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Protective and enhancing interactions among dengue viruses 1-4 and Zika virus

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Abstract

Dengue viruses 1-4 (DENV1-4) and Zika virus (ZIKV) are closely related flaviviruses transmitted by *Aedes* mosquitoes that co-circulate in Asia, the Americas, Africa, and Oceania. Here, we review recent and historical literature on *in vitro* experiments, animal models, and clinical and epidemiological studies to describe how the sequence of DENV1-4 and ZIKV infections modulates subsequent dengue and Zika disease outcome. Overall, we find these interactions are asymmetric. Immunity from a prior DENV infection or a prior ZIKV infection can enhance future severe dengue disease for some DENV serotypes while protecting against other serotypes. Further, prior DENV immunity has not been shown to enhance future uncomplicated or severe Zika and instead appears to be protective. Interestingly, secondary ZIKV infection induces type-specific ZIKV immunity but only weakly cross-neutralizing anti-DENV/ZIKV immunity, consistent with risk of future dengue disease. In contrast, secondary DENV infection induces strongly cross-neutralizing antibodies that protect against subsequent severe dengue disease. These immunologic interactions may be explained by differences in virion structure between DENV1-4 and ZIKV, which modulate thermostability, susceptibility to neutralization, and cell infectivity. Overall, these observations are important for the understanding and prediction of epidemics and development and evaluation of dengue and Zika vaccines.

Keywords

Dengue virus; Zika virus; immunity; virion structure; neutralization; enhancement; epidemiology

INTRODUCTION

Dengue viruses 1-4 (DENV1-4) and Zika virus (ZIKV) are five closely related flaviviruses transmitted by *Aedes* mosquitoes [1]. Dengue disease ranges from the debilitating but self-

limited Dengue Fever to life-threatening Dengue Hemorrhagic Fever/Dengue Shock Syndrome (DHF/DSS) and other complications associated with Severe Dengue [2–4]. ZIKV can also cause life-threatening disease, including ZIKV-associated Guillain-Barre Syndrome (GBS) in adults and congenital defects including microcephaly when infection occurs during pregnancy [5–7]. Over 3 billion people live in dengue-endemic areas in Asia, the Americas, Africa, and Oceania, with the greatest burden in Southeast Asia [8]. Just over 2 million live in areas at risk of Zika, with the highest burden in the Americas; however, the possibility for resurgence of Zika elsewhere remains [9,10]. Travelers to endemic areas are also at high risk of dengue and Zika, and in some instances can introduce these flaviviruses into non-endemic areas [11–13].

The flavivirus envelope (E) protein consists of three β-barrel domains (EDI, EDII, and EDIII). E monomers form head-to-tail homodimers, which are organized in rafts [14]. E is the major target of virus-specific neutralizing antibodies (nAbs) but also contains the immunodominant fusion loop (FL), which is conserved across flaviviruses and is susceptible to binding by antibodies that increase viral infection via antibody-dependent enhancement (ADE) [14–16]. In mature virions, the FL is generally concealed on the homodimer; however, the DENV virion can 'breathe', exposing the FL and other 'cryptic' epitopes [17,18]. The pre-membrane (pr) protein remains uncleaved from the membrane (M) protein on immature and partially immature virions, and human antibodies targeting prM can enhance DENV *in vitro* [16,19]. Pre-existing antibodies induced by prior heterotypic DENV infection or ZIKV infection can mediate ADE and enhance future risk of dengue disease, including DHF/DSS [20–23]. There is an urgent need to better understand how DENV1-4 and ZIKV interact immunologically for dengue and Zika vaccine development and evaluation as well as for epidemic preparedness.

In this review, we discuss determinants of protective and disease-enhancing interactions among DENV1-4 and ZIKV, including the sequence of infecting viruses; virus structure, stability, maturation state, and infection mechanisms; and how the epidemiology and pathogenesis of each virus may be differentially affected by pre-existing heterotypic flavivirus immunity. Further, we discuss how interactions between DENV and ZIKV may affect risk of disease and vaccine design and efficacy.

METHODS

We used PubMed to screen the titles and/or abstracts for all articles with the terms 'dengue' and 'Zika' and one or more of the following terms (serology, seroprevalence, cross protection, cross reactiv*, neutrali*, enhance*, immunity, or structure) in either the title and/or abstract (n=763, May 7, 2020). We identified 117 key studies and emphasize those published since January 2018.

DENV → DENV

Homotypic immunity—Primary DENV infection induces long-lived homotypic protection mediated in part by type-specific nAbs, which target quaternary epitopes displayed on the E homodimer or intact virion [24]. Quaternary type-specific nAbs often

target non- or partially overlapping regions on the different serotypes [25]. For some serotypes, multiple distinct quaternary type-specific nAb regions have been identified [26].

