ERRATUM POSTED 16 AUGUST 2023

INFECTIOUS DISEASES

Insights into dengue immunity from vaccine trials

Eng Eong Ooi[1,2,3]* and Shirin Kalimuddin[1,4]

The quest for an effective dengue vaccine has culminated in two approved vaccines and another that has completed phase 3 clinical trials. However, shortcomings exist in each, suggesting that the knowledge on dengue immunity used to develop these vaccines was incomplete. Vaccine trial findings could refine our understanding of dengue immunity, because these are experimentally derived, placebo-controlled data. Results from these trials suggest that neutralizing antibody titers alone are insufficient to inform protection against symptomatic infection, implicating a role for cellular immunity in protection. These findings have relevance for both future dengue vaccine development and application of current vaccines for maximal public health benefit.

INTRODUCTION

Dengue is an acute viral disease that is prevalent throughout the tropical world. It afflicts an estimated 100 million people annually (*1*). This disease is caused by infection with any one of four different dengue viruses (DENV-1 to DENV-4), which are principally but not exclusively transmitted by the mosquito *Aedes aegypti* (*2*). This mosquito is highly adapted to the urban environment, where human population density is often high. This proximity to humans and exclusivity for human blood meals renders it a highly efficient vector for DENV. As the geographic footprint of *A. aegypti* expands from the tropics to the subtropics, the global burden of dengue is projected to worsen in the coming decades (*3*). This burden is likely underappreciated, because besides acute illness, a proportion of patients with dengue experience post-acute long-term sequelae such as fatigue, poor memory, and impaired concentration (*4*–*6*). Reducing the global burden of dengue is, thus, an urgent public health priority.

A. aegypti population suppression has, until now, been the only way to prevent dengue at a population level. This approach has been remarkably successful in the Pan American *Aedes* eradication program, as well as the national vector control programs of Cuba and Singapore (*7*). However, sustaining these successes has proven challenging, not solely because of high costs involved. In Singapore, long-term reduction in DENV transmission resulted in a population with low herd immunity that is now more susceptible to epidemics, despite sustained low-vector population density (*7*). Therefore, elevating and maintaining population-level immunity through vaccination has to be a cornerstone of any successful dengue prevention program.

The endeavor to develop a dengue vaccine started with the foundational work of A. Sabin (*8*–*10*). A major challenge in the development of a safe and efficacious vaccine is the complexity of dengue pathogenesis. A lasting adaptive immune response that develops after one DENV infection is specific to that DENV; protection against the remaining three heterologous DENVs is transient (*9*, *10*). Moreover, antibodies that develop after one DENV infection can enhance a second heterologous DENV infection to increase the risk of severe dengue (*11*). The biology of such antibody-dependent enhancement (ADE) of DENV infection will be expanded upon in the later section. To fully protect against all four DENVs and prevent the risk of ADE, vaccine development efforts have focused on selecting vaccine candidates that elicit a balanced neutralizing antibody response against all four DENV types (*12*–*14*). After more than half a century since the work of Sabin, we now have two approved live-attenuated tetravalent dengue vaccines; a third vaccine has completed phase 3 clinical trials.

Although dengue is now technically a vaccine-preventable disease, there is still room for additional vaccines that elicit fewer side effects while providing even better adaptive immunity against dengue. This Review, however, is not focused on the different vaccine platforms that have been used or can be used to develop dengue vaccine candidates. It is also not meant to provide a head-to-head comparison of the current vaccines for adoption by public health agencies of dengue-endemic countries. The aim of this Review is to glean a more nuanced understanding of dengue immunity from the vaccine efficacy outcomes of the completed phase 3 clinical trials; refining our knowledge of dengue immunology could further firm the foundation for development of next-generation dengue vaccines.

DENGUE VACCINES

The developmental history of dengue vaccines (*15*–*17*) and the different constructs of those that have completed phase 3 clinical trials have been reviewed elsewhere (*18*). In summary, the first approved dengue vaccine, CYD-TDV, is a chimeric vaccine based on a yellow fever virus 17D (YF17D) backbone in which the pre-membrane (PrM) and envelope (E) genes of YF17D have been replaced by those of DENVs (Fig. 1) (*18*). Its use, however, is limited to those with prior DENV infection because of an increased risk of severe dengue when the vaccine is given to those without prior DENV infection, possibly due to ADE (*19*). The second vaccine, TAK003, uses an attenuated DENV-2 backbone with the PrM and E genes replaced with those from wild-type DENV-1, DENV-3, and DENV-4 (Fig. 1) (*18*). This vaccine recently received approval from the European Union for use in those 4 years old and above, even in those without prior DENV infection. A third vaccine, TV003, recently completed phase 3 clinical trials with promising results. This tetravalent vaccine is constructed by deleting 30

[1]Program in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore 169857, Singapore. [2]Viral Research and Experimental Medicine Centre, SingHealth Duke-NUS Academic Medical Centre, Singapore 169857, Singapore. [3]Saw Swee Hock School of Public Health, National University of Singapore, Singapore 117549, Singapore. [4]Department of Infectious Diseases, Singapore General Hospital, Singapore 169856, Singapore.
*Corresponding author. Email: engeong.ooi@duke-nus.edu.sg

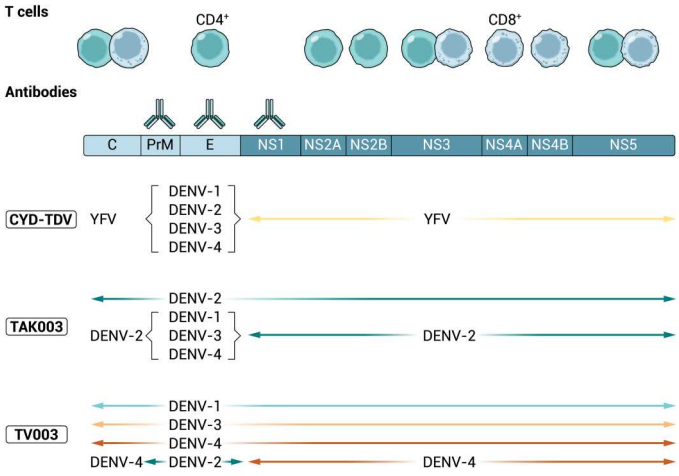

Fig. 1. Immunodominant proteins of dengue virus (DENV) and molecular constructs of three live-attenuated tetravalent dengue vaccines. DENV is a single-stranded positive-sense RNA virus comprising three structural [capsid (C), premembrane (PrM), and envelope (E)] and seven nonstructural (NS1 to NS5) proteins. The antibody response to DENV is directed primarily against the PrM, E, and NS1 proteins. The DENV-specific $CD4^+$ T cell response mainly targets the C protein, followed by E, NS3, NS2A/B, and NS5, whereas the $CD8^+$ T cell response is mainly targeted to NS3, followed by C, NS5, and NS4A/B. CYD-TDV is based on an attenuated yellow fever (YFV) backbone with the PrM and E genes replaced by those of each of four DENV serotypes. TAK003 uses an attenuated DENV-2 backbone with the PrM and E genes replaced with those from wild-type DENV-1, DENV-3, and DENV-4 strains. TV003 is constructed by deleting 30 nucleotides in the 3′ untranslated region of DENV-4 and DENV-1 (rDEN4Δ30 and rDEN1Δ30), as well as 30 and 31 nucleotides from DENV-3 (rDEN4Δ30/31). The DENV-2 component was constructed by splicing the DENV-2 PrM and E genes into the rDEN4Δ30 backbone.

nucleotides in the 3′ untranslated region of DENV-4 and DENV-1 (rDEN4Δ30 and rDEN1Δ30), as well as 30 and 31 nucleotides from DENV-3 (rDEN4Δ30/31). The DENV-2 component was constructed by splicing the DENV-2 PrM and E genes into the rDEN4Δ30 backbone (Fig. 1) (*18*).

Because the above vaccines are live, albeit attenuated, and tetravalent, vaccination should ideally cause four simultaneous DENV infections that drive B cell responses to the E protein of all four DENVs (Fig. 1) (*20*); splicing of PrM genes into the chimeric constructs was necessary to ensure proper E protein folding and virion maturation. High binding affinity and type-specific neutralizing antibodies are also more likely to develop with viremic infection. Longer duration of vaccine viremia is a direct correlate of neutralizing antibody titers to the related yellow fever vaccine, arguably the most potent vaccine available in the world (*21*). Consequently, because CYD-TDV vaccination produces mostly chimeric YF17D–DENV-4 viremia (*22*), CYD-TDV vaccination produces mostly DENV-4–specific neutralizing antibodies; neutralization of DENV-1 to DENV-3 is mostly due to cross-neutralizing antibodies (*23*). Similarly, TAK003 vaccination produces detectable DENV-2 viremia solely (*24*), resulting in the corresponding enrichment of DENV-2–specific neutralizing antibodies (*25*).

Viremia with all four DENV components can be produced from TV003 (*26*). Correspondingly, neutralizing antibodies specific for each of the four DENVs are produced after TV003 vaccination (*27*). However, in the formulation of TV003 that progressed to phase 3 clinical trial, also known as Butantan DV, vaccine viremia was detected at maximum to only two of the four DENV components in each participant (*28*). Remarkably, a phase 1 clinical trial of a proprietary formulation of TV003 by MSD (Merck in United States), known as V181, produced tetravalent viremia in most vaccinated individuals (*29*). Comparison on the outcome of future vaccine efficacy studies on V181 with those observed for Butantan DV could shed further light on how vaccine viremia shapes dengue immunity. Nonetheless, this Review will focus on the dengue immunology lessons that can be drawn from the Butantan DV formulation of TV003, because it has completed a phase 3 clinical trial.

Besides neutralizing antibodies, vaccination also produces other adaptive immune responses. Non-neutralizing antibodies can trigger protective Fc effector functions, such as complement activation and antibody-dependent cellular cytotoxicity (antibody-dependent phagocytosis will be covered in more detail in a later section) (*30*). Antibodies against the nonstructural 1 (NS1) protein, whose role in dengue pathogenesis is discussed in greater detail in a later section, are also generated from vaccination; however, these antibodies would be directed against the virus that was used as the genetic backbone of the vaccine (Fig. 1). Similarly, although $CD4^+$ T cell epitopes have been found in the E protein, most of the T cell epitopes, and especially the $CD8^+$ T cell epitopes, are located in the capsid (C) and NS proteins (Fig. 1) (*31*). Most of the vaccine-induced cellular immune response, therefore, would be directed against the NS proteins of the virus that was used as the genetic backbone and therefore may not be balanced across all four DENVs.

