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Antigenic cross-reactivity between Zika and dengue viruses: is it time to develop a universal vaccine?

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Zika and the four serotypes of dengue are closely related flaviviruses that share a high degree of structural and sequence homology and co-circulate in many regions of the world. Here, we review recent studies investigating antigenic cross-reactivity between the two viruses. We discuss the pathogenic and protective roles of cross-reactive anti-viral antibody and T cell responses, respectively, in modulating the outcome of secondary dengue or Zika infection. Based on recent findings and increased incidence of severe disease in seronegative recipients of the first dengue vaccine to be licensed, we propose that the time has come to focus on developing pan-flavivirus vaccines that protect against Zika and four dengue serotypes by eliciting protective cross-reactive T cell responses while concomitantly reducing production of cross-reactive antibodies that can exacerbate disease.

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Introduction

Zika virus (a single serotype, ZIKV) and dengue virus (four serotypes, DENV1–4) are mosquito-transmitted members of the Flaviviridae family, *Flavivirus* genus that includes West Nile, Japanese encephalitis, and yellow fever viruses. Flaviviruses contain a positive-sense single-stranded RNA genome of ~11 kb that encodes three structural proteins (capsid [C], premembrane [prM], and envelope [E]) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). ZIKV was first isolated from a sentinel rhesus monkey in Uganda in 1947, but few human infections were recorded until 2013–2014, when a large outbreak occurred in French Polynesia that affected about 28 000 patients [1]. ZIKV then spread to South America during 2014–2016 [2], and it had been reported in at least 84 countries and territories worldwide by 2017 [3]. In comparison, hundreds of millions of cases of DENV infection have been recorded annually worldwide since it was first described in Egypt and Indonesia in 1779 [4]. At present, an estimated 390 million DENV infections occur globally each year, and the virus is endemic in at least 139 countries [5]. DENV thus continues to present a significant global health problem.

While DENV and ZIKV are both spread by mosquito bites, ZIKV can also be transmitted vertically (mother-to-fetus) and by sexual contact [6]. Most ZIKV and DENV infections are asymptomatic, but both viruses can cause mild disease, characterized by fever, rash, and muscle and joint pain [6], as well as more severe life-threatening disease. ZIKV infection during pregnancy is linked to serious fetal defects, termed congenital Zika syndrome [7], and in adults, viral infection is associated with autoimmune disorders such as Guillain–Barré syndrome and immune thrombocytopenic purpura [8]. In the case of DENV infection, the most serious clinical manifestation is 'severe dengue' (formerly known as dengue hemorrhagic fever/dengue shock syndrome), the hallmark of which is plasma leakage that may progress to hypovolemic shock and death [9].

Antigenic cross-reactivity between ZIKV and DENV

DENV exists as four serotypes that differ substantially at the protein level and are antigenically distinct. Since immunity to one serotype does not fully protect against infection with a different serotype, the patient remains vulnerable to heterotypic reinfections. Paradoxically, pre-existing DENV immunity is actually the single greatest risk factor for developing severe dengue (see below) [9]. ZIKV, which exists as a single serotype [10], also shares high protein sequence homology with DENV. Thus, the emergence of ZIKV in DENV-endemic regions has raised the critical question of how the interplay between ZIKV and DENV immunity influences the clinical outcomes of sequential infection with heterologous viruses/serotypes. The principal target of the antibody (Ab) response to DENV and ZIKV is the structural E protein, which is 60–75% identical among the four DENV serotypes and 54–59% identical between ZIKV and the four DENV

serotypes at the amino acid level [9,11]. In contrast, the T cell response to DENV and ZIKV is targeted mainly to nonstructural proteins, many of which are more conserved than structural proteins among flaviviruses (Table 1). In humans and in mouse models, $CD8^+$ T cell responses to DENV are directed mainly to NS3, NS4B, and NS5 [12–15], whereas the $CD4^+$ T cell response targets NS3, NS5, and the structural C protein [16–21]. In comparison, $CD4^+$ and $CD8^+$ T cell responses to ZIKV appear to target both structural proteins (E, C, and prM) and nonstructural proteins (NS1, NS2A, NS3, NS4B, and NS5) [22,23•,24,25,26•].

