



Review

Role of the complement system in antibody-dependent enhancement of flavivirus infections

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ABSTRACT

Flavivirus infections have increased dramatically in the last decades in tropical and subtropical regions of the world. Antibody-dependent enhancement of dengue virus infections has been one of the main hypotheses to explain severity of disease and one of the major challenges to safe and effective vaccine development. In the presence of cross-reactive sub-neutralizing concentrations of anti-dengue antibodies, immune complexes can amplify viral infection in mononuclear phagocytic cells, triggering a cytokine cascade and activating the complement system that leads to severe disease. The complement system comprises a family of plasma and cellular surface proteins that recognize pathogen associated molecular patterns, modified ligands and immune complexes, interacting in a regulated manner and forming an enzymatic cascade. Pathogenic as well as protective effects of complement have been reported in flavivirus infections. This review provides updated knowledge on complement activation during flavivirus infection, including antiviral effects of complement and its regulation, as well as mechanisms of complement evasion and dysregulation of complement activity during viral infection leading to pathogenesis. Particularly, insights into classical pathway activation and its protective role on antibody-dependent enhancement of flavivirus infections are highlighted.

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Introduction

Flaviviruses are insect-borne, positive-sense single-stranded RNA viruses that include human pathogens of major health concern, including yellow fever virus (YFV), dengue virus (DENV), Zika virus (ZIKV), West Nile virus (WNV), and Japanese encephalitis virus (JEV). Flaviviruses are responsible for endemic and epidemic disease across six continents (Pierson and Diamond, 2013). DENV comprises four recognized serotypes, whereas YFV, ZIKV, WNV and JEV compose a single antigenic serotype (Pierson and Diamond, 2013). Flavivirus infections may be asymptomatic or produce a broad clinical spectrum of symptoms ranging from high fever, headache, nausea and vomiting, myalgia and arthralgia; in some cases, infection can progress into severe illness such as

hemorrhagic fever, meningoencephalitis and neurological complications (Chong et al., 2019).

At present, there are licensed effective vaccines for YFV and JEV. A tetravalent DENV vaccine (CYD-TDV) was licensed in 2016, but has been recommended only for prevention of severe secondary disease in seropositive individuals after long-term studies showed enhanced disease as vaccine antibody titers waned in seronegative individuals (Deng et al., 2020; Halstead, 2017). No vaccines are currently available for ZIKV or WNV. Careful studies of the immune response to flaviviruses may help to evolve effective strategies for vaccine development and therapeutic approaches.

The flavivirus virion accesses target cells through receptor-mediated endocytosis releasing an RNA genome that is transcribed into a single polypeptide and cleaved into three structural proteins, the capsid (C), envelope (E) and membrane/pre-membrane (M/prM) proteins and seven non-structural proteins, NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. The E protein is comprised of three distinct domains (DI–DIII) connected by flexible hinges and a transmembrane anchor. Viral replication and assembly is carried out in the cytoplasm through interactions of both cellular proteins and viral nonstructural proteins. After transport through the endoplasmic reticulum (ER), prM is processed by furin to the

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mature M protein during secretion in the *trans* Golgi compartment to produce mature infectious virions (Clyde et al., 2006). Peak viremia occurs during the acute phase of infection, while severe manifestations usually associated with defervescence occur several days after the viremic peak (2009). Acute infection is also characterized by secretion of the viral protein NS1, which is sufficient to produce endothelial hyperpermeability, even in the absence of infection (Glasner et al., 2018). Differences in ZIKV, YFV, DENV and WNV NS1 proteins may contribute to viral tropism, since these proteins promote vascular leakage in a tissue-dependent manner reflective of severe manifestations of these diseases (Puerta-Guardo et al., 2019).

Severe flavivirus disease has been associated with multiple immunopathogenic mechanisms (Halstead, 2014; Slon Campos et al., 2018). Cross-reactivity between DENV serotypes and between other flaviviruses has been linked to antibody-dependent enhancement (ADE) of flavivirus secondary infections (Chan et al., 2016; Guzman et al., 2013; Halstead et al., 1980; Katzelnick et al., 2017; Katzelnick et al., 2020). Antibody-dependent enhancement occurs when poorly neutralizing or sub-neutralizing concentrations of antibodies bind to the virion and increase the efficiency of viral attachment and entry into myeloid cells through interactions between the Fc portion of the antibody and Fcγ receptor (FcγR), resulting in increased viral replication and the promotion of immunocomplex-dependent cell signaling that increase cell permissiveness (Chan et al., 2019; Guzman et al., 2013; Pierson and Diamond, 2015). The activation of serotype cross-reactive memory T cells also plays an immunopathogenic role in secondary DENV infections, leading to dysregulated production of cytokines and other inflammatory mediators that can further increase vascular permeability (Rothman, 2011; Shrestha, 2012).