Although these vaccines have the potential to reduce the overall dengue burden, observations from the phase 3 clinical trials, as well as long-term follow-up of trial participants, have shown that they are not perfect (*19*, *32*–*37*). Vaccine efficacy against symptomatic DENV infection varies between each of the four types of DENV. Moreover, all three vaccines show greater efficacy in those with prior DENV infection (seropositive at baseline) than those who have not been infected with at least one type of DENV (seronegative at baseline). A summary of the efficacy end points of the clinical trials for CYD-TDV, TAK003, and TV003 is shown in Table 1. Questions remain about the durability of protection and the need for booster doses. The extent to which waning immunity would affect dengue pathogenesis, and by the type of DENV, remains uncertain.

CHALLENGES IN UNDERSTANDING DENGUE IMMUNITY

Knowledge of dengue immunity has had to rely on descriptive data from epidemiological observations. The quality of such data is dependent on the sensitivity of the study or surveillance methods used to detect and type dengue cases. Given the wide spectrum of clinical presentation, which could be modified by factors such as the DENV strain (*39*–*43*), ethnicity (*44*), and age (*45*), coupled with the difficulty of obtaining human samples at consistent time points during infection, it is thus unsurprising that there is considerable heterogeneity in the data. This heterogeneity hinders the identification of immune correlates of protection for dengue (*46*).

Besides the difficulty in deriving clear immune correlates of protection from epidemiological studies, the understanding of dengue immunity is also hampered by the lack of an animal model that closely recapitulates DENV infection and disease pathogenesis in

Table 1. Efficacy in protection against virologically confirmed dengue (VCD) and hospitalized VCD of the three different dengue vaccines. The time interval from completion of vaccination to measurement of efficacy differs; this table is provided for easy reference to the primary and secondary outcomes of the trials and is not intended to compare between the trials. ND, not determined due to study design; NA, not available due to either insufficient events or data that have yet to be reported.

	CYD-TDV (CYD14) (*32*)	CYD-TDV (CYD15) (*33*)	TAK003 (*35*, *36*)	TV003 (*38*)
Primary end point*:				
VCD (%)	56.5	60.8	80.2	79.6
Other efficacy end points:				
Hospitalized VCD (%)	67.2	80.3	90.4†	NA
VCD by DENV type				
DENV-1 (%)	50.0	50.3	73.7	89.5
DENV-2 (%)	35.0‡	42.3	97.7	69.6
DENV-3 (%)	78.4	74.0	62.6	NA
DENV-4 (%)	75.3	77.7	63.2‡	NA
VCD by baseline serostatus and DENV type				
Negative (overall) (%)	35.5‡	43.2‡	66.2	73.5
DENV-1 (%)	ND	ND	67.8	85.5
DENV-2 (%)	ND	ND	98.1	57.9
DENV-3 (%)	ND	ND	−68.2‡	NA
DENV-4 (%)	ND	ND	NA	NA
Positive (overall) (%)	74.3	83.7	76.1	89.2
DENV-1 (%)	ND	ND	72.0	96.8
DENV-2 (%)	ND	ND	93.7	83.6
DENV-3 (%)	ND	ND	61.8	NA
DENV-4 (%)	ND	ND	61.2‡	NA

*Efficacy of CYD-TDV was determined over a 1-year period starting at 1 month after dose 3. Efficacy of TAK003 was determined over a 1-year period after dose 2. Efficacy of TV003 was determined over a 2-year period after a single dose. †VE for hospitalized dengue was determined at 18 months after the second dose of the vaccine. ‡Not statistically significant.

humans. Mouse models that produce disease are mostly immunocompromised, particularly through knockout of genes in the interferon (IFN) signaling pathway, which is a critical part of the immune response to DENV infection in humans (*47*). Conclusions from mouse studies thus cannot be directly extrapolated to dengue in humans (*48*). Nonhuman primates (NHPs) can be infected with DENV (*49*, *50*), although most do not develop a dengue-like disease despite detectable viremia (*51*). Thus, the approximation of dengue immunity studies in animals to human immunity is uncertain.

An approach to attaining experimental and appropriately controlled data is to apply a controlled human infection model (CHIM) (*52*). The work of dengue vaccine development by Sabin relied on such experimental infection studies in human volunteers (*9*, *10*). Although conducing such challenge studies in a similar manner would now rightly be ethically unsound, it is noteworthy that Sabin used unpassaged, wild-type DENVs without observing severe dengue in any of the infected volunteers. More recent approaches in human infection studies have used partially attenuated DENVs for a greater safety margin (*53*, *54*). The resultant clinical phenotypes of such challenge infection have ranged from afebrile viral rash (*55*) to mild dengue-like, self-limiting acute illness (*56*). However, the use of partially or naturally attenuated DENV strains may underestimate the magnitude of immunity needed to protect against symptomatic infection from wild-type DENVs. For instance, TV003 vaccination in individuals not previously exposed to DENV infection conferred 100% protection against viremic outcome when challenged with the partially attenuated DENV-2 (rDEN2Δ30 Tonga/74) strain (*55*). However, rDEN2Δ30 Tonga/74 infection produced a maximum viremia of only 2.9 $\log_{10}$ plaque-forming units (PFU)/ml (*55*). Another human infection model that elicited a self-limiting febrile illness used a partially attenuated DENV-1 strain that produced peak viremia ranging from 4.1 to 4.8 $\log_{10}$ PFU/ml; the equivalent viral RNAemia in these individuals was 5.9 to 7.9 $\log_{10}$ RNA copies per ml (*56*). In contrast, RNAemia in patients with dengue is commonly in the range of 6 to 9 $\log_{10}$ viral RNA copies per ml at diagnosis (*57*). Although the inoculation dose and route of infection—subcutaneous injection versus intradermal inoculation through a mosquito bite—differ between experimental and naturally acquired DENV infection outcomes, the greater viral burden may necessitate more robust adaptive immune response from vaccination to prevent symptomatic outcome. The point estimate of efficacy of TV003 in preventing symptomatic infection in those seronegative at baseline in the phase 3 clinical trial, albeit measured at 2 years follow-up instead of a challenge infection at 6 months after vaccination, was 57.9% (*38*). Taken collectively, we submit that CHIM is an extremely useful approach to down-select vaccine candidates that are unlikely to achieve desirable degrees of efficacy from further costly clinical development, but it is not yet at a stage for us to accurately extrapolate true vaccine efficacy.

Although a detailed understanding of dengue immunity would have aided dengue vaccine development, findings from phase 3 clinical trials on dengue vaccines now provide a unique opportunity to further our understanding of dengue immunity. Clinical trials provide hypothesis-driven, placebo-controlled experimental data derived from large sample sizes; each of the four phase 3 trials of dengue vaccines has enrolled more than 15,000 participants. They are conducted in dengue-endemic regions where wild-type DENVs circulate through natural *Aedes* mosquito-human cycles. The trials have also universally used active case detection to determine vaccine efficacy; all febrile episodes were tested for DENV infection using the most sensitive diagnostic tool, reverse transcription polymerase chain reaction. Phase 3 trial findings (*32*, *33*, *35*, *36*) and long-term follow-up observations (*19*, *34*, *37*, *58*) are, thus, opportune for refining our knowledge of dengue immunity.

HUMORAL IMMUNITY

Dengue serology was developed and used to test convalescent serum samples for evidence of DENV infection in the late 1940s and 1950s (*59*–*61*). Experience gained from serological assays and studies on convalescent serum indicates that virus neutralization tests, such as plaque reduction neutralization test (PRNT) and focus reduction neutralization test, offer the greatest specificity to determine the history of primary DENV infection. Given that most secondary dengue cases are caused by infection with a DENV heterotypic to that which caused the primary infection (*11*), as well as experimental studies in NHPs (*62*, *63*), neutralizing antibodies are thought to be absolutely required for dengue immunity.

Neutralizing antibodies and PRNTs

A plaque reduction test for DENV neutralizing antibodies was first developed using cell monolayers grown in glass prescription bottles (*64*). This "macro" test used 0.4 ml of serum to react against a fixed inoculum of DENV starting at a serum dilution of 1:10. This method was eventually replaced by "micro" PRNT, which required a smaller volume of serum and cell monolayer grown in 12- or 24-well tissue culture plates (*65*–*67*). Because the "macro" test was used as a reference point for the development of "micro" PRNTs, most continued to use the same starting dilution of 1:10. Neutralization of 50% or more of the virus inoculum ($PRNT_{50}$) at the starting dilution of 1:10 was thus widely interpreted as a positive finding in convalescent serum.

Given the assumption that DENV infection produces lasting immunity to the DENV that caused the infection (*9*, *10*), a $PRNT_{50}$ titer of 10 or greater to the homotypic DENV in a convalescent patient with dengue was often assumed to be indicative of immunity. However, more recent studies suggest that there is no single protective neutralizing antibody threshold for the four DENVs. A cohort study suggested that individuals with less than a fourfold rise in neutralizing antibodies in convalescent serum compared with paired preinfection serum were vulnerable to reinfection with the same strain of DENV (*68*); detectable neutralizing antibody titers alone are, thus, insufficient to prevent even homotypic reinfection. In addition, it appears that the threshold of neutralizing antibody needed to protect against symptomatic infection is not consistent across all four DENVs. Post hoc analysis of the serological data from CYD-TDV phase 3 clinical trials indicated the need for higher neutralizing antibody titers to protect against symptomatic DENV-2 infection compared with the other three DENVs (*69*) (Table 2) (*69*); the difference in antibody titer is still striking even if comparison was limited to DENV-1 to DENV-3, where the antibodies are mostly not specific to each of these three DENVs (*23*). Likewise, a dengue cluster–based investigation found close contacts of cases who had a similar high probability of exposure to infectious mosquito bites because the cases were protected from DENV-1 and DENV-4 infection when their PRNT titers were 11 and 16, respectively; protection against DENV-2 infection, in contrast, required a PRNT titer of 323 (*70*). These findings collectively suggest that not only is there no singular threshold of neutralizing antibody titer for protection against all four DENVs but also that protection against DENV-2 requires a much higher neutralizing antibody titer than the other DENVs.