Collectively, these observations have prompted the crucial question: how does pre-existing DENV immunity modulate the immune response to and clinical outcome of a subsequent ZIKV infection, and *vice versa*? As illustrated by the following discussion, this is currently an area of intense investigation.

Pre-existing cross-reactive Abs to DENV enhance DENV and ZIKV pathogenesis

Epidemiological studies have long established that secondary DENV infection (in children or adults) and waning levels of maternal Abs in infants with primary DENV infection are risk factors for the development of severe dengue disease [27]. Ab-dependent enhancement of infection (ADE) is one of the two major hypotheses proposed to explain these epidemiologic observations. ADE occurs when a patient with pre-existing serotype-cross-reactive nonneutralizing or subneutralizing Abs is reinfected with DENV. Binding of the pre-existing Abs to the virus facilitates its infection of Fcγ receptor-bearing cells, and the increased viral burden then triggers severe dengue disease manifestations [9]. Direct evidence for the ADE hypothesis of DENV pathogenesis was obtained in 2010, almost half a century after it was first proposed, when a mouse model of ADE-mediated severe dengue disease was developed [28,29]. In recent years, several human studies have provided additional compelling evidence in support of the ADE hypothesis. A long-term pediatric cohort study in Nicaragua confirmed that ADE occurred in patients with a low titer of pre-existing anti-DENV Abs (defined by inhibition ELISA), whereas patients with high Ab titers were protected against severe dengue [30••]. Similarly, a school-based cohort study in Thailand identified a specific low titer of anti-DENV Abs (defined by hemagglutination inhibition or plaque reduction and neutralization assays) that correlated with severe dengue disease manifestations [31]. Consistent with these recent human studies and earlier epidemiological data, clinical trials of the first approved DENV vaccine Dengvaxia®, which includes DENV structural proteins but yellow fever virus (YFV) nonstructural proteins, revealed a higher incidence of severe dengue among vaccinated DENV-naïve compared with unvaccinated DENV-naïve individuals during subsequent primary DENV infection [32••], suggesting that the vaccine primed the naïve individuals for development of severe dengue disease. These recent findings have solidified ADE as a well-accepted hypothesis to explain DENV pathogenesis during primary infection of infants with maternally acquired anti-DENV Abs or secondary infection of older children and adults with heterotypic DENV.

Since the 2014–2016 ZIKV outbreak in DENV-endemic areas of the Americas, many groups have investigated whether ZIKV infection in DENV-immune individuals may exacerbate disease, analogous to the effects of secondary infection with heterotypic DENV. Within a short timeframe, several groups reported that the anti-DENV Ab response cross-reacted with ZIKV, and *vice versa* [33–37], underscoring the need for studying the consequences of ZIKV emergence in DENV-endemic countries. DENV-elicited cross-reactive Abs were found to both neutralize [36–38] and enhance [35,39,40•] ZIKV infection. E-dimer epitope (EDE)-targeting monoclonal Abs, originally isolated from DENV-infected individuals and shown to have potent neutralizing activity against DENV1–4, also effectively controlled ZIKV infection and prevented ZIKV pathogenesis in rhesus macaques [41] and in mice lacking one or more components of the interferon (IFN) system, which are highly susceptible to viral infection [36,42]. However, plasma isolated from convalescent DENV patients was able to either protect against or enhance ZIKV infection and increase disease severity in *Stat2*$^{-/-}$ mice depending on the Ab concentration [40•]. *In vitro*,

Table 1

Amino acid homology between DENV and ZIKV proteins

	Identity versus ZIKV[a]									
	C	prM/M	E	NS1	NS2A	NS2B	NS3	NS4A	NS4B	NS5
ZIKV	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
DENV1	43%	45%	58%	53%	18%	36%	66%	42%	56%	67%
DENV2	40%	42%	54%	52%	29%	43%	67%	47%	57%	68%
DENV3	40%	43%	59%	55%	29%	42%	67%	40%	58%	68%
DENV4	33%	48%	57%	53%	18%	41%	67%	39%	56%	69%

[a] The percent identities were determined using BLAST search.