The complement system has been reported to play an important role in flavivirus infections, exerting both pathogenic and protective effects (Conde et al., 2017). The complement system represents a central part of innate immunity. It is composed of more than 50 plasma proteins produced mainly by the liver and membrane-expressed receptors and regulators, which operate in plasma, in tissues and within cells (Merle et al., 2015a). Complement was first discovered because of its ability to induce

bacterial lysis. Over the years, complement's key role in homeostasis, innate-immune connection and antiviral immunity became evident. Several complement proteins normally circulate in blood as inactive precursors and collaborate as a cascade to opsonize pathogens, induce a series of inflammatory responses that modulate the activity of B and T cells, contribute to the clearance of immune complexes and maintain homeostasis (Merle et al., 2015b).

This review aims to provide a comprehensive and updated overview of the role of the complement system in flavivirus infections, with special focus on ADE of flavivirus infections.

The complement system during flavivirus infection

Activation pathways

The complement cascade can be activated through three distinct context-dependent pathways, the alternative, the classical and the lectin pathways. These pathways converge at C3 cleavage and lead to shared biological outcomes; the activation of inflammation, opsonization, lysis and priming of the adaptive immune responses (Figure 1). Diverse viral antigens are targets of each complement activation pathway (Merle et al., 2015a).

The alternative pathway is the major active complement pathway during physiological conditions. Spontaneous hydrolysis of a labile thioester bond in C3 causes conformational changes in C3 that expose a binding site for factor B. Bound factor B is cleaved by the serine protease Factor D, leading to the formation of a C3 convertase complex, C3(H₂O)Bb, that can amplify cleavage of C3 molecules to C3a and C3b. Covalently bound C3b binds factor B, which in turn is rapidly cleaved by factor D to Bb, which remains bound to C3b to form C3bBb, a C3 convertase. Similarly, this mechanism can amplify activation of the classical and lectin pathways through factor B interaction with bound C3b whose lysis was mediated through the classical or lectin pathways (Murphy and Weaver, 2017).

The classical pathway is activated when the globular heads of C1q recognize one of over 100 known C1q target molecules, including antigen-bound IgG and IgM, pathogen-associated

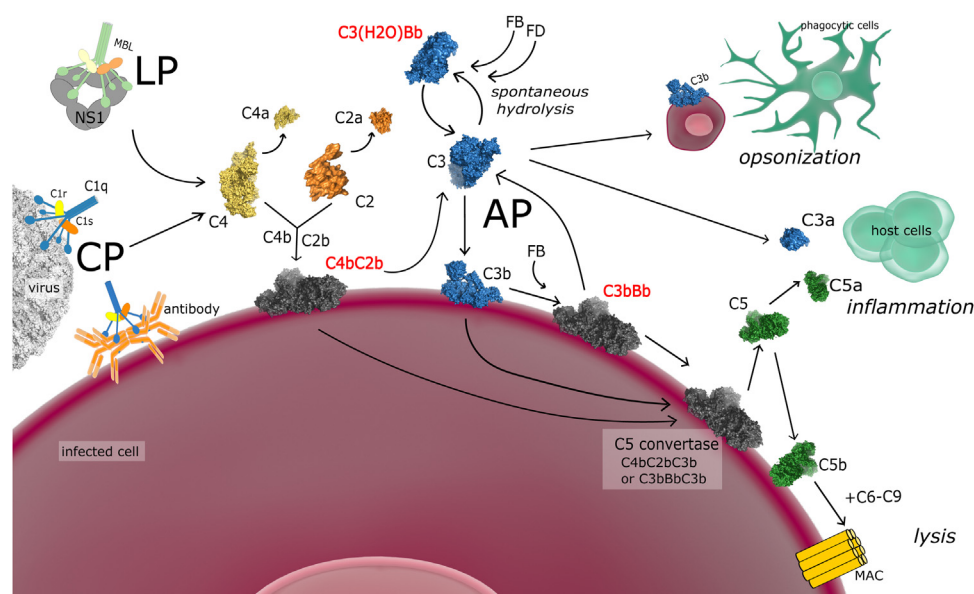


Figure 1. The classical (CP), lectin (LP) and alternative (AP) complement activation pathways converge at C3 cleavage and lead to shared biological outcomes. C3 convertases are indicated in red text. Upon cleavage of C3, C3b binds to the C3 convertase, forming the C5 convertase. C5 cleavage produces the anaphylatoxin C5a, and C5b, which instigates formation of the membrane attack complex, driving complement mediated lysis. C3a and C5a attract host immune cells and promote local inflammation. Pathogen opsonization with C3b leads to phagocytosis and antigen presentation.

