



# Enhancement of Infection by Pre-Existing Non-Neutralizing Antibodies to Cross-Reactive Flaviviruses: Ramifications for Vaccination against Dengue and Zika

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### Abstract

Dengue is a prominent mosquito-transmitted viral disease of humans that is a significant public health consideration in tropical and subtropical regions of the world in which it is endemic. Cross-reactivity between different serotypes of dengue virus activates antibody-dependent enhancement of infections, an immunopathological response that it is thought to amplify viral replication and thereby to increase disease severity. The hitherto neglected closely related flavivirus Zika has now emerged strongly in areas where dengue virus is endemic, especially in South and Central America. Recent investigations indicate that anti-dengue virus immunoglobulin G may exacerbate Zika infection in an *in vitro* model. Conversely, it appears that anti-Zika virus IgG can enhance dengue virus replication in a target cell line. Such pre-existing immunity to one flavivirus may not only obscure the diagnosis of disease caused by infection with a heterologous flavivirus but potentially adversely affect the clinical outcome. The existence of non-neutralizing antibodies to a previous dengue or Zika infection can aggravate disease manifestations of a secondary, heterologous flavivirus infection. This has far-reaching ramifications for delivery of vaccines that are at present in development, immunization with which will stimulate production of homotypic flavivirus antibodies but which may have the inadvertent harmful effect of heightening susceptibility to heterologous infection of either dengue or Zika.

### Keywords

Dengue; Zika; Serotype; Antibody; Antibody-dependent enhancement; Vaccine; Immunization

### Introduction

The arthropod-borne (arbo) viruses dengue (DENV) and Zika (ZIKV) are closely related species of enveloped, positive sense, single-stranded RNA virions of the *Flavivirus* genus [1,2]. These important human pathogens belong to the family *Flaviviridae* that also includes the causative agents of yellow fever, Japanese encephalitis and West Nile encephalitis [3]. Both are transmitted between humans by

female mosquitoes of the genus *Aedes*, principally *Ae. aegypti* and *Ae. albopictus* [4]. By virtue of the broad geographical distribution of their common vectors, DENV and ZIKV co-circulate in large parts of the tropics and subtropics [5,6]. No prophylactic vaccine or therapeutic drug with which to alleviate either infection is presently available or affordable to citizens of low income countries [7,8].

### Epidemiology

More than 2.5 billion people currently live in dengue-endemic areas [5]. Despite vector control measures in the majority of affected countries, such a mass level of exposure results in around 390 million dengue infections annually in close to 130 nations and territories [9]. The annual global incidence is estimated at 200-400 million clinical cases [10].

The previously low prevalence of Zika infection changed markedly in 2015 with a major Zika epidemic now spread to 48 nations in the Americas [6]. In Brazil alone, where the outbreak arose, 1,400,000 clinical cases were suspected [11]. Last November the World Health Organization declared Zika to be no longer an international public health emergency [12]. Rather than meaning concern over Zika is over, it is viewed as an endemic problem that will demand continuing vigilance. Sexual transmission of Zika may provide a secondary route to enable viral persistence in locations that are not endemic for *Aedes* mosquitoes [13].

### Clinical Manifestations

Dengue infections may be asymptomatic or exhibit one of three characteristic manifestations: a mild self-limiting fever lasting for 2-10 days, typically upon primary infection, from which recovery is complete; dengue with a wide range of warning signs; or severe dengue [14]. Both dengue haemorrhagic fever and dengue shock are serious, often fatal, complications that feature problems of capillary permeability and blood clotting [15]. Infection can be severely incapacitating for a person of any age.

Only around one in five adults infected with Zika show clinical symptoms [16]. The principal manifestations of mild headache, fever, myalgia, arthralgia, conjunctivitis and rash last for up to one week and are similar to but less severe than other related febrile diseases, including dengue [17]. The main possible consequence of infection, for which causality is now established [18,19], occurs via congenital transmission [17,20], the effects of which are profoundly debilitating and long-lasting [21]. In Brazil, ZIKV is associated with more than 4,000 cases of microcephaly [6], a formerly rare condition in which babies develop undersized heads and, usually, neurological impairment. Exceptionally, Zika triggers Guillain-Barré in adults, a temporary paralysis caused by neural demyelination and regarded as an autoimmune sequela of infectious disease [19,20].