Stoichiometry of DENV neutralization

The four DENV virions share a common architecture: The inner nucleocapsid is surrounded by viral E and membrane (M) proteins displayed on an endoplasmic reticulum–derived lipid membrane. Cryo–electron microscopy images reveal a total of 180 E and 180 M proteins that decorate the outer layer of the lipid membrane and are arranged into 60 asymmetrical icosahedral units (*71*, *72*). DENV is neutralized if enough E proteins are bound with antibodies, and the stoichiometry depends on how and where the antibody binds the E protein (*73*, *74*).

The stoichiometry of DENV neutralization may be influenced by the extent of virus maturation. Because DENV is trafficked from its site of replication through the trans-Golgi network for cellular egress, incomplete proteolytic cleavage of the pr peptide from the M protein could affect the extent of virus maturation (*75*, *76*). Immature virions are more likely to be produced in culture than in human infection, potentially underestimating how much antibodies are needed to neutralize DENV (*77*). However, there is no evidence that DENV-2 produces more mature virions than the other DENVs and, thus, is unlikely to explain differences in the higher neutralizing antibody threshold required for protection.

The stoichiometry requirement for DENV neutralization would theoretically favor antibodies that bind quaternary epitopes made up of two or more E proteins; fewer antibodies would be needed to neutralize each virion. Many of the DENV-type–specific monoclonal antibodies isolated from convalescent individuals bind such quaternary epitopes (*78*–*83*). Because protection against homotypic DENV reinfection is thought to be long lasting, the presence of such type-specific quaternary epitope-binding neutralizing antibodies could, by logical extension, be indicative of type-specific immunity. Efficacy outcomes from the phase 3 clinical trials provide mixed support for this logic. Although the presence of type-specific antibodies from CYD-TDV and TAK003 vaccination tracked with the respective type-specific vaccine efficacy (*23*, *25*, *84*, *85*), preliminary reports from the TV003 trial suggest a more nuanced interpretation. In CYD-TDV and TAK003, the predominant type-specific antibodies produced after vaccination were to DENV-4 (*23*) for CYD-TDV and DENV-2 (*25*, *85*) for TAK003. Correspondingly, the highest protection offered by these two different vaccines was against symptomatic infection by DENV-4 and DENV-2, respectively. In contrast, TV003 vaccination was found to produce comparable titers of type-specific antibodies to all four DENVs and especially against DENV-1 and DENV-2 (*27*, *86*, *87*). Nonetheless, in those without prior DENV infection, the efficacy of Butantan DV, which is analogous to TV003 (*28*), against symptomatic DENV-2 was lower than for DENV-1 infection (57.9% versus 85.5%) (*38*). Whether this difference is statistically significant is not known at the time of writing of this Review, because the respective 95% confidence intervals of these point estimates have yet to be reported. Thus, although each of the four DENV-specific neutralizing antibodies offer a degree of protection, the titers needed for protection against each of the four DENVs differ.

The differential threshold for type-specific DENV neutralization may reflect propensity for antibody-dependent infection

DENV infects myeloid-derived cells, such as monocytes, macrophages, and dendritic cells. Besides entry through cognate receptor–mediated endocytosis, antibody-decorated DENVs may be

Table 2. CYD-TDV elicited neutralizing antibody titers associated with 50% vaccine efficacy against symptomatic infection from each of the four DENVs. $PRNT_{50}$ titers were measured 1 month after the third dose of CYD-TDV, and VE was determined in the first year after completion of three doses.

	CYD-TDV (CYD14)*	CYD-TDV (CYD15)†
	$PRNT_{50}$ titer associated with 50% VE	
DENV-1	70	70
DENV-2	600	300
DENV-3	110	<10
DENV-4	20	<10

*Data estimated from figure S8 in (*69*). †Data estimated from figure S9 in (*69*).

taken up by these antigen-presenting cells through activating Fcγ receptors (FcγRs). Because DENVs share a sizeable proportion of their structural antigens, infection with one DENV type induces antibodies that cross-react with the other DENV serotypes. Sub-neutralizing concentrations of cross-reactive antibodies could therefore facilitate infection by offering DENV an alternative activating FcγR-mediated entry pathway (*11*, *88*). Entry through activating FcγRs also appears to be a more efficient pathway for viral entry than cognate receptor–mediated endocytosis (*88*, *89*). Such an entry pathway has been associated with increased risk of severe disease during a secondary infection with a DENV heterotypic to that which caused the primary infection (*90*). Likewise, waning concentrations of maternal neutralizing antibodies to concentrations that enhance infection have also been associated with increased risk of severe dengue (*91*–*93*). This process has been termed ADE of DENV infection.

The requirement for greater concentrations of antibodies to prevent DENV-2 infection in humans could reflect the differences in risk of ADE between the four DENVs. Although ADE has been widely attributed as a pathogenic mechanism for severe secondary dengue (*94*, *95*), it is a far more nuanced process than is perhaps appreciated. Observations from a cohort study in Nicaragua found maximal risk of severe dengue only in those with baseline preinfection antibodies that were within a restricted range of titers (*96*). Another study in Thailand, using a different serological assay, also arrived at the same conclusion (*97*). Experimentally, live attenuated yellow fever virus (the prototype virus of the flavivirus genus to which DENV belongs to) infection was only enhanced in those with a specific range of cross-reactive antibody titers generated by Japanese encephalitis vaccination (*21*).

The restricted range of antibody titers suitable for ADE is likely due to the requirement of appropriately sized virus-antibody immune complex for optimal co-ligation of appropriate immunoreceptors. High antibody concentrations aggregate DENV to form large immune complexes that co-ligate the less-abundant but inhibitory FcγRIIB. FcγRIIB contains a cytoplasmic immunoreceptor tyrosine-based inhibitory motif (ITIM), the signaling of which blocks viral entry and, hence, ADE (Fig. 2A) (*98*, *99*). Activating FcγRs, such as FcγRIIA, contain a cytoplasmic immunoreceptor tyrosine-based activating motif (ITAM) that promotes uptake of DENV-antibody complexes. It also rapidly induces expression of IFN-stimulated genes (ISGs) that are antiviral in function (*100*) and limit viral replication (Fig. 2B). For ADE to occur, DENV must elicit activating FcγR-mediated uptake without triggering ISG expression. It achieves this by binding the leukocyte immunoglobulin-like receptor B1 (LILRB1), which signals to inhibit up-regulation of ISGs in host myeloid-derived cells (Fig. 2C) (*101*, *102*). Interaction of antibody-DENV complexes at the point of entry into myeloid cells enables the virus to evade host antiviral responses that cannot be achieved through the non-ADE entry pathway alone (*103*, *104*). Given the requirement for these interactions, the four DENVs could thus have different inherent propensities for ADE.

Suspicion that the four DENVs exploit the FcγR-mediated entry pathway differently has existed for some time (*105*). Studies that found increased risk of severe dengue in infants at a time of waning maternal antibodies were conducted when DENV-2 was prevalent (*91*–*93*). In contrast, the study that did not show a similar increased risk was carried out when the predominant circulating virus was DENV-3 (*106*). A study in Khampaeng Phet, Thailand found DENV-1 and DENV-3 to be the main causes of symptomatic primary dengue. In contrast, the rate of symptomatic DENV-2 infection was elevated in infants through maternal antibody enhancement and in those with secondary dengue; the ratio of symptomatic secondary to primary infection was also higher for DENV-4, although the overall proportion of symptomatic DENV-4 infection was the smallest in this cohort (*107*). A longitudinal analysis of dengue cases reported in Bangkok from 1994 to 2006 also found similar trends: DENV-1 and DENV-3 were more often associated with primary dengue than DENV-2, whereas secondary DENV-2 was more associated with dengue hemorrhagic fever than the other DENV types (*108*). DENV-2 has also been more frequently associated with dengue shock syndrome (*109*, *110*). Last, modeling of dengue cases reported over a 6-year period in Vietnam also found DENV-2 to be the greatest risk factor for symptomatic dengue among those with secondary infection (*111*).

Perhaps the most emphatic evidence to support differential risk of severe dengue by the four DENVs in those with secondary dengue comes from a recent study that examined the risk factors for severe dengue. This study, which involved more than 5600 dengue cases, found high plasma viremia, secondary infection, and infection with DENV-2 to be factors associated with increased risk of severe dengue. Moreover, within the range of viremia commonly detected during the early febrile phase of illness (plasma of 6 to 9 $\log_{10}$ viral RNA copies/ml), risk of plasma leakage (Fig. 3A) and severe dengue (Fig. 3B) were highest in patients with secondary DENV-2 infection (*57*). Collectively, these findings suggest that DENV-2 has the highest immunological requirement for protection against infection.

Anti-NS1 antibodies

Although neutralizing antibodies primarily directed against the PrM and E proteins of DENV play an important role in vaccine efficacy, some have proposed that an effective dengue vaccine should also elicit antibodies against NS1. NS1 is the only DENV protein to be secreted from the cell during acute infection (*112*) and has been implicated in experimental models of DENV pathogenesis as a direct cause of endothelial dysfunction and vascular leakage and as a driver for the release of proinflammatory cytokines from immune cells (*112*). Correspondingly, anti-dengue NS1 antibodies

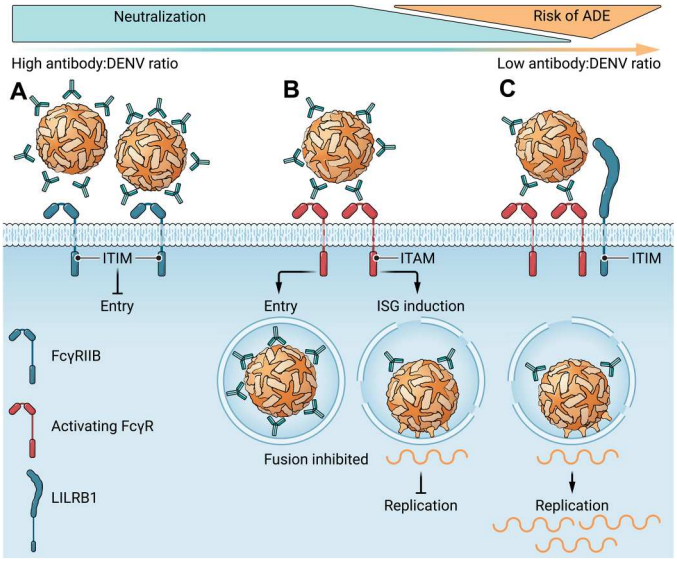

Fig. 2. Antibody-dependent enhancement (ADE) requires an optimal ratio of antibody to DENV to engage appropriate cellular receptors. A gradient of number of antibody molecules per DENV is shown from left to right. (**A**) High antibody:DENV ratios aggregate DENV to co-ligate the inhibitory Fcγ receptor IIB (FcγRIIB), which signals through its immunoreceptor tyrosine-based inhibitory motif (ITIM) cytoplasmic tail to inhibit uptake of the immune complex. (**B**) At moderate antibody:DENV ratios, the immune complex is of a size that co-ligates activating FcγR, depicted here as FcγRIIA, which has an immunoreceptor tyrosine-based activating motif (ITAM) cytoplasmic tail. In such a context, DENV infection remains inhibited, either through the antibody binding sufficient number of epitopes to prevent fusion of DENV with endosomal membranes necessary to release the RNA into the cytoplasm (left) or through ITAM-mediated signaling that up-regulates interferon-stimulated genes (ISGs) to induce an antiviral state in the myeloid-derived cell (right). (**C**) At sub-neutralizing antibody:DENV ratios, additional interaction between DENV and leukocyte immunoglobulin-like receptor B1 (LILRB1), which has an ITIM signaling cytoplasmic tail, inhibits ISG induction to promote a cytoplasmic environment that is more favorable for enhanced DENV infection.