molecular patterns such as lipopolysaccharide and proteins such as phosphatidylserine and double-stranded DNA, which may be exposed on the surface of apoptotic cells. Upon C1q binding, a conformational change takes place in the C1s C1r complex, activating the serine protease C1s. Activated C1s cleaves C4 into C4a and C4b (Merle et al., 2015a). Homologous to C3, C4 cleavage also exposes a thioester bond on the C4b component, which can bind covalently to the pathogen surface or infected cell (Murphy and Weaver, 2017). Pathogen-bound C4b then binds to C2, which is in turn cleaved by C1s, producing C2a and C2b and facilitating the formation of the C3 convertase C4bC2b (formerly known as C4b2a) (Bohlson et al., 2019). While most soluble complement proteins are produced in the liver, the major source of C1q are immature dendritic cells, monocytes and macrophages (Merle et al., 2015a).

As in the classical pathway, the lectin pathway is initiated through the binding of specific initiator molecules to mannose and other polysaccharides on bacterial and viral surfaces. There are four pattern recognition receptors that can activate the lectin pathway, mannose-binding lectin (MBL), a collectin, and ficolins 1, 2 and 3 (formerly known as M-ficolin, L-ficolin and H-ficolin) (Bohlson et al., 2019). MBL can directly bind the N-linked glycans on WNV and DENV structural proteins (Fuchs et al., 2010), and also recognizes secreted NS1 (Thiemme et al., 2016). MBL has been shown to directly bind the virions of a number of human viruses, including human immunodeficiency virus (HIV), severe acute respiratory syndrome coronavirus (SARS-CoV) and Ebola virus virions (Stoermer and Morrison, 2011). In plasma, MBL forms complexes with two serine protease zymogens, MBL-associated serine protease (MASP) 1 and 2 (Bohlson et al., 2019; Murphy and Weaver, 2017). The protease cascade initiated upon MBL binding to a pathogen surface is similar to the classical pathway: MASP-1 undergoes a conformational change and activates MASP-2, which cleaves C4 into C4a and C4b, leading to C2 cleavage. The resulting C3 convertase, C4bC2b, is identical to the classical C3 convertase (Merle et al., 2015a).

Antiviral effects of complement during flavivirus infection

Activation of the complement cascade through the classical, alternative or lectin pathway leads to cleavage of C3 into C3a, an anaphylatoxin, and C3b, a classical opsonin. Covalent binding of C3b amplifies complement activation, flags viral particles and infected cells for clearance by phagocytosis and promotes assembly of the C5 convertase. C5 cleavage initiates formation of the membrane attack complex (MAC), comprised of the C5–C9 complement components, and release of C5a, an anaphylatoxin (Merle et al., 2015a). Complement-mediated inflammation, through release of the anaphylatoxins C3a and C5a, recruits inflammatory cells to the site of complement activation, and increases vascular permeability, facilitating activation of cellular and humoral immune response (Merle et al., 2015b). MAC has been shown to lyse virus infected cells and inactivate enveloped viruses (Carr et al., 2020; Schiela et al., 2018).

In addition to their role as initiators of the classical and lectin pathways, respectively, C1q and MBL have direct antiviral effects. MBL has been shown to block fusion of DENV and WNV through a mechanism dependent on C3 and C4 deposition (Fuchs et al., 2010). Likewise, C1q binding of the DENV virion leads to lower viral titers and decreased expression of inflammatory molecules by monocytes *in vitro* (Douradinha et al., 2014).

Complement activation provides an important link between the innate immune response and B-cell responses to viral infection. Recognition of C3b-coated antigens by complement receptors on B cells strongly enhances B cell responses to those antigens. In WNV infection, C3, C4 and complement receptors 1 and 2 (CR1/CR2) were required for normal IgM responses in mice, whereas these

components and C1q were required for a normal IgG response (Mehlhof and Diamond, 2006).