### Immunity and Immunopathology

Dengue may be caused by any one of four antigenically distinct serotypes of dengue virus, DENV 1-4 [22,23]. A fifth, more distantly related serotype is mooted to exist [24]. Primary infection with one serotype generates neutralizing homotypic immunoglobulin (Ig)G that provides life-long immunity to that serotype but confers only

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transient protection against others [25-27]. Importantly, subsequent infection with a heterotypic serotype generates cross-reactive non-neutralizing antibodies whose presence in the peripheral circulation increases the risk of antibody-dependent enhancement (ADE) of infection, a form of immunopathology. IgG induced by a primary DENV challenge recognizes but does not neutralize a further heterologous DENV serotype. Instead, it binds to the virion and facilitates the virus-antibody complex to enter Fcγ receptor-expressing antigen-presenting cells, causing increased viraemia and thus disease exacerbation [28]. Distinct dengue serotypes differ in their ability to produce severe illness but a causal association is undefined [29]. In many regions of endemicity, including the Indian subcontinent [30], multiple DENV serotypes circulate.

Similarly to dengue, human immunity to a Zika primary infection is notable for production of virus-specific IgG [31]. Unlike for DENV, however, there is only a single recognised ZIKV serotype. After secondary exposure to homologous infection a person mounts an effective anamnestic response. Zika was a neglected tropical disease before its global emergence, so details of host immunity to infection remain sketchy. However, consequent to the extensive investment of resources into Zika vaccine development in the last year [32], the knowledge gap in regard to immunity and immunopathogenesis is narrowing appreciably [33].

## Vaccine Development

Since efficacious vaccines exist against the fellow flaviviruses that cause yellow fever and Japanese encephalitis, a similar therapeutic is anticipated for both dengue and Zika [34]. The challenge for dengue vaccine design is to attain multi-serotype immunity but not provoke associated pathology [35,36]. Tetravalent vaccines that aim for balanced immunity to DENV 1-4 are undergoing field trials [37]. However, if post-vaccination IgG decreases, ADE may become a problem. Thus, recurrent infection is the leading risk factor for severe dengue. Babies immunized passively via maternal antibodies from a dengue pre-immune mother are at high risk of severe dengue [28,36]. Encouragingly, the latest clinical vaccine trial, that of a recombinant, live attenuated, tetravalent DENV 1-4 chimera scaffolded on a yellow fever 17D backbone, was completely effective, albeit in a small-scale volunteer challenge study of only the DENV-2 serotype performed under tightly controlled conditions [38]. This has led just recently to the first licensure for commercial use of any dengue vaccine (CYD-TDV; Dengvaxia®, Sanofi Pasteur) [39]. Yet, because of enduring concerns about ADE major challenges to vaccine effectiveness and long-term safety in the immunized population persist.

As a spin-off from the success of Dengvaxia® it is planned for a modified version of the live attenuated construct to be developed to combat ZIKV. This may expedite the generation of a candidate Zika vaccine for which there was no call before the current outbreak. However, as for all infectious diseases vaccine production is lengthy, arduous and costly [40]. This initiative has received considerable ring-fenced funds from global government and charitable organizations [32], but for a vaccine to reach the marketplace may take several years [41,42]. While conducting ethically approved tests of vaccine safety and efficacy in humans at all times requires due diligence and caution, any candidate vaccine administered to expectant mothers is subject to extremely rigorous evaluation [43,44]. This relates particularly to ZIKV since the gravest clinical manifestation, microcephaly, and affects pregnancy. It is against this background that vaccine developers, clinical trial managers and public health administrators

should take care not to add to the wealth of misinformation regarding Zika by implying, however guardedly, that a vaccine is within immediate reach [45,46].