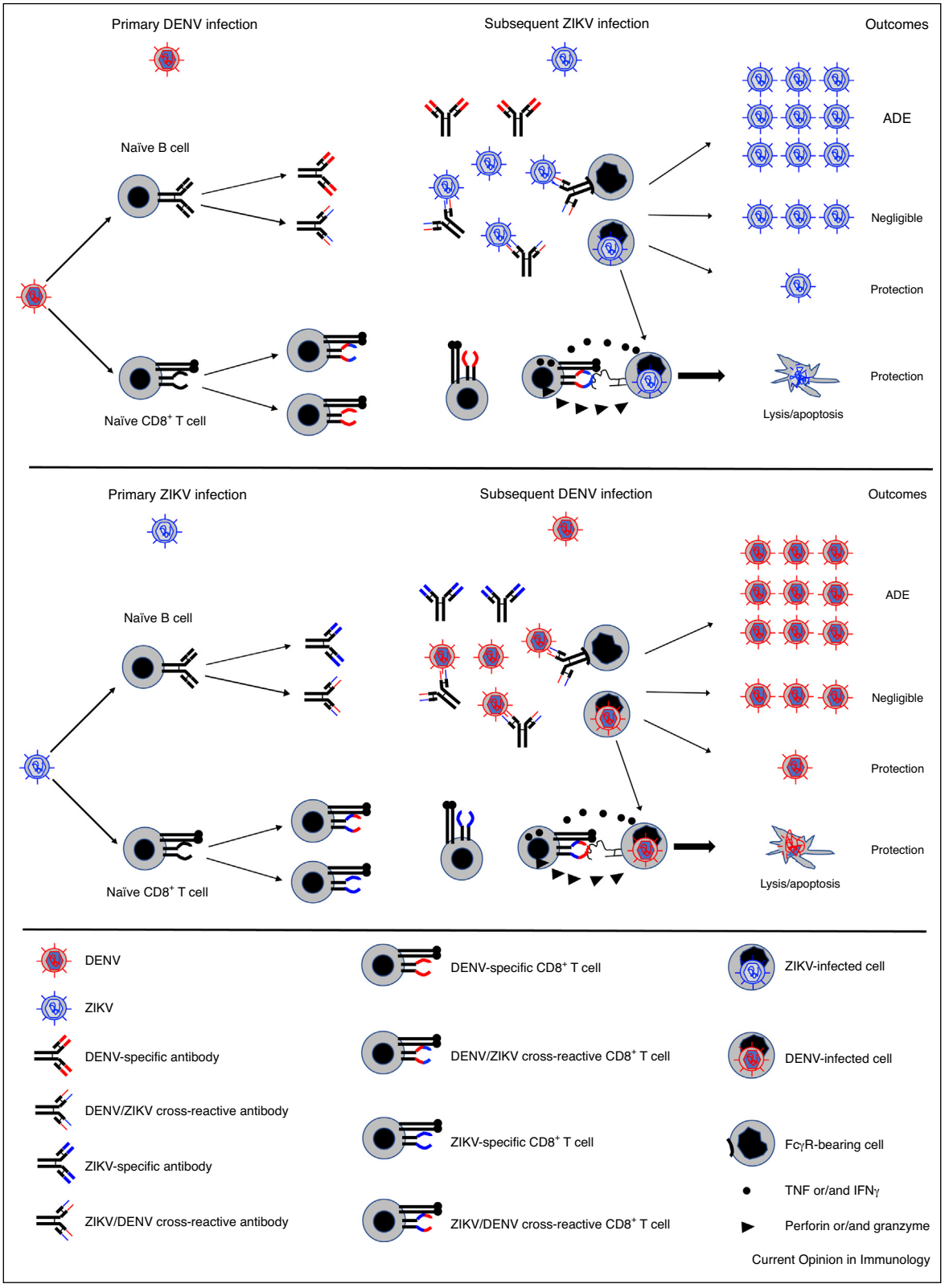
monoclonal Abs isolated from DENV-infected patients increased ZIKV infection of human placental macrophages and explants [43]. In contrast to these observations, maternally derived anti-DENV Abs showed little to no protective effect against ZIKV infection in *LysMCre*$^{+}$*Ifnar1*$^{fl/fl}$ mice, which lack the type I IFN receptor on myeloid cells [44]. Similarly, other studies have shown that pre-existing DENV immunity of 1–3 years duration had no effect on ZIKV infection in macaques [45,46] and that the cross-neutralizing capacity of the human anti-DENV Ab response became less potent over time [47,48]. Collectively, the studies in humans and mouse models demonstrate that pre-existing DENV Abs can (i) contribute to cross-protection, (ii) mediate ADE, or (iii) have little to no effect during subsequent ZIKV infection (Figure 1), which is analogous to the scenario during secondary infection with heterotypic DENV. Future epidemiologic studies will be crucial in determining whether pre-existing DENV Abs in humans protect against or enhance ZIKV pathogenesis. If evidence continues to mount that pre-existing DENV Abs increase ZIKV disease severity via ADE, a pan-flavivirus vaccine targeting both DENV and ZIKV will be required. Since there is clear evidence that anti-DENV Abs can cross-protect against ZIKV infection, it seems likely that a universal vaccine could be designed to simultaneously protect against ZIKV and all four DENV serotypes via induction of cross-neutralizing Abs. However, it will first be necessary to understand the precise qualitative and quantitative features of the pre-existing DENV Ab response that dictate whether it has a cross-protective, enhancing, or neutral effect during ZIKV infection.