The importance of complement in the outcome of viral infections is highlighted by the ubiquity of viral mechanisms for the evasion of complement, often through the production of viral proteins homologous to complement regulatory proteins or by encoding proteins that sequester or directly degrade complement components (Marinho et al., 2014; Merle et al., 2015b). In flavivirus infections, secreted NS1 modulates complement activation by direct binding, antagonizing complement components from the lectin and classical pathways, C4 and C1s, and leading to reduced activity of the classical C3 convertase and reduced C3b deposition (Avirutnan et al., 2010; Avirutnan et al., 2006). Flavivirus NS1 has also been implicated as an inhibitor of MAC formation by binding to vitronectin, a terminal complement protein regulator, resulting in decreased C9 polymerization (Conde et al., 2016), though MAC may have a limited role in the complement-mediated antiviral response (Mehlhof et al., 2009a). DENV NS1 has also been reported to bind to clusterin and inhibit MAC formation (Kurosu et al., 2007). Yet another mechanism of complement evasion is the direct recruitment of host complement regulatory proteins to the virion (Stoermer and Morrison, 2011).

Regulation of complement during flavivirus infection

Complement activation has immediate local effects that are mediated through the relatively short half-lives of soluble complement proteins and designated soluble and surface molecules that negatively regulate the complement cascade. These factors limit inappropriate activation and potential tissue damage that complement-mediated immunity can cause and enforce the locality of complement-mediated effector mechanisms (Merle et al., 2015a).

Much of the C3b present at physiologic levels due to spontaneous hydrolysis in the alternative pathway is deactivated through hydrolysis before it can form a covalent bond to the pathogen surface (Murphy and Weaver, 2017). But even covalently bound C3b is controlled by regulatory surface proteins, such as membrane cofactor protein (MCP or CD46) and CD35, as well as recruitable soluble proteins, such as factor H, that protect host cells from complement activation. MAC inactivation is built into its structure through the dual role of C8, which binds C5b–C7 in the absence of membrane binding, inducing a conformational change in the complex that results in the loss of lytic activity. MAC-inhibitory protein (MAC-IP or CD59) protects host cells from MAC-induced lysis, while clusterin and vitronectin bind to soluble MAC products, altering its ability to bind membranes (Stoermer and Morrison, 2011).

Complement-driven pathogenesis in flavivirus infections

The balance between protection and pathogenesis in the immunological response to pathogens is delicate and despite abundant regulatory processes, complement-mediated mechanisms of pathogenesis have been described in viral infections, often mediated by the same components that drive protection. In flavivirus infections, the failure of complement regulatory mechanisms leads to a powerful inflammatory response that has been associated with pathological effects particularly in severe manifestations of DENV infection. Plasma leakage can lead to shock, the principal cause of death in fatal dengue (Aye et al., 2014), and has been associated with hyperactivation of inflammatory proteins from the lectin and alternative pathways (C3a, C4a and C5a) and dysregulation of complement regulatory proteins (factors D, H, CD46, CD59) (Avirutnan et al., 2006; Bokisch et al., 1973; Marinho et al., 2014; Nascimento et al., 2009; Ubol et al., 2008).

Furthermore, maximum vascular leakage coincides with peak circulation of soluble complement proteins (Anon, 1973; Avirutnan et al., 2006).

Increased consumption of plasma C3 and C5, leading to increased levels of the anaphylatoxins C3a and C5a as well as increased C1q metabolism have been directly observed in children suffering from shock due to DENV infection (Bokisch et al., 1973), and this increase in C3 consumption is significantly associated with disease severity in patients (1973; Nishioka, 1974). Notably, C3a and C5a have vasoactive properties that may be associated with vascular leakage observed in DENV-infected patients (Carr et al., 2020). Deposition of multiple complement components were found in the tissues of fatal pediatric Dengue patients, including MAC C9 deposition in hepatocytes, Kupffer cells and macrophages while C1q, C3b and C9 deposits were found in germinal centers (Aye et al., 2014). Additionally, lower FB, higher FD and lower FH,

complement regulatory proteins of the alternative pathway, have been reported in Dengue patients with severe hemorrhagic manifestations and shock syndrome compared with patients with dengue fever (Nascimento et al., 2009; Yamanaka et al., 2013).

