization	DENV-1	Human	1F4 and 14C10	EDI/II Hinge
	DENV-2	Human	2D22	Centered around EDIII
	DENV-3	Human	5J7	EDI/II Hinge
ADE	DENV-4	Mouse	1G6	EDIII
	DENV1-4	Human	4D10	prM

Source: (Sarker et al., 2023; Xu et al., 2017).

The amount of antibodies bound to virus particles is related to viral neutralization and ADE. Neutralization occurs when antibody concentrations surpass the stoichiometric threshold for neutralization and the minimal quantity of antibody required for stable attachment of viruses to cells. ADE occurs in the presence of antibodies below the stoichiometric threshold for neutralization and less than the minimal amount required for stable attachment of viral particles to cells. The stoichiometry of antibody binding is determined by the antibody's affinity and the total number of accessible epitopes on the virion (Dowd & Pierson, 2011). In DENV infection, neutralizing antibodies can neutralize the virus, but at the same time can also facilitate the entry of the virus into host cells. The balance between neutralizing antibodies and ADE can be influenced by 2 contexts, namely the affinity and amount of these antibodies (Dowd & Pierson, 2011; Wilder-Smith et al., 2019). The writing of this article aims to determine the balance of neutralizing antibodies with antibody dependent enhancement (ADE) in DENV infection. This article informs the reader that ADE are not only antibodies that recognize ADE epitopes, but can also recognize neutralizing epitopes at levels below the neutralization threshold (sub-neutralization), therefore the number of epitopes recognized by antibodies must be balanced so that there is no worsening of DENV infection as a result of ADE.

NEUTRALIZING ANTIBODIES IN DENV INFECTION

Antibody neutralization is the phenomenon of antibodies that can bind to viral antigens to block the target antigen from attaching to cell receptors and ultimately failure of infection from the virus (Langerak et al., 2019). Antibody neutralization is useful to see the clearing of DENV that infects the body and antibodies in the body can protect against DENV infection (Abbas A, Litchman A, 2019). Neutralization of DENV requires the involvement of more than one antibody. Neutralization of antibodies to DENV can occur in primary and secondary infections. In primary infection, neutralization occurs when the neutralization titer is high so that antibodies can block and bind to the binding site (epitope) on the DENV E protein which results in DENV not being able to bind to host cell receptors. After successfully binding the epitope, DENV particles will be eliminated by macrophages through FcγR and Fc binding of antibodies so that phagocytosis and DENV destruction will occur. The elimination of DENV will provide protective protection against DENV infection. Not much different from antibody neutralization in primary infection, neutralization in secondary DENV infection will occur because antibodies have previously been formed in primary infection, but the DENV serotype must be the same as the serotype in primary infection (Abbas A, Litchman A, 2019).

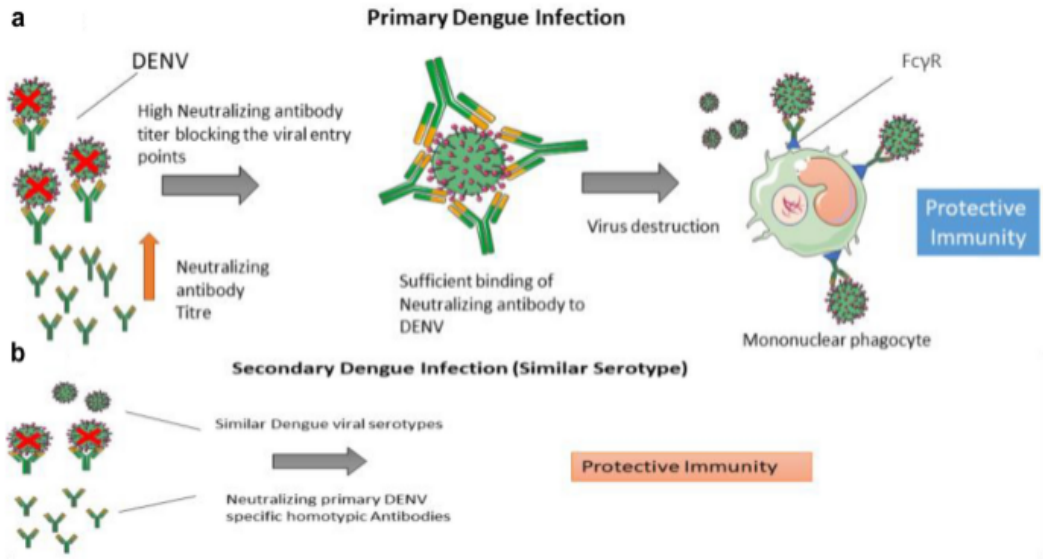

Figure 1. Antibody neutralization in primary and secondary DENV infection
Source: (Luo et al., 2015).