Heterotypic immunity—Primary DENV infection also induces weakly-neutralizing anti-DENV antibodies targeting the FL, prM protein, and other epitopes [16,19]. Such antibodies bind heterologous DENV virions and can mediate ADE by promoting virus infection of immune cells via Fcγ receptors, activating the cells, increasing viremia and levels of pathogenic NS1 protein, and initiating an immune cascade that leads to vascular leakage and severe disease [27–30]. Early identification of those at risk of progression to severe dengue disease is urgently needed for patient triage and treatment, but point-of-care biomarkers that reliably predict severe disease remain elusive [31,32]. In contrast, high levels of binding and neutralizing antibody titers are protective against symptomatic secondary dengue [21,22,33,34]. Further, after secondary DENV infection, individuals are at lower risk of subsequent symptomatic disease, although not in all studies [35–37]. Protection after secondary DENV infection has been attributed to the induction of enduring, protective antibodies that target cross-reactive epitopes, including the EDE epitope, the A-strand in EDIII, and the be loop near the FL [38–40].

Sequence of infecting DENV serotypes—Pre-existing immunity may be differentially associated with protection and disease-enhancement, depending on the infecting serotypes. Hospital-based studies in Thailand and Nicaragua showed that a greater fraction of symptomatic and severe dengue patients infected with DENV1 and DENV3 are primary infections, whereas nearly all symptomatic and severe dengue patients infected with DENV2 and DENV4 are secondary [41–43]. The exception is severe primary DENV2 in infants, who often experience disease in the presence of maternal antibodies [42]. Further, the sequence of infecting DENV serotypes has long been considered an important epidemiological determinant of subsequent disease severity, especially the sequence DENV1→DENV2 [28,44–46]. However, the sequence DENV2→DENV1 has been associated with severe secondary dengue in French Polynesia [47]. In Vietnam, the order DENV1→DENV2, DENV1→DENV4, DENV2→DENV3, and DENV4→DENV3 were associated with increased risk of experiencing a future symptomatic DENV infection [48]. The level of pre-existing antibodies is also important. While high pre-existing nAb titers against DENV3 were protective against severe DENV3 disease, high titers against DENV2 were not associated with protection against severe disease caused by DENV2 [49]. Further, the postvaccination nAb titer required for protection against DENV2 is higher than that for other serotypes [50]. In support of these observations, we recently found in a cohort study in Nicaragua that high pre-existing anti-DENV antibody titers are protective against symptomatic and severe DENV1 and DENV3, but not DENV2, infections. In contrast, intermediate levels of anti-DENV antibodies are associated with enhancement of symptomatic and severe DENV2 and DENV3 infections, but not DENV1 infections [23] (Figure 1).

DENV → ZIKV

In vitro *studies and animal models*—As ZIKV emerged across the Americas, researchers asked whether pre-existing anti-DENV immunity modified Zika disease.

Primary DENV infection induces low or undetectable levels of cross-reactive nAbs against ZIKV, while some secondary DENV infections do generate cross-reactive nAbs to ZIKV [51–53]. After both primary and secondary DENV infection, a large proportion of memory B cells (MBCs) are cross-reactive, but secondary DENV infection induces a broader response that is also ZIKV-cross-reactive [54]. Consistent with these findings, some monoclonal antibodies (mAbs) isolated from patients following secondary DENV infection potently neutralize and protect against ZIKV infection by targeting quaternary epitopes on the virion [51,55–57]. Two such antibodies, the anti-DENV E dimer (EDE) and SIgN-3C mAbs, each have distinct mechanisms of neutralization against DENV and ZIKV [55,58].

Early studies during the Zika pandemic focused on the ability of anti-DENV antibodies to enhance ZIKV infection (Figure 2). DENV-immune serum from pregnant and non-pregnant individuals as well as mAbs isolated following primary and secondary dengue enhanced ZIKV infection in FcγRII-expressing K562 cells, PBMC-derived CD14$^+$ monocytes, and monocyte-derived immature dendritic cells [59–63]. DENV-immune serum also enhanced ZIKV infection and reduced induction of pro-inflammatory cytokines and antiviral responses in primary human macrophages, placental macrophages (Hofbauer cells), and other cells in placental explants [64–66]. Further, DENV-immune serum and mAbs targeting EDI/II enhanced ZIKV infection and morbidity in Stat2$^{-/-}$ and AG129 mice [63,67]. However, in three independent studies in rhesus macaques previously infected with DENV1, DENV2, DENV3, or DENV4, enhancement was not observed in magnitude or kinetics of ZIKV viral load nor in the immune cell response compared to naive animals [68–70]. Additionally, anti-DENV CD8$^+$ and CD4$^+$ T cells can protect against ZIKV in mice [71–73]. Thus while *in vitro* and mouse models suggest DENV can protect against and enhance ZIKV, ADE is not observed in non-human primate (NHP) studies.