Classical pathway activation and antibody interaction

The classical pathway can be activated by C1q interaction with IgG or IgM. During primary infection, classical pathway activation is principally mediated by C1q interaction with natural IgM antibodies (Stoermer and Morrison, 2011). The C1q binding sites of IgM, a pentamer, are exposed only when antigen is bound (Czajkowsky and Shao, 2009; Diebolder et al., 2014).

While C1q binding sites are exposed on monomeric IgG, C1q has low avidity for individual IgG Fc and complement activation depends on antigen-driven clustering of IgG. In the presence of

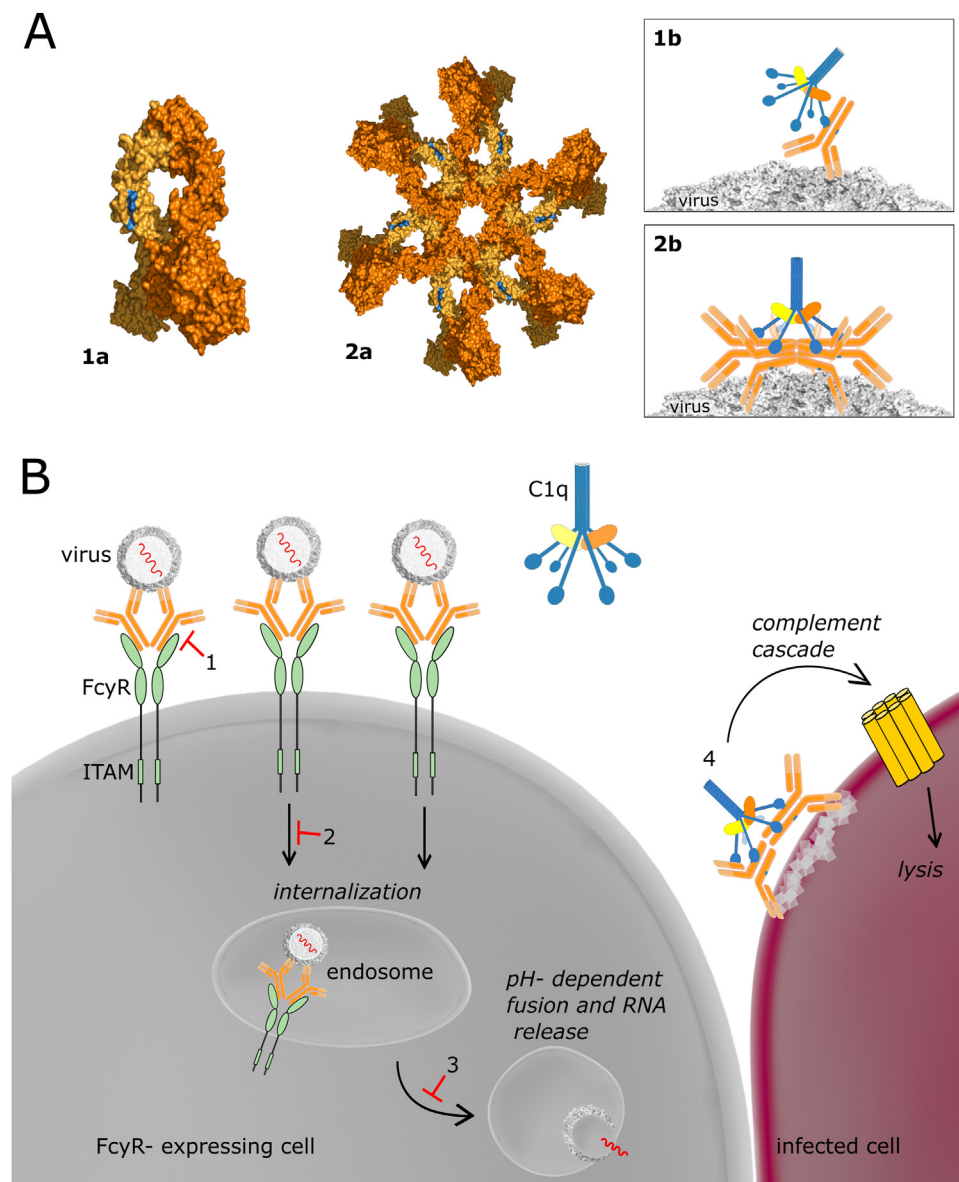


Figure 2. (A) IgG forms hexamers on the antigen surface that promote multivalent C1q binding and the activation of complement. Key residues of the C1q binding site on IgG1 are indicated in blue (E318, K320, K322) on a human IgG1 monomer (1a) and hexamer (2a) (PDB 1HZH). Low (1b) and high (2b) avidity IgG1 antibody interactions with C1q. Hexamer formation depends on antigen availability and antibody isotype. (B) Potential mechanisms of C1q reduction of ADE of flavivirus infections. In the presence of sub-neutralizing concentrations of anti-flavivirus antibodies, opsonized viral particles are engaged by FcγR and internalized to the host cell. C1q may (1) exert steric interference in FcγR and Fc interactions, (2) attenuate cell signaling through the FcγR that leads to internalization, (3) restrict E protein movements needed for pH-dependent fusion in endosomes, or (4) activate complement proteins that lead to MAC dependent lysis of the infected cell.

antigen, IgG forms hexamers on the antigen surface that promote multivalent C1q binding and the activation of complement (Diebolder et al., 2014; Gaboriaud et al., 2004; Wang et al., 2016) (Figure 2A). The hexameric conformation is dependent on antigen binding but is mediated by Fc:Fc interactions, one Fab arm of each IgG binds the antigen while the second Fab arm extends upward into the plane of the C1q stem, allowing the six Fc portions to form a platform that leaves key C1q binding residues exposed, providing a high avidity binding site for hexavalent C1q. Wang et al. found that the Fab arms are not necessary for complement activation but serve to stabilize the hexamer and play a regulatory role (Diebolder et al., 2014; Wang et al., 2016).