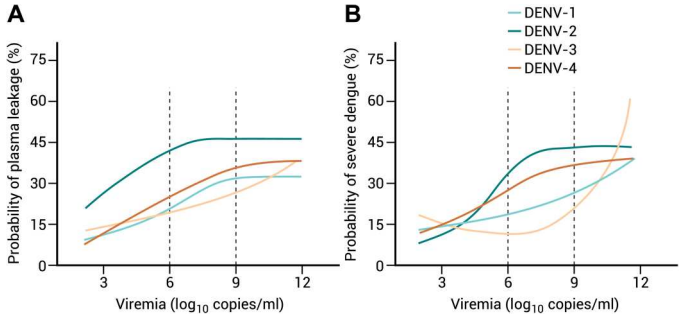

Fig. 3. Secondary DENV-2 infection is associated with the highest risk of vascular leak and severe dengue. Plots are adapted from figure 4 in (*57*). (**A**) The probability of plasma leakage in patients with dengue experiencing a secondary DENV-1, DENV-2, DENV-3, or DENV-4 infection is shown. (**B**) The probability of severe dengue in patients experiencing a secondary DENV-1, DENV-2, DENV-3, or DENV-4 infection is shown. The area between the dashed lines in each graph represents the amount of viremia commonly detected in patients with dengue in the febrile phase of illness.

appear to play a protective role, at least in mouse models of infection. Mice vaccinated against NS1 are protected from lethal virus challenge (*113*), and more recently, two monoclonal antibodies against flavivirus NS1 were shown to reduce viremia, endothelial dysfunction, and vascular leak, leading to increased survival in DENV-infected mice (*114*, *115*). Importantly, unlike cross-reactive or sub-neutralizing antibodies to the E protein, anti-NS1 antibodies are not able to induce ADE.

The evidence for the pathogenic role of NS1 in humans is less clear. Although some studies have found an association between higher NS1 antigenemia and severe disease (*116*), others have shown conflicting results (*117*). Patients with secondary DENV-2 are more likely to have undetectable NS1 concentrations than those with primary infection, although this same study also found that high NS1 concentrations were associated with the nadir of thrombocytopenia (*118*). Although the pathogenic role of NS1 and the protective function of anti-NS1 antibodies have yet to be proven clinically, there nevertheless may be some benefit for dengue vaccines to elicit anti-NS1 antibodies to potentially modulate disease severity in breakthrough symptomatic infections.

CELLULAR IMMUNITY

To date, dengue vaccine development has primarily been focused on inducing high and balanced titers of neutralizing antibodies to prevent symptomatic illness from DENV. However, neutralizing antibodies are only one component of the adaptive immune response. Cellular immunity, driven by $CD4^+$ and $CD8^+$ T cells, also plays an important role in viral control and prevention of disease (*119*). Helper $CD4^+$ T cells support other components of the immune system such as B cells and macrophages, through secretion of cytokines such as IFN-γ, tumor necrosis factor–α (TNF-α), and interleukin-2 (IL-2) (*120*, *121*), whereas cytotoxic $CD8^+$ T cells directly kill virus-infected cells (*121*). Thus, both neutralizing antibodies and the cellular immune response play complementary roles to control infection and mitigate disease severity.

The role of T cells in DENV infection has been a somewhat contentious issue, with both pathological and protective roles being proposed. Previously, it was thought that antigen-specific T cells induced during a primary DENV infection would result in the expansion of low-affinity, cross-reactive memory T cells during secondary heterotypic infection, resulting in increased immunopathology due to a lower ability to clear virus and the secretion of inflammatory cytokines, a phenomenon known as original antigenic sin (*122*, *123*). A study in Thai children hospitalized with dengue found that DENV-specific T cells in secondary infection were of low affinity to the infecting serotype, with stronger affinity to other serotypes likely responsible for prior infection (*124*). These T cell studies were done in the acute stage of infection in children with severe dengue. Consequently, this "original antigenic sin" explanation may only represent a subset of patients with secondary dengue. Unlike the acute phase of illness, studies in those who have recovered from dengue found strong cross-reactive T cell responses (*125*). It is now increasingly thought that T cells likely play a protective role in dengue, with DENV-specific T cell responses being associated with protective efficacy (*31*). Stronger and more polyfunctional antigen-specific $CD8^+$ T cell responses have been found in individuals with human leukocyte antigen (HLA) alleles that are associated with a reduced susceptibility to severe disease (*126*). In a similar vein, DENV infection induces cytolytic DENV-

specific $CD4^+$ T cells that are correlated with protective HLA DR alleles (*127*). In a nested case–control study of school age children in Thailand, children with higher frequencies of IFN-γ–, IL-2–, and TNF-α–secreting DENV-specific T cells were more likely to develop asymptomatic rather than symptomatic dengue DENV infection (*128*). Such observations suggest a protective role for T cells in dengue.

$CD8^+$ T cell responses to DENV target mainly the NS3 protein, followed by C, NS5, and NS4A/B, whereas $CD4^+$ responses target mainly C, followed by E, NS3, NS2A/B, and NS5 (*31*). Cellular immunity from these DENV-reactive T cells may be particularly important for rapid clearance of infection when neutralizing antibodies are not present at sufficiently high titers to sterilize infection. The functional role of T cells has been demonstrated in allogeneic kidney transplant recipients, who were given T cell–suppressive therapies to prevent graft rejection (*129*, *130*). With low T cell counts, these patients had a protracted course of DENV infection and eventually only cleared the infection when the T cell counts recovered to near the lower limit of normal values (*129*).

T cell responses elicited by dengue vaccination

CYD-TDV uses a yellow fever (YF17D) backbone and, thus, lacks DENV NS proteins that contain a large proportion of $CD8^+$ T cell epitopes (Fig. 1) (*126*, *131*, *132*). Although there is some degree of cross-reactivity to DENV with T cells induced by YF17D vaccination, these tend to be limited and of much lower magnitude (*125*). For example, although CYD-TDV vaccination in baseline dengue-naïve individuals induced strong $CD8^+$ T cell responses against YF17D NS3, cross-reactive $CD8^+$ responses against DENV NS3 were muted (*133*). This lack of a strong DENV-specific T cell response could be one of the contributory reasons for the poor efficacy of CYD-TDV against DENV-1 and DENV-2.

TAK003 instead uses an attenuated DENV-2 virus, PDK53, as its backbone for the DENV-1, DENV-3, and DENV-4 constructs. Unsurprisingly, in dengue-naïve and dengue-exposed adolescents vaccinated with TAK003, the highest frequencies of IFN-γ–secreting $CD4^+$ and $CD8^+$ T cells were directed against DENV-2 NS1, with detectable, although lower, cross-reactive responses observed against DENV-1, DENV-3, and DENV-4 NS proteins (*134*). Similar findings have been reported in flavivirus-naïve adults (*135*). The T cell responses induced by TAK003 mirror its efficacy profile, with the highest clinical efficacy against DENV-2 compared with the other three serotypes: 97.7% for DENV-2, 73.7% for DENV-1, and 62.6% against DENV-3; the efficacy results for DENV-4 were inconclusive because of insufficient cases (*35*–*37*).

TV003 induces a broad and polyfunctional $CD8^+$ T cell response mirroring that of natural secondary DENV infection; more than 90% of those responses were directed against conserved DENV sequences, suggesting that TV003-induced T cell responses should be able to recognize epitopes from all four serotypes (*136*). This tetravalent vaccination outcome in T cell responses contrasts with those induced after monovalent vaccination; conserved sequences only accounted for 46% in DEN1Δ30, 16% in DEN2/4Δ30, 54% in DEN3Δ30/31, and 7% in DEN4Δ30 vaccination. Moreover, T cells elicited by monovalent vaccination targeted both structural and NS proteins (*136*), although differences in $CD8^+$ T cell immunodominance patterns exist across the four DENVs (*137*). For example, 51% of the $CD8^+$ T cell responses after DENV-3 infection targeted structural proteins, whereas only 19% targeted structural proteins after DENV-2 infection. These differences could be due to variations in antigen processing and presentation during infection with the different DENVs. Thus, although almost all the $CD8^+$ T cell responses elicited by tetravalent vaccination were observed to be directed against the NS proteins (primarily NS3 and NS5), similar to the response after secondary infection, it remains unclear whether T cells targeting conserved epitopes alone would be equally protective across the different serotypes.

Measuring cellular immunity routinely

Although it is now clear that an effective dengue vaccine should be capable of inducing DENV-specific cellular immunity, it remains unknown what this minimum threshold needs to be. Cellular immunity is seldom evaluated as a correlate of protection in DENV vaccine development, with both vaccine developers and regulators relying almost exclusively on antibody titers to identify vaccine candidates. A contributing factor is the absence of standardized T cell assays, which are simple to perform and interpret. DENV-specific T cells constitute only a tiny proportion of all T cells in the body and usually require complex functional assays to be detected (*31*). Currently used assays such as IFN-γ enzyme-linked immunospot (ELISpot) and flow cytometry–based assays are labor intensive, time consuming, and require highly specialized technical expertise and equipment (*138*). In addition, such assays are usually performed on peripheral blood mononuclear cells, which require large blood volumes; this makes them unsuitable for large-scale deployment, especially in pediatric populations. Fortunately, more user-friendly T cell assays are now on the horizon. For example, a recently developed rapid cytokine release assay requiring only small volumes of whole blood (<1 ml) demonstrated good sensitivity and specificity in assessing severe acute respiratory syndrome coronavirus 2–specific T cell responses (*139*). The use of such standardized assays could be expanded to DENV vaccine development and would enable correlates of protection that integrate both antibody and T cell measurements to be defined (*140*).