Clinical and epidemiological studies—Consistent with findings in NHPs, ZIKV viremia, cytokine expression, innate immune profile, and/or clinical score did not differ between DENV-exposed and DENV-naive patients with Zika in Brazil and Nicaragua [74,75]. In Puerto Rico, secondary DENV2 and DENV3 infections had significantly higher viral load than primary DENV2 and DENV3 infections, but no significant difference was observed in viremia of primary and secondary Zika cases [76]. Instead, in a Nicaraguan pediatric cohort, prior DENV infection and higher pre-existing anti-DENV and anti-ZIKV antibody levels were associated with reduced risk of uncomplicated Zika when adjusting for age, sex, and recent DENV infection [23,77]. Further, a study in Brazil showed that the level of pre-existing anti-NS1 DENV antibodies was protective against ZIKV infection and disease [78]. To date, studies of infant outcomes of maternal ZIKV infection do not differ significantly by maternal disease severity, viremia, or acute-phase anti-DENV IgG antibody titers [79]. Higher PRNT titers to ZIKV at birth are seen in women with infants born with microcephaly compared to controls, likely due to greater ZIKV viral load, but prior DENV status did not distinguish cases and controls [80,81]. At the population level, one study observed that areas with large dengue epidemics within the last six years had lower rates of microcephaly, suggesting a protective role of recent DENV infection, while areas with major epidemics >6 years ago were at greater risk for microcephaly epidemics [82]. Finally, case-control studies showed that ZIKV exposure but not prior DENV exposure was strongly

associated with Guillain-Barre Syndrome [6]. Thus, to date, ADE has not been observed in clinical or epidemiological studies of uncomplicated ZIKV nor in studies of severe Zika, including congenital Zika syndrome.

ZIKV → DENV

In the first two years after the Zika epidemic, there was little DENV transmission in the Americas. This phenomenon was linked to transient cross-protection against dengue epidemics as a result of the Zika epidemic [83]. However, several years later, large epidemics of dengue returned as predicted, and the question arose as to whether ZIKV may enhance subsequent risk of dengue disease (Figure 2) [83].

Early B cell and antibody responses—During and soon after ZIKV infection, those with prior DENV infection (secondary ZIKV infection) displayed distinct adaptive immune responses from ZIKV-infected DENV-naïve individuals (primary ZIKV infection). While anti-ZIKV IgM and IgA antibodies expanded at similar rates, anti-ZIKV IgG antibody responses were stronger and occurred earlier in secondary ZIKV infection in humans and NHPs, consistent with an anamnestic response [68,74]. In secondary ZIKV infection, plasmablasts were clonally related and somatically mutated, DENV/ZIKV cross-reactive but with stronger binding to DENV (especially the FL), and capable of enhancing DENV2 and/or ZIKV, overall pointing to a bias toward DENV [84,85]. In contrast, plasmablasts in primary ZIKV infection were clonally unrelated, displayed much less somatic hypermutation, were ZIKV-specific, targeted the whole virion and not ZIKV E protein (suggesting binding to quaternary epitopes), and were not capable of enhancing DENV2 [84,85]. At 14 days post-symptom onset, those with a history of DENV also had a stronger DENV-ZIKV cross-reactive MBC responses compared with those experiencing primary ZIKV infection [86].

Late B cell and antibody responses—By late convalescence (5 to 8 months post-infection), potent, type-specific anti-ZIKV nAbs and MBCs were produced in humans and NHPs following both primary and secondary ZIKV infection [53,68,74,86]. These type-specific nAbs were unaffected by depletion of anti-DENV1-4 antibodies [52,86]. Even in secondary ZIKV infection, potent anti-ZIKV nAbs were derived from MBCs with somatic mutation levels consistent with *primary* ZIKV infection [84].

In contrast, neither primary nor secondary ZIKV infection consistently produced broadly neutralizing cross-reactive anti-DENV-ZIKV B cell responses. In secondary ZIKV infection, the most highly mutated MBCs had higher affinity for ZIKV E than the ZIKV-specific potently neutralizing mAbs (derived from MBCs), but mAbs from these affinity matured MBCs only weakly neutralized DENV1-4 and ZIKV and predominantly targeted the FL [84]. Following primary and secondary ZIKV infection, mAbs toward EDI/II were highly DENV-ZIKV cross-reactive and capable of ADE *in vitro* and in a DENV mouse model [67]. Further, the degree of DENV-ZIKV MBC cross-reactivity was similar for ZIKV-infected individuals with 0, 1, or >1 prior DENV infections [86]. Finally, even in secondary ZIKV infection, almost none of the ZIKV serum nAb response is attributable to DENV-ZIKV cross-neutralizing antibodies, whereas after secondary DENV infection, the majority of the

neutralizing antibody response to DENV1-4 was cross-reactive [86,87]. Thus, secondary ZIKV infection in humans appears to produce a cross-reactive polyclonal MBC and antibody response that is poorly neutralizing and may be capable of ADE.