The capacity of the four human IgG isotypes to bind C1q and activate complement varies greatly and is dependent on Fc domain flexibility and conformation to the extent that these factors permit hexamer formation and exposure of the C1q binding site. The relative flexibility of human Ig hinge regions, IgG3 > IgG1 > IgG4 > IgG2, contributes to the ability of these subclasses to bind C1q and activate complement via the classical pathway: IgG3 > IgG1 > IgG2 > IgG4. IgG3 and IgG1, with comparatively long and flexible hinge regions, interact efficiently with C1q (Vidarsson et al., 2014). Reduced binding of C1q by IgG2 is due to the alanine at residue 235, which is leucine in the other subtypes. IgG4 completely fails to bind C1q, and fails to activate complement via the classical pathway. Vidarsson et al. shows that while IgG4 may form immune complex hexamers, rotation of loops in CH2 domain due to subclass-specific sequence variations shield C1q binding residues and prevent C1q binding (Brekke et al., 1994; Tao et al., 1993; Vidarsson et al., 2014).

Fc glycosylation in human IgG1 and mouse IgG2a at the CH2–CH3 junction influences the complement activating capacity of human IgG subtypes (Vidarsson et al., 2014). N297 aglycosyl mutants abrogate classical complement activation (Jefferis, 2012; Lee et al., 2017). Wang et al. found that the effects of IgG deglycosylation on complement activation was mediated by IgG hexamer abundance, and that mutants that retained the ability to form hexamers also retained the ability to bind C1q and activate complement (Wang et al., 2016). This suggests that the mechanism of aglycosyl abrogation of complement activation may be the prevention of Fc:Fc interactions that permit stable hexamer formation.

Lilienthal et al. found that murine IgG1, like its human analog IgG4, not only fails to bind C1q, but prevents complement activation by mouse IgG2a. Authors suggest that human IgG4 and mouse IgG1 can prevent complement activation by inhibiting Fc:Fc interactions of C1q binding subclasses through antigen binding competition and steric inhibition (Lilienthal et al., 2018).

Antibody-dependent enhancement of infection and complement

Structural perspectives of neutralization and antibody-dependent enhancement in flaviviruses

ADE has been demonstrated *in vitro* for many viruses, including alphaviruses, coronaviruses, HIV and coxsackieviruses (Taylor et al., 2015), but the role of ADE *in vivo* and in clinical settings is best described in flaviviruses. There is considerable antigenic cross-reactivity between flaviviruses and ADE has been demonstrated extensively. Immune serum from YFV, JEV and other flaviviruses have been shown to enhance DENV infection *in vitro* (Halstead et al., 1980), and even sub-neutralizing concentrations of virus and type-specific antibody can induce ADE of WNV and DENV infections (Pierson et al., 2007; Yamanaka et al., 2008). In DENV infections, the most important factor for severe disease is secondary infection with a heterologous serotype (Guzman