THE FUTURE OF DENGUE VACCINES

A lesson to be learned from the phase 3 clinical trials is the need for dengue vaccines to elicit the full spectrum of adaptive immune responses. Neutralizing antibodies can protect but require titers that may be too high to be attained in those without prior DENV infection. Moreover, although neutralizing antibodies prevent infection, sustained titers of such antibodies may require repeated exposure to DENVs (*141*). Engendering humoral responses to the NS1 protein, as well as $CD8^+$ T cell responses to the C and NS proteins, could produce the multilayered immune response that may be necessary for the prevention of dengue and severe dengue. The lack of such a multilayered response may explain the increased risk of hospitalization and severe dengue observed in DENV-naïve vaccinees who received CYD-TDV (*19*). In contrast, it may have been such a multilayered DENV-directed response that, at least in part, averted increased rates of individuals hospitalized with dengue, despite the lack of efficacy against symptomatic DENV-3 infection in DENV-naïve TAK003 vaccinees (*37*). Phase 4 clinical studies of TAK003 should shed further light on the respective roles of these adaptive immune responses in protecting against dengue and

severe dengue, especially against those caused by DENV-3 in those without prior DENV infection.

Ideally, any dengue vaccine should prevent both symptomatic infection and DENV transmission. Such a vaccine would need to robustly elicit both neutralizing antibodies and T cells, given their complementary roles in immunity. However, even if a dengue vaccine is not able to achieve high titers of neutralizing antibodies across all four serotypes, one that is able to induce a broad and sustained T cell response may still be highly effective in protecting against hospitalized dengue and severe disease. Similar lessons can be drawn from our experience with coronavirus disease 2019 (COVID-19) vaccines; although current COVID-19 mRNA vaccines are not able to sustainably protect against infection by the Omicron variant because of antibody escape mutations, protection against severe disease is maintained through preserved virus-specific T cell responses (*142*–*147*). Furthermore, vaccination also brings benefit at the population level: Modeling of CYD-TDV vaccination, carried out before the World Health Organization's recommendation for pre-vaccination to ensure vaccination of only those with prior DENV infection, suggested cost-effectiveness of this vaccine if priced competitively, for countries with high dengue endemicity (*148*). Improved overall outcomes with TAK003 and TV003 vaccination are thus likely to bring even greater cost-effectiveness to dengue prevention for endemic countries. Thus, a vaccine that fully protects against neither symptomatic infection nor transmission but prevents hospitalization and severe dengue may not be perfect but would, nonetheless, be a useful solution for population-level dengue control.

Last, we suggest that, until a single-dose vaccine that fully protects against disease from all four DENVs is available, a heterologous prime-boost approach could exploit the strengths, and at the same time overcome the limitations, of each of these vaccines. Clinical observations indicate that after two episodes of infection, the third and fourth episodes of DENV infection are mostly asymptomatic (*149*). Hence, priming with a single dose of TAK003 followed by boosting with CYD-TDV, for instance, could simulate sequential infection because vaccine viremia studies indicate different types of infection from these vaccines; TAK003 showed measurable DENV-2 vaccine viremia (*24*), whereas vaccine viremia was detected predominantly from the DENV-4 followed by the DENV-3 components of CYD-TDV (*22*). Similar combinations could also be envisaged with TV003. A heterologous prime-boost approach could, thus, negate the need for years of vaccine development before dengue is no longer merely a theoretically, but rather a truly, vaccine-preventable disease.

REFERENCES AND NOTES

1. S. Bhatt, P. W. Gething, O. J. Brady, J. P. Messina, A. W. Farlow, C. L. Moyes, J. M. Drake, J. S. Brownstein, A. G. Hoen, O. Sankoh, M. F. Myers, D. B. George, T. Jaenisch, G. R. W. Wint, C. P. Simmons, T. W. Scott, J. J. Farrar, S. I. Hay, The global distribution and burden of dengue. *Nature* **496**, 504–507 (2013).
2. A. Wilder-Smith, E.-E. Ooi, O. Horstick, B. Wills, Dengue. *Lancet* **393**, 350–363 (2019).
3. J. P. Messina, O. J. Brady, N. Golding, M. U. G. Kraemer, G. R. W. Wint, S. E. Ray, D. M. Pigott, F. M. Shearer, K. Johnson, L. Earl, L. B. Marczak, S. Shirude, N. Davis Weaver, M. Gilbert, R. Velayudhan, P. Jones, T. Jaenisch, T. W. Scott, R. C. Reiner Jr., S. I. Hay, The current and future global distribution and population at risk of dengue. *Nat. Microbiol.* **4**, 1508–1515 (2019).
4. S. Kalimuddin, Y. E. Teh, L. E. Wee, S. Paintal, R. Sasisekharan, J. G. Low, S. K. Sheth, E. E. Ooi, Chronic sequelae complicate convalescence from both dengue and acute viral respiratory illness. *PLoS Negl. Trop. Dis.* **16**, e0010724 (2022).
5. D. T. H. Tam, H. Clapham, E. Giger, N. T. T. Kieu, N. T. Nam, D. T. T. Hong, B. T. Nuoi, N. T. H. Cam, N. T. H. Quyen, H. C. Turner, T. Jaenisch, C. P. Simmons, P. K. Lam, B. Wills, Burden of postinfectious symptoms after acute dengue, Vietnam. *Emerg. Infect. Dis.* **29**, 160–163 (2023).
6. D. C. Tiga, E. A. Undurraga, J. Ramos-Castañeda, R. A. Martínez-Vega, C. A. Tschampl, D. S. Shepard, Persistent symptoms of dengue: Estimates of the incremental disease and economic burden in Mexico. *Am. J. Trop. Med. Hyg.* **94**, 1085–1089 (2016).
7. E.-E. Ooi, K. T. Goh, D. J. Gubler, Dengue prevention and 35 years of vector control in Singapore. *Emerg. Infect. Dis.* **12**, 887–893 (2006).
8. A. B. Sabin, R. W. Schlesinger, Production of immunity to dengue with virus modified by propagation in mice. *Science* **101**, 640–642 (1945).
9. A. B. Sabin, Research on dengue during World War II. *Am. J. Trop. Med. Hyg.* **1**, 30–50 (1952).
10. G. E. Snow, B. Haaland, E. E. Ooi, D. J. Gubler, Review article: Research on dengue during World War II revisited. *Am. J. Trop. Med. Hyg.* **91**, 1203–1217 (2014).
11. S. B. Halstead, S. Nimmannitya, S. N. Cohen, Observations related to pathogenesis of dengue hemorrhagic fever. IV. Relation of disease severity to antibody response and virus recovered. *Yale J. Biol. Med.* **42**, 311–328 (1970).
12. F. Guirakhoo, K. Pugachev, J. Arroyo, C. Miller, Z. X. Zhang, R. Weltzin, K. Georgakopoulos, J. Catalan, S. Ocran, K. Draper, T. P. Monath, Viremia and immunogenicity in nonhuman primates of a tetravalent yellow fever-dengue chimeric vaccine: Genetic reconstructions, dose adjustment, and antibody responses against wild-type dengue virus isolates. *Virology* **298**, 146–159 (2002).
13. J. E. Blaney Jr., J. M. Matro, B. R. Murphy, S. S. Whitehead, Recombinant, live-attenuated tetravalent dengue virus vaccine formulations induce a balanced, broad, and protective neutralizing antibody response against each of the four serotypes in rhesus monkeys. *J. Virol.* **79**, 5516–5528 (2005).
14. B. D. Kirkpatrick, A. P. Durbin, K. K. Pierce, M. P. Carmolli, C. M. Tibery, P. L. Grier, N. Hynes, S. A. Diehl, D. Elwood, A. P. Jarvis, B. P. Sabundayo, C. E. Lyon, C. J. Larsson, M. Jo, J. M. Lovchik, C. J. Luke, M. C. Walsh, E. A. Fraser, K. Subbarao, S. S. Whitehead, Robust and balanced immune responses to all 4 dengue virus serotypes following administration of a single dose of a live attenuated tetravalent dengue vaccine to healthy, flavivirus-naive adults. *J Infect Dis* **212**, 702–710 (2015).
15. S. J. Thomas, The necessity and quandaries of dengue vaccine development. *J. Infect. Dis.* **203**, 299–303 (2011).
16. A. P. Durbin, Historical discourse on the development of the live attenuated tetravalent dengue vaccine candidate TV003/TV005. *Curr. Opin. Virol.* **43**, 79–87 (2020).
17. J. R. Pinheiro-Michelsen, R. d. S. O. Souza, I. V. R. Santana, P. d. S. da Silva, E. C. Mendez, W. B. Luiz, J. H. Amorim, Anti-dengue vaccines: From development to clinical trials. *Front. Immunol.* **11**, 1252 (2020).
18. J. Pintado Silva, A. Fernandez-Sesma, Challenges on the development of a dengue vaccine: A comprehensive review of the state of the art. *J. Gen. Virol.* **104**, 001831 (2023).
19. S. Sridhar, A. Luedtke, E. Langevin, M. Zhu, M. Bonaparte, T. Machabert, S. Savarino, B. Zambrano, A. Moureau, A. Khromava, Z. Moodie, T. Westling, C. Mascareñas, C. Frago, M. Cortés, D. Chansinghakul, F. Noriega, A. Bouckenooghe, J. Chen, S.-P. Ng, P. B. Gilbert, S. Gurunathan, C. A. DiazGranados, Effect of dengue serostatus on dengue vaccine safety and efficacy. *N. Engl. J. Med.* **379**, 327–340 (2018).
20. D. R. Martinez, S. W. Metz, R. S. Baric, Dengue vaccines: The promise and pitfalls of antibody-mediated protection. *Cell Host Microbe* **29**, 13–22 (2021).
21. K. R. Chan, X. Wang, W. A. A. Saron, E. S. Gan, H. C. Tan, D. Z. L. Mok, S. L. X. Zhang, Y. H. Lee, C. Liang, L. Wijaya, S. Ghosh, Y. B. Cheung, S. R. Tannenbaum, S. N. Abraham, A. L. St John, J. G. H. Low, E. E. Ooi, Cross-reactive antibodies enhance live attenuated virus infection for increased immunogenicity. *Nat. Microbiol.* **1**, 16164 (2016).
22. J. Torresi, P. C. Richmond, L. G. Heron, M. Qiao, J. Marjason, L. Starr-Spires, D. van der Vliet, J. Jin, T. A. Wartel, A. Bouckenooghe, Replication and excretion of the live attenuated tetravalent dengue vaccine CYD-TDV in a flavivirus-naive adult population: Assessment of vaccine viremia and virus shedding. *J. Infect. Dis.* **216**, 834–841 (2017).
23. S. Henein, J. Swanstrom, A. M. Byers, J. M. Moser, S. F. Shaik, M. Bonaparte, N. Jackson, B. Guy, R. Baric, A. M. de Silva, Dissecting antibodies induced by a chimeric yellow fever-dengue, live-attenuated, tetravalent dengue vaccine (CYD-TDV) in naive and dengue-exposed individuals. *J. Infect. Dis.* **215**, 351–358 (2017).
24. V. Tricou, J. G. Low, H. M. Oh, Y.-S. Leo, S. Kalimuddin, L. Wijaya, J. Pang, L. M. Ling, T. H. Lee, M. Brose, Y. Hutagalung, M. Rauscher, A. Borkowski, D. Wallace, Safety and immunogenicity of a single dose of a tetravalent dengue vaccine with two different serotype-2 potencies in adults in Singapore: A phase 2, double-blind, randomised, controlled trial. *Vaccine* **38**, 1513–1519 (2020).
25. L. J. White, E. F. Young, M. J. Stoops, S. R. Henein, E. C. Adams, R. S. Baric, A. M. de Silva, Defining levels of dengue virus serotype-specific neutralizing antibodies induced by a live