ZIKV neutralizing epitopes—Potent type-specific anti-ZIKV mAbs target epitopes on EDIII as well as other quaternary epitopes [67,84,85,88,89]. A recent study showed that a chimeric virus with ZIKV EDIII introduced into the DENV4 E protein did not become susceptible to neutralization by human ZIKV-immune serum, suggesting that key epitope(s) targeted by ZIKV nAbs are outside EDIII [90]. Other potent anti-ZIKV nAbs have been identified, including intradimer epitopes in EDI/EDII, EDII, and EDI/EDIII, and an interdimer epitope in EDII -- some of which have been shown to be protective against ZIKV in mouse models [88,89]. Notably, some studies have identified DENV/ZIKV cross-neutralizing epitopes, including for DENV1/ZIKV (EDIII lateral ridge), DENV2/ZIKV (EDI/EDIII linker region), and DENV3/ZIKV (unknown epitope), suggesting that broad neutralization of DENV/ZIKV may be possible [84,86,91,92].

ADE in skin explants and mouse models—Primary ZIKV infection induces anti-DENV antibodies capable of enhancing DENV2 infection in various models. One study used a skin explant model to show that primary anti-ZIKV human serum mediates ADE of DENV2 infection in FcγR-b earing cells in the skin, including macrophages, dendritic cells, and Langerhans cells. More FcγR-bearing cells were infected and produced a greater quantity of DENV2 in the presence of ZIKV-immune serum than cells infected in the presence of naive serum. Adding anti-FcγRI and anti-FcγRII antibodies fully blocked the enhancing effect. In contrast, epidermal keratinocytes, which lack FcγR, were similarly infected by DENV2, with or without heterotypic serum. The authors also showed that similar enhancement effects were observed for DENV2 and ZIKV infection in the presence of anti-DENV3 antibodies [93]. In addition, ZIKV infection and inactivated ZIKV vaccines induce cross-reactive antibodies to DENV in mice that enhance DENV2 infection and disease [94,95]. LysMCre[+]Ifnar1^{fl/fl} mice (lacking the IFNα/β receptor in myeloid cells) born to ZIKV-infected mothers and naive mice that received passively transferred anti-ZIKV maternal antibodies displayed elevated viral load, clinical severity, and mortality when challenged with DENV2 [96]. In contrast, in the reverse experiment, mice born to DENV2-immune mothers did not experience greater disease severity upon challenge with ZIKV than naive mice. While ZIKV induced antibodies that bound but did not neutralize DENV2, DENV2 infection did not induce antibodies that bound to ZIKV, helping explain the asymmetric enhancing interactions [96].

Non-human primate and human studies—Prior ZIKV infection can also enhance DENV viremia in NHPs [70,97], although not in all studies [98]. One study showed that macaques with prior ZIKV infection had increased viral load, thrombocytopenia, leukopenia, and neutropenia following DENV2 challenge [97]. A follow-up study showed IgG1 antibodies were associated with DENV2 enhancement [99]. Another study showed that macaques previously infected with ZIKV had elevated viremia but not disease following DENV2 challenge compared to controls [70]. However, one study in macaques did not observe that ZIKV infection led to ADE of DENV2 [98].

We have found that children with one prior ZIKV infection are at significantly greater risk of symptomatic and severe dengue disease caused by DENV2 in pediatric cohorts in Nicaragua [23]. Additionally, children with one prior DENV plus one prior ZIKV infection are also at elevated risk of symptomatic and severe DENV2 infection, consistent with previous studies of MBCs and serum antibodies showing that secondary ZIKV infection does not induce broadly neutralizing anti-DENV antibodies [84–86]. Further, intermediate pre-existing levels of anti-DENV or anti-ZIKV antibodies are associated with enhancement of symptomatic and severe dengue. However, individuals with two or more prior DENV infections with or without a subsequent ZIKV infection and those with high anti-DENV or anti-ZIKV antibodies are at much lower risk of symptomatic and severe DENV2, with rates similar to DENV-naïve individuals.