et al., 2013). Infants born to dengue-immune mothers are at increased risk of severe disease during primary infection due to waning titers of placentally-acquired maternal antibodies (Kliks et al., 1988). A long-term study of a pediatric cohort in Nicaragua found that risk of severe disease was linked to a specific range of pre-existing anti-DENV antibody titers (Katzelnick et al., 2017). In a clinical trial of live-attenuated YFV vaccine, subjects with specific titers of pre-existing cross-reactive JEV antibodies showed prolonged YF-vaccine viremia and numerous markers of antibody-enhanced vaccination (Chan et al., 2016). Following the 2017 outbreak of ZIKV across DENV-endemic regions of the Americas, the spectre of cross-reactive enhancement in DENV raised many concerns. Early results in patients with a history of prior flavivirus infection reveals a complex landscape. Pre-existing dengue immunity was shown to decrease the rate of symptomatic ZIKV infections in children (Gordon et al., 2019), while prior ZIKV infection increases the risk of severe disease in subsequent DENV infections (Katzelnick et al., 2020).

Antibodies provide protection against secondary viral infections through neutralization; inhibiting infection through repeatedly crosslinking virions to large antibody-virion aggregates that are more efficiently cleared from circulation (Chan et al., 2011), epitope-specific effects that inhibit viral attachment or fusion (Vogt et al., 2009) or Fc-mediated effector mechanisms (Nimmerjahn and Ravetch, 2008). ADE occurs in the stoichiometric window in which the number of bound antibodies support a stable attachment of the immune complex to the FcγR but does not reach the neutralization threshold (Pierson and Diamond, 2015). The neutralization capacity of an antibody depends on affinity, which determines the fraction of the epitopes bound to antibody, and epitope accessibility, which can limit the neutralization capacity of high affinity antibodies (Dowd and Pierson, 2011).

There are aspects of flavivirus biology and the antibody repertoire generated during primary infections that govern antibody affinity and epitope accessibility and therefore the interplay between protection and pathogenesis. The E protein is the principal antigenic target and antibodies may recognize epitopes that are differentially accessible along three distinct axes of symmetry, imposing steric constraints on antibody recognition. Furthermore, antibodies may recognize epitopes composed of more than one protein (Dowd and Pierson, 2011).

The antigenic landscape is made more complex by the inefficiency of furin processing (Junjhon et al., 2010). Distribution of prM cleavage is non-random, leaving partially mature virions with immature patches of uncleaved prM, rather than in an even distribution across the virion surface. Evidence suggests that these patches of uncleaved prM arise in portions of the virion that do not come into contact with the membrane-associated furin during transport through the trans-Golgi network (Rey et al., 2018). This failure of furin processing is dependent on cell type and, vitally, produces a bias toward immature and partially mature virions produced in commonly used cell lines, in stark contrast to the fully mature virions found in human plasma (Raut et al., 2019). The proportion of partially mature virions is closely linked to susceptibility to neutralization; fully mature virions are less susceptible to neutralization with cross reactive and heterotypic immune sera than partially mature variants likely due to the differences in epitope exposure (Beltramello et al., 2010; Mukherjee et al., 2016; Raut et al., 2019).

Notably, the antibody repertoire after natural infection with DENV and WNV includes poorly neutralizing antibodies associated with the immature conformation (Beltramello et al., 2010; Dejnirattisai et al., 2010; Lai et al., 2008; Throsby et al., 2006). Antibodies against two such epitopes play an important role in enhancement; anti-prM antibodies are poorly neutralizing and enhance infection over a wide range of concentrations

(Dejnirattisai et al., 2010; Rodenhuis-Zybert et al., 2010; Wang et al., 2017). Likewise, fusion loop epitopes, which recognize a hydrophobic pocket between DII and DIII that is exposed in immature virions but is buried in mature virions (de Alwis et al., 2014; Barba-Spaeth et al., 2016; Rey et al., 2018) are also strong mediators of ADE. Transient changes in virus structure, known as “virion breathing” also play a role in the accessibility of the fusion loop and other cryptic epitopes. Non-neutralizing antibodies against secreted NS1 hexamers can protect against the plasma leakage associated with high levels of plasma NS1, but have also shown cross reactivity with human fibrinogen and endothelial cells, leading to tissue damage (Falconar, 1997; Liu et al., 2011; Reyes-Sandoval and Ludert, 2019). Virion heterogeneity, derived from these and other characteristics such as intra-serotype variation, and serotype based-E protein differences, may lead to escape from neutralization and influence disease progression.