attenuated tetravalent dengue vaccine (TAK-003). *PLoS Negl. Trop. Dis.* **15**, e0009258 (2021).
26. S. S. Whitehead, A. P. Durbin, K. K. Pierce, D. Elwood, B. D. McElvany, E. A. Fraser, M. P. Carmolli, C. M. Tibery, N. A. Hynes, M. Jo, J. M. Lovchik, C. J. Larsson, E. A. Doty, D. M. Dickson, C. J. Luke, K. Subbarao, S. A. Diehl, B. D. Kirkpatrick, In a randomized trial, the live attenuated tetravalent dengue vaccine TV003 is well-tolerated and highly immunogenic in subjects with flavivirus exposure prior to vaccination. *PLoS Negl. Trop. Dis.* **11**, e0005584 (2017).
27. U. K. Nivarthi, J. Swanstrom, M. J. Delacruz, B. Patel, A. P. Durbin, S. S. Whitehead, B. D. Kirkpatrick, K. K. Pierce, S. A. Diehl, L. Katzelnick, R. S. Baric, A. M. de Silva, A tetravalent live attenuated dengue virus vaccine stimulates balanced immunity to multiple serotypes in humans. *Nat. Commun.* **12**, 1102 (2021).
28. E. G. Kallas, A. R. Precioso, R. Palacios, B. Thomé, P. E. Braga, T. Vanni, L. M. A. Campos, L. Ferrari, G. Mondini, M. da Graça Salomão, A. da Silva, H. M. Espinola, J. do Prado Santos, C. L. S. Santos, M. d. C. S. T. Timenetsky, J. L. Miraglia, N. M. F. Gallina, D. Weiskopf, A. Sette, R. Goulart, R. T. Salles, A. Maestri, A. M. E. Sallum, S. C. L. Farhat, N. K. Sakita, J. C. O. A. Ferreira, C. G. T. Silveira, P. R. Costa, I. Raw, S. S. Whitehead, A. P. Durbin, J. Kalil, Safety and immunogenicity of the tetravalent, live-attenuated dengue vaccine Butantan-DV in adults in Brazil: A two-step, double-blind, randomised placebo-controlled phase 2 trial. *Lancet Infect. Dis.* **20**, 839–850 (2020).
29. K. L. Russell, R. E. Rupp, J. O. Morales-Ramirez, C. Diaz-Perez, C. P. Andrews, A. W. Lee, T. S. Finn, K. S. Cox, A. Falk Russell, M. M. Schaller, J. C. Martin, D. M. Hyatt, S. Gozlan-Kelner, A. Bili, B.-A. G. Coller, A phase I randomized, double-blind, placebo-controlled study to evaluate the safety, tolerability, and immunogenicity of a live-attenuated quadrivalent dengue vaccine in flavivirus-naïve and flavivirus-experienced healthy adults. *Hum. Vaccin. Immunother.* **18**, 2046960 (2022).
30. A. G. Dias Jr., C. Atyeo, C. Loos, M. Montoya, V. Roy, S. Bos, P. Narvekar, T. Singh, L. C. Katzelnick, G. Kuan, D. A. Lauffenburger, A. Balmaseda, G. Alter, E. Harris, Antibody Fc characteristics and effector functions correlate with protection from symptomatic dengue virus type 3 infection. *Sci. Transl. Med.* **14**, eabm3151 (2022).
31. Y. Tian, A. Grifoni, A. Sette, D. Weiskopf, Human T cell response to dengue virus infection. *Front. Immunol.* **10**, 2125 (2019).
32. M. R. Capeding, N. H. Tran, S. R. S. Hadinegoro, H. I. H. J. M. Ismail, T. Chotpitayasunondh, M. N. Chua, C. Q. Luong, K. Rusmil, D. N. Wirawan, R. Nallusamy, P. Pitisuttithum, U. Thisyakorn, I.-K. Yoon, D. van der Vliet, E. Langevin, T. Laot, Y. Hutagalung, C. Frago, M. Boaz, T. A. Wartel, N. G. Tornieporth, M. Saville, A. Bouckenooghe; CYD14 Study Group, Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: A phase 3, randomised, observer-masked, placebo-controlled trial. *Lancet* **384**, 1358–1365 (2014).
33. L. Villar, G. H. Dayan, J. L. Arredondo-García, D. M. Rivera, R. Cunha, C. Deseda, H. Reynales, M. S. Costa, J. O. Morales-Ramírez, G. Carrasquilla, L. C. Rey, R. Dietze, K. Luz, E. Rivas, M. C. Miranda Montoya, M. Cortés Supelano, B. Zambrano, E. Langevin, M. Boaz, N. Tornieporth, M. Saville, F. Noriega; CYD15 Study Group, Efficacy of a tetravalent dengue vaccine in children in Latin America. *N. Engl. J. Med.* **372**, 113–123 (2015).
34. S. R. Hadinegoro, J. L. Arredondo-García, M. R. Capeding, C. Deseda, T. Chotpitayasunondh, R. Dietze, H. I. Muhammad Ismail, H. Reynales, K. Limkittikul, D. M. Rivera-Medina, H. N. Tran, A. Bouckenooghe, D. Chansinghakul, M. Cortés, K. Fanouillere, R. Forrat, C. Frago, S. Gailhardou, N. Jackson, F. Noriega, E. Plennevaux, T. A. Wartel, B. Zambrano, M. Saville; CYD-TDV Dengue Vaccine Working Group, Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. *N. Engl. J. Med.* **373**, 1195–1206 (2015).
35. S. Biswal, H. Reynales, X. Saez-Llorens, P. Lopez, C. Borja-Tabora, P. Kosalaraksa, C. Sirivichayakul, V. Watanaveeradej, L. Rivera, F. Espinoza, L. K. Fernando, R. Dietze, K. Luz, R. V. da Cunha, J. Jimeno, E. López-Medina, A. Borkowski, M. Brose, M. Rauscher, I. L. Fevre, S. Bizjajeva, L. Bravo, D. Wallace; TIDES Study Group, Efficacy of a tetravalent dengue vaccine in healthy children and adolescents. *N. Engl. J. Med.* **381**, 2009–2019 (2019).
36. S. Biswal, C. Borja-Tabora, L. M. Vargas, H. Velásquez, M. T. Alera, V. Sierra, E. J. Rodriguez-Arenales, D. Yu, V. P. Wickramasinghe, E. D. Moreira Jr., A. D. Fernando, D. Gunasekera, P. Kosalaraksa, F. Espinoza, E. López-Medina, L. Bravo, S. Tuboi, Y. Hutagalung, P. Garbes, I. Escudero, M. Rauscher, S. Bizjajeva, I. L. Fevre, A. Borkowski, X. Saez-Llorens, D. Wallace; TIDES study group, Efficacy of a tetravalent dengue vaccine in healthy children aged 4-16 years: A randomised, placebo-controlled, phase 3 trial. *Lancet* **395**, 1423–1433 (2020).
37. L. Rivera, S. Biswal, X. Sáez-Llorens, H. Reynales, E. López-Medina, C. Borja-Tabora, L. Bravo, C. Sirivichayakul, P. Kosalaraksa, L. M. Vargas, D. Yu, V. Watanaveeradej, F. Espinoza, R. Dietze, L. K. Fernando, P. Wickramasinghe, E. D. Moreira Jr., A. D. Fernando, D. Gunasekera, K. Luz, R. V. Cunha, M. Rauscher, O. Zent, M. Liu, E. Hoffman, I. L. Fevre, V. Tricou, D. Wallace, M. T. Alera, A. Borkowski; TIDES study group, Three-year efficacy and safety of Takeda's dengue vaccine candidate (TAK-003). *Clin. Infect. Dis.* **75**, 107–117 (2022).
38. Instituto Butantan, Butantan's dengue vaccine has 79.6% efficacy, partial results from 2-year follow-up show (2022); https://butantan.gov.br/noticias/butantan%27s-dengue-vaccine-has-79.6-efficacy-partial-results-from-2-year-follow-up-show.
39. R. Rico-Hesse, Microevolution and virulence of dengue viruses. *Adv. Virus Res.* **59**, 315–341 (2003).
40. R. Rico-Hesse, L. M. Harrison, R. A. Salas, D. Tovar, A. Nisalak, C. Ramos, J. Boshell, M. T. R. de Mesa, R. M. R. Nogueira, A. T. d. Rosa, Origins of dengue type 2 viruses associated with increased pathogenicity in the Americas. *Virology* **230**, 244–251 (1997).
41. A. Steel, D. J. Gubler, S. N. Bennett, Natural attenuation of dengue virus type-2 after a series of island outbreaks: A retrospective phylogenetic study of events in the South Pacific three decades ago. *Virology* **405**, 505–512 (2010).
42. G. Manokaran, E. Finol, C. Wang, J. Gunaratne, J. Bahl, E. Z. Ong, H. C. Tan, O. M. Sessions, A. M. Ward, D. J. Gubler, E. Harris, M. A. Garcia-Blanco, E. E. Ooi, Dengue subgenomic RNA binds TRIM25 to inhibit interferon expression for epidemiological fitness. *Science* **350**, 217–221 (2015).
43. A. Syenina, D. Vijaykrishna, E. S. Gan, H. C. Tan, M. M. Choy, T. Siriphanitchakorn, C. Cheng, S. G. Vasudevan, E. E. Ooi, Positive epistasis between viral polymerase and the 3′ untranslated region of its genome reveals the epidemiologic fitness of dengue virus. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 11038–11047 (2020).
44. M. Oliveira, W. Lert-Itthiporn, B. Cavadas, V. Fernandes, A. Chuansumrit, O. Anunciação, I. Casademont, F. Koeth, M. Penova, K. Tangnararatchakit, C. C. Khor, R. Paul, P. Malasit, F. Matsuda, E. Simon-Lorière, P. Suriyaphol, L. Pereira, A. Sakuntabhai, Joint ancestry and association test indicate two distinct pathogenic pathways involved in classical dengue fever and dengue shock syndrome. *PLoS Negl Trop Dis* **12**, e0006202 (2018).
45. J. G. H. Low, A. Ong, L. K. Tan, S. Chaterji, A. Chow, W. Y. Lim, K. W. Lee, R. Chua, C. R. Chua, S. W. S. Tan, Y. B. Cheung, M. L. Hibberd, S. G. Vasudevan, L.-C. Ng, Y. S. Leo, E. E. Ooi, The early clinical features of dengue in adults: Challenges for early clinical diagnosis. *PLoS Negl. Trop. Dis.* **5**, e1191 (2011).
46. L. C. Katzelnick, E. Harris; Participants in the Summit on Dengue Immune Correlates of Protection, Immune correlates of protection for dengue: State of the art and research agenda. *Vaccine* **35**, 4659–4669 (2017).
47. R. E. Chen, M. S. Diamond, Dengue mouse models for evaluating pathogenesis and countermeasures. *Curr. Opin. Virol.* **43**, 50–58 (2020).
48. R. M. Zellweger, S. Shresta, Mouse models to study dengue virus immunology and pathogenesis. *Front. Immunol.* **5**, 151 (2014).
49. S. B. Halstead, H. Shotwell, J. Casals, Studies on the pathogenesis of dengue infection in monkeys. I. Clinical laboratory responses to primary infection. *J Infect Dis* **128**, 7–14 (1973).
50. S. B. Halstead, H. Shotwell, J. Casals, Studies on the pathogenesis of dengue infection in monkeys. II. Clinical laboratory responses to heterologous infection. *J. Infect. Dis.* **128**, 15–22 (1973).
51. C. A. Sariol, L. J. White, Utility, limitations, and future of non-human primates for dengue research and vaccine development. *Front. Immunol.* **5**, 452 (2014).
52. S. J. Thomas, Dengue human infection model. *Hum. Vaccin. Immunother.* **9**, 1587–1590 (2013).
53. W. Sun, K. H. Eckels, J. R. Putnak, A. G. Lyons, S. J. Thomas, D. W. Vaughn, R. V. Gibbons, S. Fernandez, V. J. Gunther, M. P. Mammen, J. D. Statler, B. L. Innis, Experimental dengue virus challenge of human subjects previously vaccinated with live attenuated tetravalent dengue vaccines. *J. Infect. Dis.* **207**, 700–708 (2013).
54. M. P. Mammen, A. Lyons, B. L. Innis, W. Sun, D. McKinney, R. C. Y. Chung, K. H. Eckels, R. Putnak, N. Kanesa-thasan, J. M. Scherer, J. Statler, L. V. Asher, S. J. Thomas, D. W. Vaughn, Evaluation of dengue virus strains for human challenge studies. *Vaccine* **32**, 1488–1494 (2014).
55. B. D. Kirkpatrick, S. S. Whitehead, K. K. Pierce, C. M. Tibery, P. L. Grier, N. A. Hynes, C. J. Larsson, B. P. Sabundayo, K. R. Talaat, A. Janiak, M. P. Carmolli, C. J. Luke, S. A. Diehl, A. P. Durbin, The live attenuated dengue vaccine TV003 elicits complete protection against dengue in a human challenge model. *Sci. Transl. Med.* **8**, 330ra336 (2016).
56. T. P. Endy, D. Wang, M. E. Polhemus, R. G. Jarman, L. E. Jasper, G. Gromowski, L. Lin, R. A. De La Barra, H. Friberg, J. R. Currier, M. Abbott, L. Ware, M. Klick, K. M. Paolino, D. C. Blair, K. Eckels, W. Rutvisuttinunt, S. J. Thomas, A phase 1, open-label assessment of a dengue virus-1 live virus human challenge strain. *J. Infect. Dis.* **223**, 258–267 (2021).
57. N. L. Vuong, N. T. H. Quyen, N. T. H. Tien, N. M. Tuan, D. T. H. Kien, P. K. Lam, D. T. H. Tam, T. van Ngoc, S. Yacoub, T. Jaenisch, R. B. Geskus, C. P. Simmons, B. A. Wills, Higher plasma viremia in the febrile phase is associated with adverse dengue outcomes irrespective of infecting serotype or host immune status: An analysis of 5642 Vietnamese cases. *Clin. Infect. Dis.* **72**, e1074–e1083 (2021).
58. H. Salje, M. T. Alera, M. N. Chua, T. Hunsawong, D. Ellison, A. Srikiatkhachorn, R. G. Jarman, G. D. Gromowski, I. Rodriguez-Barraquer, S. Cauchemez, D. A. T. Cummings, L. Macareo, I.-K. Yoon, S. Fernandez, A. L. Rothman, Evaluation of the extended efficacy of the