Neutralization will occur if the number of antibodies bound to the virion can exceed the threshold number of antibodies needed to neutralize the virus. Not only the number of antibodies but the affinity and accessibility of epitopes on DENV also play an important role. Neutralizing antibodies bound to the E protein of DENV can inhibit attachment to cell receptors, internalization into cells, and fusion with the endosome membrane which causes no replication of DENV in the cell (A et al., 2016; Guzman & Vazquez, 2010). Specific antibodies are required in relatively high concentrations to bind hard-to-reach epitopes while neutralization of easy-to-reach epitopes only requires low antibody concentrations and antibody affinities (Shukla et al., 2020b).

ANTIBODY DEPENDENT ENHANCEMENT (ADE) IN DENGUE INFECTION

ADE is a phenomenon where antibodies can facilitate the virus to enter the cell easily. This is due to the antibody-DENV complex, the Fc of the antibody will bind to the FcγRs on the cell so that the virus replicates and results in increased virus production in the cell. ADE occurs during secondary DENV infection, if the serotype of the virus is different from the serotype in the primary DENV infection, it will trigger the ADE phenomenon. The phenomenon is caused by non-neutralizing heterotypic antibodies from primary infection that will bind to different serotypes of DENV. The Fc antibody can bind to FcγR on monocytes or DCs, which is the initial target of DC infection. The interaction of antibodies and FcγR causes monocytes or DC cells to become infected and mediates an increase in replication and release outside the cell so that it can infect other cells. Dengue patients who develop ADE are at increased risk for developing dengue hemorrhagic fever (DHF), dengue shock syndrome (DSS), neurologic sequelae, and mortality (A et al., 2016; Guzman et al., 2010). It has been explained that the number of specific antibodies against DENV is too low to bind the binding site (epitope) of DENV virion that plays a role in attachment to target cells (Rothman, 2011). DENV internalization assisted by FcγR can suppress the antiviral response (type-1 IFN). In addition, there is an increase in IL-10 cytokine production which causes Th-2 to be unable to eliminate the virus. The release of cytokines from DENV-infected immune cells mediated by ADE can cause vascular endothelial cell dysfunction and increase vascular permeability (Shukla et al., 2020).

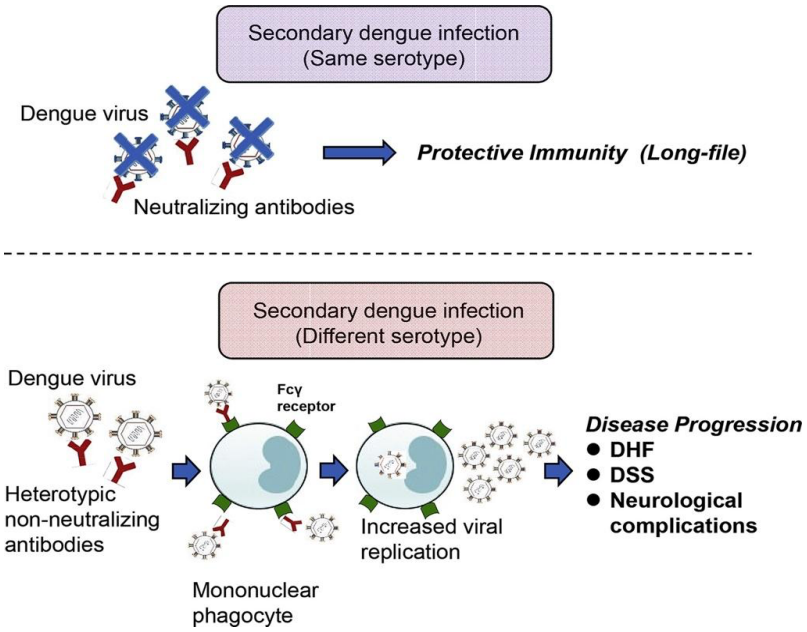

Figure 2. ADE phenomenon in secondary infection of DENV with different serotypes
Source: (Sarker et al., 2023).