The classical pathway and antibody-dependent enhancement

The presence of C1q has been shown to modulate ADE of homologous and heterologous DENV infection *in vitro* (Yamanaka et al., 2008) and of WNV *in vitro* and in mouse models (Mehlhop et al., 2007; Mehlhop et al., 2009b). There are multiple potential mechanisms of C1q reduction of ADE, including steric interference in FcγR and Fc interactions, attenuation of cell signaling through the FcγR that leads to internalization, restriction of E protein movements needed for fusion, or downstream complement-mediated effects such as MAC-dependent lysis (Figure 2B).

Yamanaka et al. found that enhancement of DENV infection decreased in a dose-dependent manner in the presence of fresh human serum. The authors found that C1q- or C3-depleted serum did not reduce ADE, and that the addition of these complement factors to depleted serum reduced ADE at physiological levels of C1q and high levels of C3 (Yamanaka et al., 2008).

C1q is necessary and sufficient to restrict ADE of WNV mediated by anti-flavivirus monoclonal antibodies (mAbs) of subclasses that bind C1q with high affinity (Mehlhop et al., 2007). The addition of C1q permits neutralization by mAbs against epitopes displayed at too low a frequency to permit neutralization in complement-free conditions, suggesting that C1q modulates ADE by lowering the neutralization threshold to a level that eclipses the concentrations of antibody that support ADE (Mehlhop et al., 2009b). These data suggest that antibody-bound E proteins, further stabilized in the immune complex by C1q, are unable to make the conformational changes required for entry and fusion.

Antibody subtype becomes a determinant of protection or pathogenesis when C1q is present; Mehlhop et al. found that C1q enhanced neutralization of WNV by humanized IgG1 and IgG3 to a far greater extent than the IgG2 and IgG4, though IgG class did not affect the neutralization capacity of humanized E16 antibody in the absence of C1q (Mehlhop et al., 2009b). Due to the overlapping FcγR and C1q binding sites, competition between FcγR and C1q binding may be determined by the varying affinities of different FcγRs (Boesch et al., 2014) for IgG subtypes (Lilienthal et al., 2018). While Mehlhop et al. found that C1q binding to WNV did not inhibit cellular attachment, it remains unclear whether this attachment is FcγR-mediated (Mehlhop et al., 2009b). C1q may block FcγR engagement of IgG subtypes with high affinity for C1q. The role of human IgG2 and IgG4 immune complexes is unclear, these subtypes have low affinity for C1q but are also comparatively poor mediators of other effector functions. In fact, the presence of human IgG4 can inhibit hexamer formation and therefore stable C1q engagement by other IgG subtypes (Lilienthal et al., 2018), which may explain the limitations of C1q protection *in vivo*. Yamanaka and Konishi found that the presence of mouse IgG1 (homologous to human IgG4 and lacking

C1q binding capacity) interrupted the complement-mediated effects of other IgG subtypes (Yamanaka and Konishi, 2016). Human IgG4 and mouse IgG1 are the lowest abundance IgG subtypes, but their presence may block C1q mediated reduction of ADE.

Contrary to the protective effect described in flavivirus studies, a recent report found that the presence of C1q drives an FcγR-independent mechanism of ADE of Ebola virus in human kidney cells mediated by cross-linking of virus-antibody-C1q complexes to cell surface C1q receptors, leading to increased attachment and enhanced viral entry into cells (Furuyama et al., 2020).

Conclusions

Although flavivirus infections cause diseases of great public health concern, no antivirals, specific treatments or vaccines exist to date for most of them. ADE is a major barrier to dengue vaccine development and is an important consideration for ZIKV vaccination in dengue endemic regions. Studying how to reduce ADE by interactions with C1q or other complement factors may contribute to develop safe vaccine approaches and therapeutic strategies. ADE is a phenomenon that is abundant and widespread *in vitro* that has been recapitulated in clinically under specific conditions. As the factors that drive neutralization and enhancement are unraveled *in vitro*, it is important to revisit the transferability of these models to *in vivo* and human applications. Complement plays a central role in homeostasis and innate immunity, which, like antibodies, can drive protection or pathogenesis in flavivirus infections through complex pathways. Defining the conditions under which these vital components of the immune system interact with immune complexes and consequently limits ADE may lead the development of vaccines that promote humoral immune responses where protective interactions predominate.

Contributors

Both authors contributed to the conception, writing and revision of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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Ethical approval

None.

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