Dengvaxia vaccine against symptomatic and subclinical dengue infection. *Nat. Med.* **27**, 1395–1400 (2021).
59. A. B. Sabin, I. Young, A complement fixation test for dengue. *Proc. Soc. Exp. Biol. Med.* **69**, 478–480 (1948).
60. B. H. Sweet, A. B. Sabin, Properties and antigenic relationships of hemagglutinins associated with the dengue viruses. *J. Immunol.* **73**, 363–373 (1954).
61. L. C. Rowan, An outbreak of dengue-like fever, North Queensland, 1954; serological findings with the virus neutralization and complement fixation tests. *Med. J. Aust.* **46**, 323–328 (1959).
62. S. B. Halstead, J. Casals, H. Shotwell, N. Palumbo, Studies on the immunization of monkeys against dengue. *Am. J. Trop. Med. Hyg.* **22**, 365–374 (1973).
63. S. B. Halstead, In vivo enhancement of dengue virus infection in rhesus monkeys by passively transferred antibody. *J. Infect. Dis.* **140**, 527–533 (1979).
64. P. K. Russell, A. Nisalak, P. Sukhavachana, S. Vivona, A plaque reduction test for dengue virus neutralizing antibodies. *J. Immunol.* **99**, 285–290 (1967).
65. A. T. De Madrid, J. S. Porterfield, A simple micro-culture method for the study of group B arboviruses. *Bull. World Health Organ.* **40**, 113–121 (1969).
66. N. Fujita, M. Tamura, S. Hotta, Dengue virus plaque formation on microplate cultures and its application to virus neutralization (38564). *Proc. Soc. Exp. Biol. Med.* **148**, 472–475 (1975).
67. D. M. Morens, S. B. Halstead, L. K. Larsen, Comparison of dengue virus plaque reduction neutralization by macro and 'Semi-Micro' methods in LLC-MK2 cells. *Microbiol. Immunol.* **29**, 1197–1205 (1985).
68. J. J. Waggoner, A. Balmaseda, L. Gresh, M. K. Sahoo, M. Montoya, C. Wang, J. Abeynayake, G. Kuan, B. A. Pinsky, E. Harris, Homotypic dengue virus reinfections in Nicaraguan children. *J. Infect. Dis.* **214**, 986–993 (2016).
69. Z. Moodie, M. Juraska, Y. Huang, Y. Zhuang, Y. Fong, L. N. Carpp, S. G. Self, L. Chambonneau, R. Small, N. Jackson, F. Noriega, P. B. Gilbert, Neutralizing antibody correlates analysis of tetravalent dengue vaccine efficacy trials in asia and latin America. *J. Infect. Dis.* **217**, 742–753 (2018).
70. D. Buddhari, J. Aldstadt, T. P. Endy, A. Srikiatkhachorn, B. Thaisomboonsuk, C. Klungthong, A. Nisalak, B. Khuntirat, R. G. Jarman, S. Fernandez, S. J. Thomas, T. W. Scott, A. L. Rothman, I.-K. Yoon, Dengue virus neutralizing antibody levels associated with protection from infection in thai cluster studies. *PLoS Negl. Trop. Dis.* **8**, e3230 (2014).
71. W. Zhang, P. R. Chipman, J. Corver, P. R. Johnson, Y. Zhang, S. Mukhopadhyay, T. S. Baker, J. H. Strauss, M. G. Rossmann, R. J. Kuhn, Visualization of membrane protein domains by cryo-electron microscopy of dengue virus. *Nat. Struct. Biol.* **10**, 907–912 (2003).
72. X. Zhang, P. Ge, X. Yu, J. M. Brannan, G. Bi, Q. Zhang, S. Schein, Z. H. Zhou, Cryo-EM structure of the mature dengue virus at 3.5-Å resolution. *Nat. Struct. Mol. Biol.* **20**, 105–110 (2013).
73. T. C. Pierson, M. S. Diamond, A game of numbers: The stoichiometry of antibody-mediated neutralization of flavivirus infection. *Prog. Mol. Biol. Transl. Sci.* **129**, 141–166 (2015).
74. T. C. Pierson, Q. Xu, S. Nelson, T. Oliphant, G. E. Nybakken, D. H. Fremont, M. S. Diamond, The stoichiometry of antibody-mediated neutralization and enhancement of West Nile virus infection. *Cell Host Microbe* **1**, 135–145 (2007).
75. I.-M. Yu, W. Zhang, H. A. Holdaway, L. Li, V. A. Kostyuchenko, P. R. Chipman, R. J. Kuhn, M. G. Rossmann, J. Chen, Structure of the immature dengue virus at low pH primes proteolytic maturation. *Science* **319**, 1834–1837 (2008).
76. J. Junjhon, T. J. Edwards, U. Utaipat, V. D. Bowman, H. A. Holdaway, W. Zhang, P. Keelapang, C. Puttikhunt, R. Perera, P. R. Chipman, W. Kasinrerk, P. Malasit, R. J. Kuhn, N. Sittisombut, Influence of pr-M cleavage on the heterogeneity of extracellular dengue virus particles. *J. Virol.* **84**, 8353–8358 (2010).
77. L. Premkumar, B. Segovia-Chumbez, R. Jadi, D. R. Martinez, R. Raut, A. J. Markmann, C. Cornaby, L. Bartelt, S. Weiss, Y. Park, C. E. Edwards, E. Weimer, E. M. Scherer, N. Rouphael, S. Edupuganti, D. Weiskopf, L. V. Tse, Y. J. Hou, D. Margolis, A. Sette, M. H. Collins, J. Schmitz, R. S. Baric, A. M. de Silva, The receptor binding domain of the viral spike protein is an immunodominant and highly specific target of antibodies in SARS-CoV-2 patients. *Sci. Immunol.* **5**, eabc8413 (2020).
78. E. P. Teoh, P. Kukkaro, E. W. Teo, A. P. C. Lim, T. T. Tan, A. Yip, W. Schul, M. Aung, V. A. Kostyuchenko, Y. S. Leo, S. H. Chan, K. G. C. Smith, A. H. Y. Chan, G. Zou, E. E. Ooi, D. M. Kemeny, G. K. Tan, J. K. W. Ng, M. L. Ng, S. Alonso, D. Fisher, P.-Y. Shi, B. J. Hanson, S.-M. Lok, P. A. MacAry, The structural basis for serotype-specific neutralization of dengue virus by a human antibody. *Sci. Transl. Med.* **4**, 139ra183 (2012).
79. R. de Alwis, S. A. Smith, N. P. Olivarez, W. B. Messer, J. P. Huynh, W. M. P. B. Wahala, L. J. White, M. S. Diamond, R. S. Baric, J. E. Crowe Jr., A. M. de Silva, Identification of human neutralizing antibodies that bind to complex epitopes on dengue virions. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 7439–7444 (2012).
80. G. Fibriansah, J. L. Tan, S. A. Smith, A. R. de Alwis, T.-S. Ng, V. A. Kostyuchenko, K. D. Ibarra, J. Wang, E. Harris, A. de Silva, J. E. Crowe Jr., S.-M. Lok, A potent anti-dengue human antibody preferentially recognizes the conformation of E protein monomers assembled on the virus surface. *EMBO Mol. Med.* **6**, 358–371 (2014).
81. G. Fibriansah, J. L. Tan, S. A. Smith, R. de Alwis, T.-S. Ng, V. A. Kostyuchenko, R. S. Jadi, P. Kukkaro, A. M. de Silva, J. E. Crowe, S.-M. Lok, A highly potent human antibody neutralizes dengue virus serotype 3 by binding across three surface proteins. *Nat. Commun.* **6**, 6341 (2015).
82. E. N. Gallichotte, T. J. Baric, B. L. Yount, D. G. Widman, A. Durbin, S. Whitehead, R. S. Baric, A. M. de Silva, Human dengue virus serotype 2 neutralizing antibodies target two distinct quaternary epitopes. *PLOS Pathog.* **14**, e1006934 (2018).
83. E. N. Gallichotte, D. G. Widman, B. L. Yount, W. M. Wahala, A. Durbin, S. Whitehead, C. A. Sariol, J. E. Crowe Jr., A. M. de Silva, R. S. Baric, A new quaternary structure epitope on dengue virus serotype 2 is the target of durable type-specific neutralizing antibodies. *MBio* **6**, e01461–e01415 (2015).
84. E. N. Gallichotte, R. S. Baric, A. M. de Silva, The molecular specificity of the human antibody response to dengue virus infections. *Adv. Exp. Med. Biol.* **1062**, 63–76 (2018).
85. J. A. Swanstrom, S. Henein, J. A. Plante, B. L. Yount, D. G. Widman, E. N. Gallichotte, H. J. Dean, J. E. Osorio, C. D. Partidos, A. M. de Silva, R. S. Baric, Analyzing the human serum antibody responses to a live attenuated tetravalent dengue vaccine candidate. *J. Infect. Dis.* **217**, 1932–1941 (2018).
86. S. A. Smith, R. de Alwis, N. Kose, A. P. Durbin, S. S. Whitehead, A. M. de Silva, J. E. Crowe Jr., Human monoclonal antibodies derived from memory B cells following live attenuated dengue virus vaccination or natural infection exhibit similar characteristics. *J Infect Dis* **207**, 1898–1908 (2013).
87. J. A. Swanstrom, U. K. Nivarthi, B. Patel, M. J. Delacruz, B. Yount, D. G. Widman, A. P. Durbin, S. S. Whitehead, A. M. de Silva, R. S. Baric, Beyond neutralizing antibody levels: The epitope specificity of antibodies induced by national institutes of health monovalent dengue virus vaccines. *J. Infect. Dis.* **220**, 219–227 (2019).
88. S. B. Halstead, E. J. O'Rourke, Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. *J. Exp. Med.* **146**, 201–217 (1977).
89. S. B. Halstead, E. J. O'Rourke, Antibody-enhanced dengue virus infection in primate leukocytes. *Nature* **265**, 739–741 (1977).
90. M. G. Guzman, D. J. Gubler, A. Izquierdo, E. Martinez, S. B. Halstead, Dengue infection. *Nat. Rev. Dis. Primers* **2**, 16055 (2016).
91. T. N. B. Chau, N. T. Hieu, K. L. Anders, M. Wolbers, L. B. Lien, L. T. M. Hieu, T. T. Hien, N. T. Hung, J. Farrar, S. Whitehead, C. P. Simmons, Dengue virus infections and maternal antibody decay in a prospective birth cohort study of Vietnamese infants. *J. Infect. Dis.* **200**, 1893–1900 (2009).
92. S. C. Kliks, S. Nimmanitya, A. Nisalak, D. S. Burke, Evidence that maternal dengue antibodies are important in the development of dengue hemorrhagic fever in infants. *Am. J. Trop. Med. Hyg.* **38**, 411–419 (1988).
93. C. P. Simmons, T. N. B. Chau, T. T. Thuy, N. M. Tuan, D. M. Hoang, N. T. Thien, L. B. Lien, N. T. Quy, N. T. Hieu, T. T. Hien, C. McElnea, P. Young, S. Whitehead, N. T. Hung, J. Farrar, Maternal antibody and viral factors in the pathogenesis of dengue virus in infants. *J Infect Dis* **196**, 416–424 (2007).
94. D. H. Libraty, P. R. Young, D. Pickering, T. P. Endy, S. Kalayanarooj, S. Green, D. W. Vaughn, A. Nisalak, F. A. Ennis, A. L. Rothman, High circulating levels of the dengue virus nonstructural protein NS1 early in dengue illness correlate with the development of dengue hemorrhagic fever. *J. Infect. Dis.* **186**, 1165–1168 (2002).
95. D. W. Vaughn, S. Green, S. Kalayanarooj, B. L. Innis, S. Nimmannitya, S. Suntayakorn, T. P. Endy, B. Raengsakulrach, A. L. Rothman, F. A. Ennis, A. Nisalak, Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *J. Infect. Dis.* **181**, 2–9 (2000).
96. L. C. Katzelnick, L. Gresh, M. E. Halloran, J. C. Mercado, G. Kuan, A. Gordon, A. Balmaseda, E. Harris, Antibody-dependent enhancement of severe dengue disease in humans. *Science* **358**, 929–932 (2017).
97. H. Salje, D. A. T. Cummings, I. Rodriguez-Barraquer, L. C. Katzelnick, J. Lessler, C. Klungthong, B. Thaisomboonsuk, A. Nisalak, A. Weg, D. Ellison, L. Macareo, I.-K. Yoon, R. Jarman, S. Thomas, A. L. Rothman, T. Endy, S. Cauchemez, Reconstruction of antibody dynamics and infection histories to evaluate dengue risk. *Nature* **557**, 719–723 (2018).
98. K. R. Chan, S. L.-X. Zhang, H. C. Tan, Y. K. Chan, A. Chow, A. P. C. Lim, S. G. Vasudevan, B. J. Hanson, E. E. Ooi, Ligation of Fc gamma receptor IIB inhibits antibody-dependent enhancement of dengue virus infection. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 12479–12484 (2011).
99. K. Boonnak, B. M. Slike, G. C. Donofrio, M. A. Marovich, Human FcγRII cytoplasmic domains differentially influence antibody-mediated dengue virus infection. *J. Immunol.* **190**, 5659–5665 (2013).
100. K. M. Dhodapkar, D. Banerjee, J. Connolly, A. Kukreja, E. Matayeva, M. C. Veri, J. V. Ravetch, R. M. Steinman, M. V. Dhodapkar, Selective blockade of the inhibitory Fcgamma receptor