T cell responses—Differences in T cell specificity also likely play a role in protective and possibly pathogenic interactions between DENV and ZIKV, as reviewed in detail elsewhere [100,101]. Anti-DENV CD8+ T cells predominantly target non-structural proteins (NS3, NS4B, and NS5) with the exception of DENV3, which also targets structural proteins [102]. Anti-DENV CD4+ T cells target structural (C, E) and non-structural 5 (NS5) proteins [100]. Notably, a large proportion of CD8+ and CD4+ T cells from DENV vaccine recipients and blood donors in Nicaragua and Sri Lanka, all collected before the emergence of Zika, were stimulated by ZIKV peptides from the whole ZIKV proteome. Some CD8+ and CD4+ cells reacted with epitopes on ZIKV non-structural proteins that were fully or mostly conserved with DENV1-4 [103]. Consistent with these findings, anti-DENV CD8+ T cells as well as anti-DENV CD4+ T cells have been shown to be protective against ZIKV in mice and in a pregnant mouse model [71–73].

As for studies of B cells, secondary ZIKV infection induced earlier and higher magnitude T cell responses that were declining by convalescence, while primary ZIKV T cell responses were increasing at convalescence. Further, secondary ZIKV induced CD8+ T cells that upregulated granzyme B and PD1 [103]. However, a later study showed that both primary and secondary ZIKV infection induced multifunctional ZIKV-specific CD8+ T cells by late convalescence [104]. In contrast to DENV, ZIKV CD4+ T cells mostly target structural proteins, and following primary and secondary ZIKV infection, a low proportion of the CD4+ T cell response was reactive to DENV E protein [67,103]. Some studies suggest ZIKV may skew the CD4+ T cell response and bias future DENV infections toward pathogenesis in mice and severe outcomes, including microcephaly, in humans [105,106].

Structural differences between DENV1-4 and ZIKV

Structural differences between ZIKV and DENV1-4 have been observed to modify exposure of epitopes targeted by enhancing antibodies and potently neutralizing antibodies and to modulate virion binding to key cell receptors and attachment factors. Such differences may help explain asymmetric immune interactions between DENV1-4 and ZIKV (Figure 3).

Virion breathing—Differences between strains may alter virion 'breathing', i.e. the structural conformations sampled by the virion that can influence stoichiometry of antibody binding. Notably, ZIKV is more thermally stable than DENV but not WNV [107,108].

Further support for increased stability of ZIKV is that a mutation in the E protein at amino acid position 198 alters breathing of DENV and WNV, but not ZIKV [109]. A nearby mutation on DENV at E position 204 also modifies breathing of DENV1 strains, and this modulates exposure of EDIII and susceptibility to an anti-EDIII DENV1/ZIKV cross-neutralizing mAb [17,110]. However, how breathing modifies infection and susceptibility to antibodies *in vivo* is unknown.

CD-loop on EDIII—Virion stability is also reinforced by a feature in the CD-loop of EDIII. The E protein of neurotropic flaviviruses, including ZIKV, contains an extended CD-loop compared to non-neutrotropic flaviriruses such as DENV1-4, and this has been directly associated with ZIKV thermostability [111]. Deletion of the extra amino acid (346) destabilized the ZIKV virion, even at room temperature [111].

Glycan loop glycan at N153 or N154—Differences in the glycan loop in EDI (N154 for ZIKV, N153 for DENV) are also important for virion stability and susceptibility to neutralization. On mature ZIKV virions, as compared to DENV, the loop containing the glycosylation site N154 extends toward DII on adjacent E proteins, improving E homodimer formation, covering the FL, and eliminating susceptibility to weakly-neutralizing, often enhancing anti-DENV FL antibodies [107,112,113]. Artificial mutation of positions in the N154 environment modify the early stage of ZIKV infection, with some mutations increasing fusion with the endosomal membrane, possibly due to increased exposure of the FL, and others blocking glycosylation and eliminating virion binding to cells expressing DC-SIGNR [114,115]. Further, the ZIKV glycan at N154 contains a more complex sugar than DENV, which improves binding to DC-SIGNR-expressing cells including placental villi [115,116].

N67 glycan—Unlike ZIKV and other flaviviruses, the DENV E protein also contains an N glycan at position 67. The N67 glycan has high affinity for DC-SIGN, which is expressed on dendritic cells and a subset of macrophages. Improved binding to DC-SIGN leads to greater virus tropism for dendritic cells and monocyte/macrophage-derived cells [117–119]. Interaction with DC-SIGN is related to high-mannose glycan, a simple sugar, whereas complex sugars improve binding to DC-SIGNR. WNV and JEV do not naturally contain N67, but substitution with this glycosylation site increases WNV and JEV interaction with DC-SIGN [119]. Further, potently neutralizing EDE mAbs neutralize ZIKV differently from DENV2 because of the N67 glycan. EDE1 mAbs are N67-independent and potently neutralize ZIKV and DENV2. In contrast, EDE2 mAbs depend on the N67 glycan for binding, resulting in potent neutralization of DENV2 and lack of neutralization but rather enhancement of ZIKV [55,60].