Pre-existing cross-reactive T cell immunity to DENV protects against DENV and ZIKV infection

In addition to Abs, T cells may also contribute to the pathogenesis of severe dengue upon secondary infection of older children and adults. Early studies demonstrated that patients with severe dengue could mount only a weak cross-reactive CD8^{+} T cell response against the infecting DENV heterotype, comprise inefficient, low-affinity cells that expressed high levels of cytokines such as IFNγ and TNF, low levels of the cytotoxic degranulation marker CD107a, and were prone to apoptosis [49,50]. However, more recent studies with both humans and mouse models suggest that DENV serotype cross-reactive T cells can protect against DENV infection and severe disease [12,13,51–54]. Comprehensive studies in two DENV-endemic countries, Sri Lanka and Nicaragua, have revealed that the magnitude and breadth of the epitope-specific CD8^{+} T cell response to DENV is HLA-linked and positively correlates with resistance to severe dengue [12,13]. Similarly, the magnitude and functional capacity of epitope-specific cytotoxic CD4^{+} T cells are also associated with HLA alleles linked to resistance to severe dengue [52]. Studies in *Ifnar1*$^{-/-}$ mice support these results and provide direct evidence that DENV serotype cross-reactive CD8^{+} T cells can protect against heterotypic DENV reinfection [55,56]. In fact, in a short-term model, CD8^{+} T cells could protect against severe dengue even in the presence of subneutralizing levels of cross-reactive Abs [56], suggesting that interplay between the cross-reactive Ab and T cell responses may determine the outcome of subsequent infection. Mouse models in which different vaccination strategies and maternal Ab-mediated ADE were investigated also demonstrated that CD8^{+} T cells could prevent ADE and reduce DENV pathogenesis [57–59]. Consistent with these findings, the unequal protection and enhancement problems of Dengvaxia® may be due to the lack of DENV-specific CD8^{+} T cell responses, as this vaccine contains YFV but not DENV nonstructural proteins [60,61]. Taken together, these recent studies in humans and mouse models indicate that a DENV vaccine should ideally elicit serotype-cross-protective T cells, particularly CD8^{+} T cells, to abrogate ADE resulting from suboptimal or waning Ab responses induced by the vaccine (Figure 2).