PHENOMENA OF NEUTRALIZATION AND ADE IN VITRO AND IN VIVO

A. *In Vitro*

Kotaki et al. 2021 generated human monoclonal antibody clones (1C5, 1E5, 3E1, 1F11, 2C2, 2G2, 3E1, and 3G9) derived from Indonesian patients infected with DENV and tested for antibody neutralization and ADE. DENV-2 was neutralized at high concentrations on HuMAb clones while DENV-1, DENV-3, and DENV-4 were not neutralized by targeting the E protein of DENV-2, but it was not specified which domain to target. One antibody, 1E5, even at a high concentration could not neutralize all test concentrations. This is because the antibody targets a different epitope that does not target the neutralization epitope, but the mutant FLE epitope that induces ADEs (Kotaki et al., 2021).

Luo Y et al. 2015 performed neutralization and ADE testing on polyclonal antibodies targeting the Pr4 epitope which is the epitope of PrM. antibodies infected with DENV-2 virus at a high concentration dilution of 1:10 were only able to reduce 60% plaque, DENV-4 reduced 40% plaque, DENV-3 reduced 30% plaque, DENV-1 reduced 33% plaque and Imature DENV-2 reduced 45% plaque. Polyclonal antibodies targeting Pr4 can neutralize the virus but are weak and can react to 4 serotypes and imDENV-2 (immature DENV-2). After that, the antibody was tested for ADE and results were obtained by flow cytometry testing, namely at the highest dilution (1:10) the percentage of infected cells ranged from 50-20% of cells in DENV serotypes 1-4 and immature DENV-2. Then at the next dilution the percentage of infected cells increased and indicated the presence of ADE, namely at a dilution of 1:20-1:160 for immature DENV2 (87.6%), DENV2 (80%) and DENV1 (78%), while for DENV3 it increased to 1:320 with 70% infected cells and DENV4 at a dilution of 1:640 with 82% infected cells. Based on the test results, it can be seen that there is ADE activity in the serum. Anti-Pr4 antibody plays a role in ADE and can increase DENV infection with a percentage varying from 6.3-87.6% (Luo et al., 2015).

B. *In Vivo*

The phenomenon of neutralization of DENV infection was explored in a Thai study with 40 children who had predominantly secondary DENV-2 infection. 33 children were reported to have mild infection and 7 children had severe disease. Children with mild DENV infection had DENV-2 neutralizing antibodies from previous heterotypic DENV infection and children with severe dengue disease did not have neutralizing antibodies, but antibody dependent enhancement (Guzman et al., 2007).

The DENV-2 outbreak in 1981 (previously a mild epidemic of DENV-1 in 1977) that occurred in Cuba recorded 300,000 cases with 10,000 severe cases that induced dengue hemorrhagic fever and dengue shock syndrome (Guzmán et al., 1987). The severity of dengue fever/DSS did not occur in children born after the 1977 outbreak, so the 1981 outbreak only had a mild risk of primary DENV infection because the body can produce specific antibodies related to DENV-2 that can neutralize the infection. In addition, residents who experience secondary DENV-2 infection can cause the severity of dengue infection (Guzman et al., 2007). The presence of DENV-1 epitope-specific antibodies formed from primary infection cannot neutralize the secondary infection and cause ADE, this happens because the antibodies do not reach the threshold for neutralization and cause ADE. In addition, plasmablasts producing serotype-specific and cross-reactive antibodies will mostly target the dominant epitope, the fusion loop epitope (FLE) that mediates viral fusion into host cells. FLE is an ADE-inducing epitope (Sarker et al., 2023; Xu et al., 2017).

In the case of dengue vaccine failure (Dengvaxia, Sanofi) that occurred in 19 children who were confirmed to have died from severe dengue infection in 2017 in the Philippines in the context of dengue vaccination given to 800,000 in 2016 school children was initiated by the presence of ADE (McGill Journal of Global Health, 2022). When children are vaccinated for the first time and have never been infected with dengue before (seronegative) then this can induce ADE. The existence of the vaccine makes the body will induce specific antibodies against one of the DENV serotypes (e.g. DENV-1) and cross-reactive antibodies against other serotypes so that it will result in primary infection, an imbalance between homotypic / serotype-specific and heterotypic antibodies to the four DENV serotypes causes ADE in seronegative children (Halstead, 2016). When DENV infection occurs with a serotype different from DENV-1, previously formed antibodies cannot bind properly to serotypes other than DENV-1(Centers for Disease Control and Prevention, 2022). The lack of antibodies that can bind and neutralize DENV infection (below the threshold) will cause ADE which results in an ineffective immune response, induction of proinflammatory cytokines and causes the severity of DENV infection (A et al., 2016).

BALANCE OF NEUTRALIZING ANTIBODY AND ANTIBODY DEPENDENT ENHANCEMENT (ADE) IN DENV INFECTION

The balance between neutralizing antibodies and ADE can be influenced by antibody affinity, antibody amount, and the amount of epitope accessibility on the virion (Dowd & Pierson, 2011; Wilder-Smith et al., 2019). Antibody affinity itself will determine the fraction of epitope that will be bound by the antibody at a certain concentration.