Virion maturation—Anti-prM and anti-FL antibodies are able to bind immature and partially immature DENV virions and facilitate ADE [120]. Maturation state is determined by the degree of furin cleavage, which can vary by cell type and the sequence of pr-M furin cleavage site. Although DENV1-4 virions are partially immature when grown in common cell lines, DENV1 virions in blood of patients with primary DENV infection were mature *in vivo* and not susceptible to binding by prM antibodies [121]. However, the virion maturation

state of other DENV strains and serotypes and the maturation state during secondary infection are unknown. Of interest, DENV1-4 display amino acid variation near the pr-M cleavage site that is not present in other flaviviruses and that modulates the production of immature virions [122]. The evolutionary and functional importance of prM for DENV1-4 and ZIKV and its role modulating maturation state is of great interest.

Dengue and Zika vaccines

Immunological interactions among DENV1-4 and ZIKV are a critical concern for dengue and Zika vaccines. The only licensed dengue vaccine, Dengvaxia®, was introduced in mass vaccination programs and then confirmed to elevates incidence of DHF/DSS in those without prior DENV infection and thus is only recommended for individuals with a documented history of DENV infection [123,124]. Several other DENV vaccines are in Phase 3 clinical trials and may have different efficacy and safety profiles. However, two tetravalent dengue vaccine candidates have shown serotype-specific differences in protection [50,125], which may be attributable to differences in vaccine formulation and T cell responses, but also to differences in how anti-DENV immunity modulates disease caused by each serotype. Further, leading DENV vaccines were developed before the introduction of ZIKV. How DENV vaccines perform in the context of ZIKV immunity requires further study.

Dozens of candidate Zika vaccines were rapidly developed during the epidemic to protect at-risk populations. The possibility that a ZIKV vaccine could enhance DENV has been a prominent concern for many ZIKV vaccine developers, and approaches to avoid inducing ADE include ZIKV EDIII vaccines, stabilized ZIKV dimer vaccines, and ZIKV NS1 vaccines [89]. The finding that natural ZIKV infection can enhance future dengue disease severity further highlights the importance finding safe approaches to ZIKV vaccination [23]. Notably, a recent study has shown even ZIKV-specific protection differs dramatically across constructs: ZIKV vaccine candidates that induce potent nAbs to mature ZIKV virions, and not just high nAb titers overall, are most protective in challenge studies [126]. This finding suggests that methods for measuring immune correlates that take into account key structural features of ZIKV, and flaviviruses in general, will be most valuable for evaluating clinical studies.

CONCLUSION

Overall, the literature to date indicates that DENV1-4 and ZIKV can reciprocally modify disease outcomes. However, these interactions are asymmetric (Figure 1). Some sequences of infection have been mostly associated with cross-protection, including DENV1-4 infection followed by ZIKV infection. Other sequences have been observed to result in enhanced disease, including ZIKV infection followed by DENV2 infection. It has long been observed that specific DENV serotypes differentially modulate subsequent DENV disease severity in a serotype-dependent manner, and recent studies suggest such differences extend to ZIKV. Further studies of the structural and immunological differences between DENV1-4, ZIKV, and possibly other flaviviruses, and how immunity to one modulates disease with

another, will provide important insights for safe and effective flavivirus vaccine development and use as well as preparation for future epidemics.