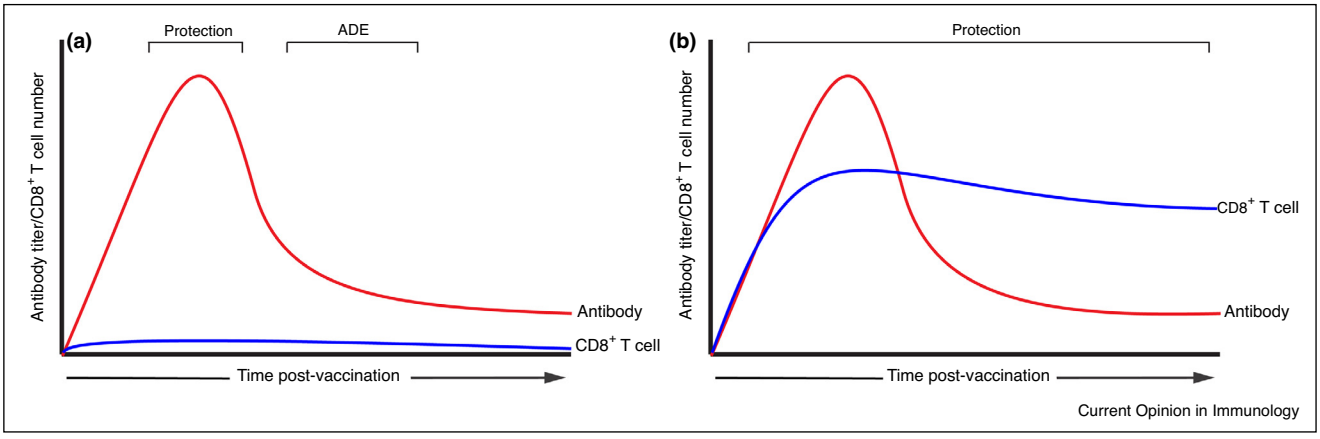
The recent emergence of ZIKV in DENV-endemic regions has prompted investigators to address whether the outcome of secondary infection with ZIKV is also influenced by pre-existing T cell immunity to DENV. ZIKV epitopes, particularly those derived from the conserved nonstructural proteins such as NS3, are recognized by human CD4^{+} and CD8^{+} T cells elicited by prior DENV infection or vaccination [62•,63–65]. In fact, ZIKV-cross-reactive T cells from DENV-immune individuals expressed higher levels of activation markers, such as granzyme B and PD-1, and exhibited a more vigorous response compared with T cells from DENV-naïve individuals [62•]. In agreement with this observation in humans, DENV-immune HLA transgenic mice displayed a more robust and earlier cross-reactive CD8^{+} T cell response to ZIKV infection than did naïve transgenic mice [23•]. This study is of particular importance because it showed that immunization with DENV epitopes that are relevant to the response in humans could protect against ZIKV infection, suggesting that similar vaccination strategies to elicit cross-protective CD8^{+} T cell responses might be beneficial in humans. In support of this, mouse studies modeling sequential DENV and ZIKV infection demonstrated CD8^{+} T cell-mediated short-term cross-protection against ZIKV in both non-pregnant and pregnant mice [66••,67]. Taken together, these studies firmly establish that pre-existing DENV-primed CD8^{+} T cells provide at least transient cross-protection against subsequent infection with either ZIKV or heterotypic DENV (Figure 1). The transient nature of cross-protection mediated by DENV-primed CD8^{+} T cells against ZIKV is consistent with the short duration of cross-protection, ranging from one to two weeks to three years, that is observed during secondary DENV infections in humans [9]. Although further studies are

Figure 1



The proposed roles of the pre-existing cross-reactive immunity to DENV during subsequent ZIKV infection, and *vice versa*. Cross-reactive Abs mediate neutralizing, enhancing or negligible effects during subsequent infection with heterologous virus. Cross-reactive $CD8^+$ T cells provide protection against subsequent infection with heterologous virus.

Figure 2



The characteristics of an antibody-centric DENV or ZIKV vaccine and a pan-flavivirus vaccine that elicits both Ab and $CD8^+$ T cell responses. **(a)** Ab-centric DENV or ZIKV vaccines induce strong antibody but weak or negligible $CD8^+$ T cell responses. **(b)** The proposed pan-flavivirus vaccines induce both Ab and $CD8^+$ T cell responses.

needed to determine whether DENV-primed $CD8^+$ T cells also mediate cross-protection against ZIKV and heterotypic DENV in humans, these recent mouse studies suggest that a universal vaccine could be designed to elicit cross-protective $CD8^+$ T cell responses against ZIKV and the four DENV serotypes.