Viral neutralization and ADE associated with the number of antibodies bound to viruses. Viral neutralization occurs when antibodies are present at concentrations greater than the minimal amount required for the stable attachment of virions to cells. ADE occurs at antibody concentrations lower than the stoichiometric threshold for neutralization and lower than the

minimal quantity of antibody required to sustain the stable attachment of virions to cells (Figure 1) (Dowd & Pierson, 2011).

In a study using 180 epitopes of protein E with antibodies at a concentration equal to its Kd will be able to bind 90 epitopes, if the antibody concentration is doubled then the antibody can bind 60 epitopes, if the concentration is increased 2x then the antibody can bind 120 epitopes. It has been demonstrated that antibody affinity correlates with in vitro neutralizing activity. High-affinity interaction. Antibodies that have high affinity will increase the number of virions bound to the antibody and determine the fractional occupancy of the virion epitope or the number of epitopes that can be accessed by the antibody to bind (accessible epitope). In testing with flaviviruses that display 180 epitopes, more than 30 accessible epitopes are required for neutralization to occur (Figure 1A) (based on experiments with two E-DIII-LR WNV-specific mAbs) (Dowd & Pierson, 2011).

Engagement of virions if the quantity of antibodies attached to virions falls short of the stoichiometric level for neutralization, antibody-dependent enhancement of infection (ADE) may take place. Antibody affinity and epitope accessibility both affect how much antibody is attached to each virion. To exceed the stoichiometric threshold in flaviviruses, 180 accessible epitopes would result in an occupancy of 17% so as to exceed the neutralization threshold. Therefore, while having a high affinity for the epitope, antibodies binding to cryptic epitopes that are hard to reach for antibody recognition may not be able to produce enough stoichiometry to surpass the threshold necessary for neutralization. At low occupancy, antibodies that bind highly accessible epitopes may exceed the neutralization stoichiometric threshold (Figure 1C) (A et al., 2016).

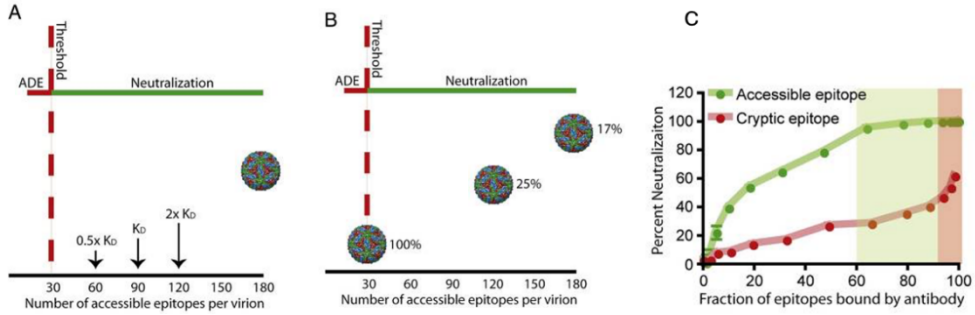

Figure 3. Balance of Neutralization and ADE in DENV infection.
A) Antibody affinity can determine the fraction of epitope bound by an antibody molecule at a given concentration. B) Epitope accessibility plays a role in setting the occupancy requirement for neutralization. C) Easily accessible epitopes can neutralize infections at lower amounts of the fraction of epitopes bound by antibodies compared to epitopes that are difficult to access
Source: (Dowd & Pierson, 2011).

Antibodies can bind to residues on nearby proteins or carbohydrates or epitopes determined by amino acid contacts of a particular viral structural protein. These antibodies have relatively low occupancy requirements for neutralization since they only need to bind a small portion of the epitopes to prevent infection. In contrast, high fractional occupancy requirements for neutralization are characteristic of antibodies that bind uncommon or difficult to access determinants (A et al., 2016).

Factors that can affect the neutralization balance in flaviviruses include dynamical structure, viral maturation and the role of complement (Dowd & Pierson, 2011). Virus movement dynamics may play a role in epitope accessibility in flaviviruses. This dynamic was observed in mAb 1A1D-2. The mAb is a DENV sub-complex specific mAb that recognizes epitopes on the A-strand of E-

DIII and is able to neutralize DENV serotypes 1, 2, and 3 (Lok et al., 2008; Roehrig et al., 1998). It was explained that Cryo-EM reconstruction of 1A1D-2 that binds to DENV showed a rotation of the E protein away from the virion surface with a comparison of the E protein arrangement in the mature virion state (Figure 2) (Dowd & Pierson, 2011; Lok et al., 2008; Roehrig et al., 1998).