Acknowledgments

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antibodies to mature ZIKV virions and those that were maturation-insensitive were better predictors of *in vivo protection.*
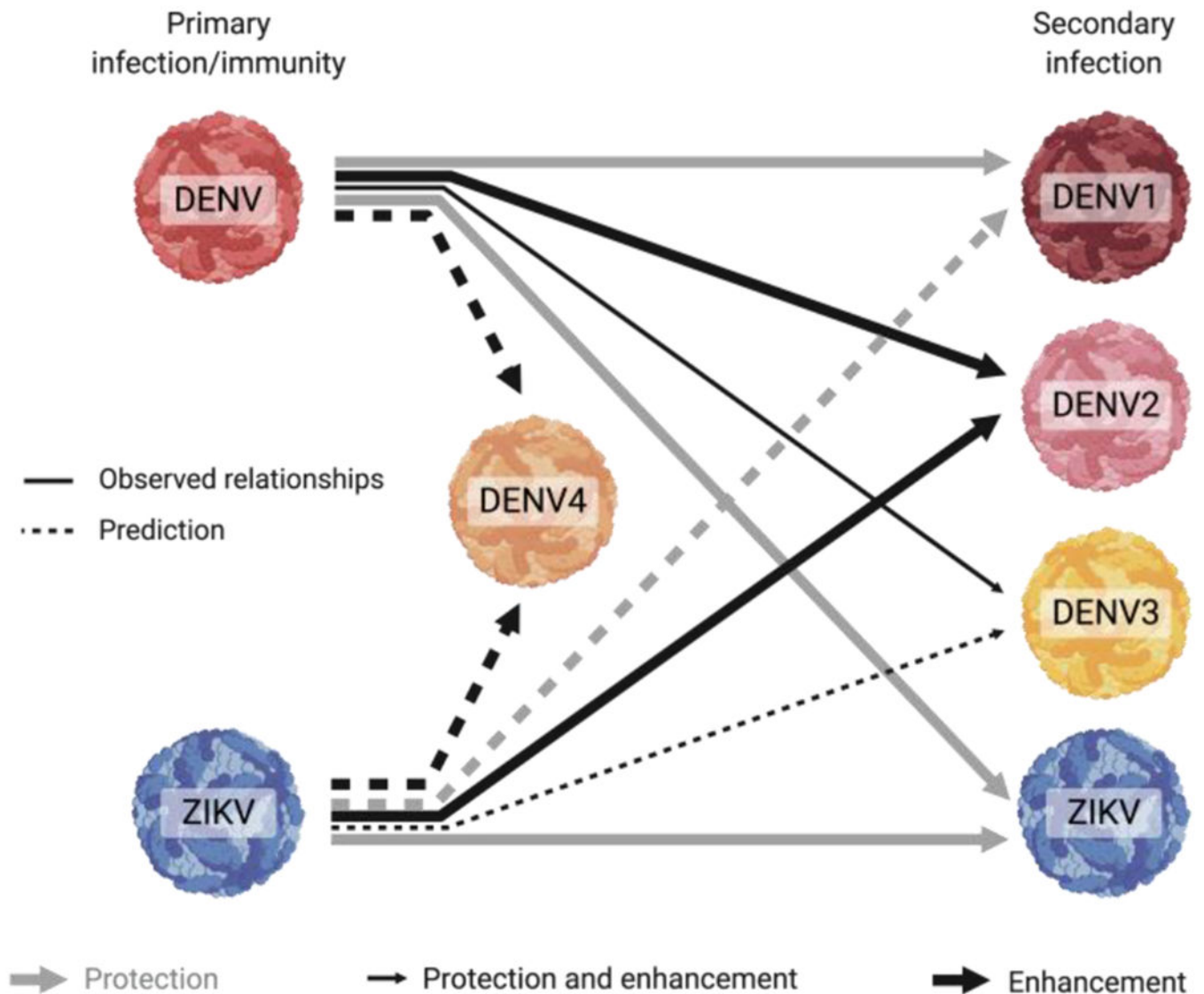
Fig. 1. Observed and hypothesized protective and enhancing interactions between DENV1-4 and ZIKV.
Primary DENV immunity, at high pre-existing antibody concentrations, is observed to protect against secondary symptomatic DENV1, DENV3, and ZIKV infection, but not DENV2 infection. In contrast, intermediate antibody levels generated from primary DENV infection increase symptomatic infection and enhance disease severity caused by DENV3 and DENV2 infection, but not by DENV1 or ZIKV infection. Primary ZIKV infection is also observed to enhance subsequent DENV2 infection and disease severity. We hypothesize that the effect of prior ZIKV infection on secondary infection with each DENV serotype will be similar to how primary DENV infection affects secondary infection with each DENV serotype. Grey arrows indicate protection, large black arrows indicate enhancement, and small black arrows indicate protection and enhancement. Solid lines indicate observed relationships, dotted lines are predicted relationships. The effect of prior DENV or ZIKV immunity on subsequent DENV4 disease has not been fully described.
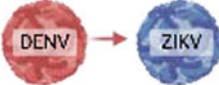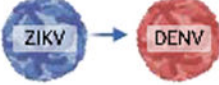