## ADE of homologous infection

DENV and ZIKV share around 60% nucleotide identity [2]. As the two viruses are so closely related, there is significant homology in surface epitope antigenicity [3,47]. Through ADE anti-DENV IgG against one serotype can enhance further infectivity of other serotypes for monocytes and macrophages, triggering increased viraemia that correlates with severe dengue [28]. Likewise, *in vitro* ZIKV provokes ADE in response to sub-neutralizing concentrations of homologous antiserum, and, notably, in response to heterologous antisera to other flaviviruses, including DENV [31,48]. So far, however, *in vivo* ADE promoting severe disease has been reported only for dengue and heterologous DENV IgG [28,47]. Epidemiological studies and animal models are required to confirm the part played by ADE in the development of congenital and neurological complications linked to ZIKV infections.

## ADE of heterologous infection

The effect on ZIKV of broadly neutralizing human anti-DENV monoclonal antibodies and DENV immune sera was examined recently using neutralization and ADE assays [49,50]. Anti-DENV IgG cross-reacted, failed to neutralize, and heightened noticeably ZIKV infection *in vitro*. DENV immune sera showed differing capacities to neutralize ZIKV and similarly to elevate ZIKV infection. It is supposed that pre-existing immunity to DENV enhances ZIKV infection *in vivo* and may increase disease severity [47,50]. In a contemporaneous study, serum samples from dengue-immune pregnant women exacerbated ZIKV infection [51].

If anti-DENV antibodies augment ZIKV replication begs the question whether or not a ZIKV-induced IgG response enhances dengue infection. Well, this does appear to be true, perhaps unsurprisingly given the considerable degree of antigenic overlap of the related flaviviruses. To date, it has been shown that antibodies to ZIKV are able to enhance DENV-2 replication *in vitro* [52]. Moreover, mice exposed to Zika virus produced anti-ZIKV IgG that enhanced DENV replication. Polyclonal serum was strongly ZIKV-neutralizing but demonstrated a DENV-sub-neutralizing capacity and hence the potential to increase dengue severity [52].

## Non-neutralizing antibody cross-reactivity

In most countries in which ZIKV now exists DENV is already endemic [5,6], so the viruses co-circulate. Since they share primary vectors, *Ae. aegypti* and *Ae. albopictus*, conditions that support DENV transmission also promote ZIKV [4]. Hence, many patients who contract ZIKV will likely have been exposed before to one, if not more, DENV serotypes. Similarly, many individuals who are exposed to at least one of the DENV serotypes will, at present and in future, encounter ZIKV [53]. It is reasonable to anticipate that ZIKV will spread ultimately to all habitats of competent *Aedes* mosquitoes. While in these sites DENV may remain the predominant flavivirus, ZIKV may represent the primary flavivirus threat for a significant proportion of the community. Those persons will mount an IgG response to DENV and ZIKV, respectively, in the absence of a history of exposure to other flaviviruses.

## Conclusions

In locations where DENV and ZIKV co-circulate appreciating how primary infection with one virus affects susceptibility to

secondary exposure to the other underpins applications for disease therapy, prevention and control. A complication arises due to the intense circulation of DENV and ZIKV in endemic areas, when a person's immunity will be boosted naturally by multiple exposures to the same serotype, resulting in an altered antibody profile. Since anti-flavivirus IgGs are highly cross-reactive this may affect not only antibody specificity, such as cross-neutralization capacity, but also the range of susceptibility. The potential exists either to protect or, via ADE, to worsen infection. Elucidating the dynamics of these immune interactions will better enable the making of correct diagnoses, accurate clinical outcomes and improved vaccine design and delivery. At a time when the first DENV vaccine will soon be available to purchase [39], and a new ZIKV vaccine candidate shows promise [54], attention must be paid to the real risk for vaccination against one virus to enhance a natural infection with the other. The possible clinical consequences of such artificially induced ADE of infection are serious. In the context of community vaccination programs strong public engagement and clear communication will be key factors in increasing knowledge of dengue and Zika and in addressing concerns over prevention versus susceptibility to disease [55].

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