Pre-existing cross-reactive Abs to ZIKV enhance DENV pathogenesis

Several vaccine candidates against ZIKV are currently in development, and their planned deployment in DENV-endemic countries underscores the urgent need to understand how pre-existing immunity to ZIKV affects the outcome of subsequent DENV infection. Several studies have already shed light on the interplay between ZIKV immunity and DENV pathogenesis. In AG129 ($Ifnar^{-/-}$) mice, injection of monoclonal Abs isolated from ZIKV-infected patients or vaccination with inactivated ZIKV both enhanced disease severity following DENV infection [34]. Similarly, $LysMCre^{+}Ifnar1^{fl/fl}$ pups with maternally acquired anti-ZIKV Abs, but not pups born to naïve mothers developed severe dengue disease [44]. These findings are in line with a report that prior ZIKV infection significantly enhanced peak DENV viremia in macaques [68]. Collectively, these studies indicate that pre-existing ZIKV Abs can enhance DENV pathogenesis (Figure 1). The precise qualities of the pre-existing anti-ZIKV Ab response that induce ADE during subsequent DENV infection should be defined to ensure the design of ZIKV vaccines that avoid ADE-induce severe dengue. One potential strategy is to engineer mutations in the E protein, as suggested by a mouse study in which a ZIKV vaccine containing fusion loop mutations in the E protein reduced cross-reactive Ab production and circumvented ADE-mediated disease development upon DENV infection [69]. Clearly, ongoing and future clinical trials of ZIKV vaccine candidates in DENV-endemic countries should be carefully monitored for vaccine-induced enhancement of dengue disease severity.

Pre-existing cross-reactive T cell immunity to ZIKV protects against DENV infection

In contrast to the multiple studies demonstrating the pathogenic role of pre-existing cross-reactive ZIKV Abs during DENV infection, relatively few studies have examined the role of pre-existing T cell immunity during DENV infections. Two mouse studies reported that ZIKV-elicited $CD8^+$ T cells were cross-reactive with DENV, but in only one of these studies, the investigators evaluated the role of this response, which conferred cross-protection against DENV infection [66••,70•]. Nevertheless, these findings, in combination with the studies described above, do suggest that $CD8^+$ T cell responses to ZIKV and DENV are mutually cross-protective (Figure 1). Although further research will be necessary to validate these findings in humans and to evaluate the precise features (e.g. magnitude, specificity, phenotype, and location) of the cross-protective $CD8^+$ T cell response in animal models and humans, the studies to date support the feasibility of a pan-flavivirus vaccine to elicit cross-protective $CD8^+$ T cell responses against both viruses.

Concluding remarks and future perspectives

Bearing in mind that DENV and ZIKV co-circulate in a number of countries, the need to understand the protective versus pathogenic impact of pre-existing DENV immunity on subsequent ZIKV infection, and *vice versa*, is made more urgent by the fact that a recently introduced DENV vaccine is suboptimal/inefficient and that

ZIKV vaccines are under rapid development. Emerging research suggests that DENV-elicited or ZIKV-elicited cross-reactive humoral immunity contributes to the pathogenesis of the reciprocal virus, whereas cross-reactive T cell immunity, particularly that mediated by $CD8^+$ T cells, is mutually protective. Studies are now needed to (i) determine whether cross-reactive $CD4^+$ T cells also contribute to protection and (ii) define the precise attributes of the cross-protective versus pathogenic immune responses during ZIKV and DENV infections. Studies in mouse models suggest that DENV or ZIKV vaccines that elicit cross-reactive Abs alone may be more dangerous than those inducing both cross-reactive Ab and $CD8^+$ T cell responses. Therefore, the ideal universal vaccine may be one that elicits robust long-lasting protection against both viruses via a combination of Ab and $CD8^+$ T cell responses. This would not only avoid ADE mediated by an inefficient or waning vaccine-induced cross-reactive Ab response but also harness the protective capacity of cross-reactive $CD8^+$ T cell responses. Importantly, a universal vaccine would also be desirable to provide cost-effective protection for resource-poor countries.

Conflict of interest statement

Nothing declared.

Author contributions

JW and SS wrote the review.

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- • of special interest
- •• of outstanding interest

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