Fig. 2. *In vitro***, animal models, and clinical studies showing protective and enhancing interactions between DENV → DENV, DENV → ZIKV, and ZIKV → DENV.**
Columns show studies conducted in *in vitro* assays, mouse models, non-human primate challenge studies, human epidemiological and clinical studies, and human pregnancy/infant studies. Rows show the sequence of infecting viruses: primary DENV followed by secondary DENV infection with a different serotype, primary DENV infection followed by secondary ZIKV infection, and primary ZIKV infection followed by secondary DENV infection. Green "P" indicates protection, red "E" indicates enhancement, and question marks indicate unknown relationships.
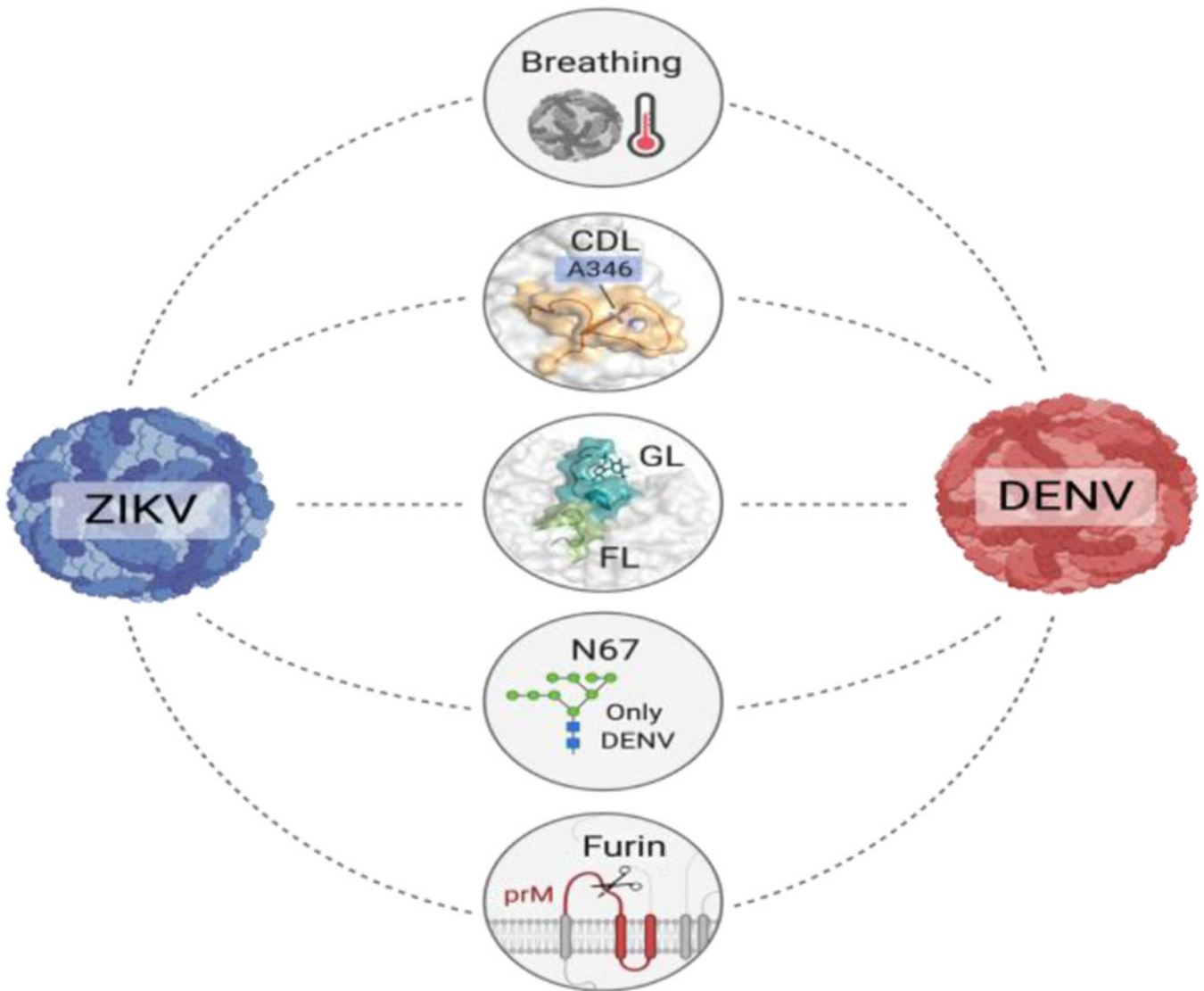
Fig. 3. Key structural differences between ZIKV and DENV.
Circles, from the top: (1) ZIKV is more thermally stable and undergoes less virion 'breathing' than DENV. (2) The ZIKV EDIII CD loop contains an additional amino acid at position 346 compared to DENV, which modifies virion thermostability. (3) The ZIKV glycan loop at N154 helps mediate homodimer formation and covers the FL, making ZIKV nonsusceptible to anti-FL antibodies, whereas anti-FL antibodies can weakly neutralize and enhance DENV. (4) DENV contains a glycan at position 67 that is not present on ZIKV and alters susceptibility to broadly neutralizing antibodies. (5) The furin cleavage site may differ in efficiency between DENV1-4 and ZIKV, altering virion maturation state.