Neutralizing antibody potency is significantly affected by changes in epitope accessibility. Structural changes can result in changes in the shape or conformation of DENV so that epitopes present on DENV can be easily accessed by antibodies and antibody neutralization will be easier (Dowd & Pierson, 2011). The virion maturation process also affects the accessibility of neutralizing antibody-bound epitopes. Virion maturation can reduce the accessibility of certain antibody classes to levels where neutralization is no longer possible. Immature virions contain more prM (pre-membrane) proteins where these proteins become epitopes that will bind to specific antibodies so that they will support antibody neutralization (Figure 3). In the partially mature state, some prM proteins have been successfully cleaved by furin to produce mature M proteins. prM in partially mature is not as much as in the immature state. In mature virions, the existing prM protein has been completely cleavaged. pr is cleavaged from the M protein by furin so that the M protein remains bound and the pr protein is released and not bound in the mature virus membrane. The absence of prM protein reduces the number of epitopes accessible to neutralizing antibodies thus favoring the presence of ADEs. Furin binds to the M protein and cleaves the soluble pr from the M protein, removing the pr protein from the mature viral membrane (A et al., 2016; Dowd & Pierson, 2011).

The neutralization potential can be enhanced by the interaction with complement protein C1q, this enhancement also applies specifically to determinants that are difficult to access. Complement C1q enhances neutralization by reducing the threshold required for neutralization. This enhancement mechanism is carried out by C1q by binding to IgM (single pentametric immunoglobulin) molecules on the surface of the pathogen. C1q can also interact with IgG monomers but the interaction affinity is quite low. Interaction with IgG molecules requires multivalent interactions with antibodies that bind to epitopes on the surface of the pathogen. Isotype influences how this interaction occurs, Although C1q can attach well to a single pentameric IgM molecule linked to the pathogen's surface in humans, the affinity of the monomeric association with IgG is rather low. Effective contact with antibody molecules arrayed on the surface of the pathogen requires multivalent interactions and happens in a way that depends on the isotype and subclass. Human IgG3 and IgG1 efficiently interact with C1q, whereas IgG2 and IgG4 subtypes have weaker C1q binding (Dowd & Pierson, 2011) .

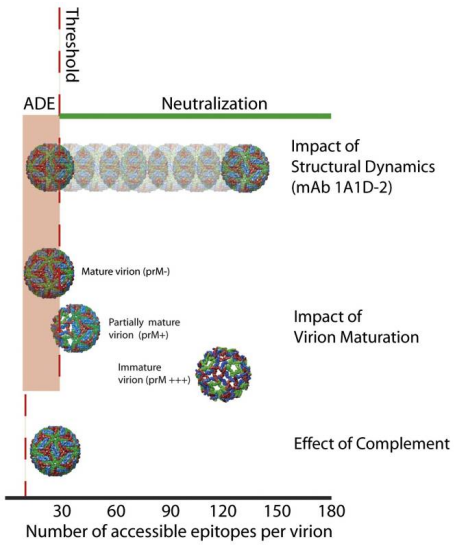

Figure 4. Factors that regulate the activity of anti-flavivirus antibodies.
Dynamic movement of DENV may regulate the exposure of epitopes that can be recognized by mAb 1A1D-2. Virion maturation affects the accessibility of epitopes that can be bound by neutralizing antibodies. Interaction with complement C1q may enhance neutralization
Source: (Dowd & Pierson, 2011).

CONCLUSION

The balance between neutralizing antibodies and ADE can be influenced by 2 factors, namely the affinity and amount of antibodies. Antibody affinity determines the proportion of epitopes bound by a given concentration of antibody molecule. A high-affinity antibody binds a larger fraction of its epitope than a low-affinity antibody with the same specificity at a given antibody concentration. A decrease in epitope accessibility increases the proportion of epitopes required for the neutralization of a virus. Epitope accessibility significantly impacts the potency of neutralizing antibodies. Epitope accessibility can be influenced by dynamic structural changes of DENV that can modulate epitope accessibility, virion maturity (related to prM protein), and C1q complement that can increase the potency of neutralizing antibodies by shifting the threshold for neutralization. Further studies are needed to elucidate the factors that determine the ratio of mature to immature virion particles, the amount of complement C1q required to shift the neutralization threshold and the affinity of neutralizing and non-neutralizing antibodies in patients with known severity of DENV infection.

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