(FcgammaRIIB) in human dendritic cells and monocytes induces a type I interferon response program. *J. Exp. Med.* **204**, 1359–1369 (2007).

101. K. R. Chan, E. Z. Ong, H. C. Tan, S. L. X. Zhang, Q. Zhang, K. F. Tang, N. Kaliaperumal, A. P. C. Lim, M. L. Hibberd, S. H. Chan, J. E. Connolly, M. N. Krishnan, S. M. Lok, B. J. Hanson, C.-N. Lin, E. E. Ooi, Leukocyte immunoglobulin-like receptor B1 is critical for antibody-dependent dengue. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 2722–2727 (2014).
102. F. Nimmerjahn, A. Lux, LILR-B1 blocks activating FcγR signaling to allow antibody dependent enhancement of dengue virus infection. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 2404–2405 (2014).
103. E. Z. Ong, S. L. Zhang, H. C. Tan, E. S. Gan, K. R. Chan, E. E. Ooi, Dengue virus compartmentalization during antibody-enhanced infection. *Sci. Rep.* **7**, 40923 (2017).
104. C. Y. Y. Chan, J. Z. H. Low, E. S. Gan, E. Z. Ong, S. L.-X. Zhang, H. C. Tan, X. Chai, S. Ghosh, E. E. Ooi, K. R. Chan, Antibody-dependent dengue virus entry modulates cell intrinsic responses for enhanced infection. *mSphere* **4**, e00528-19 (2019).
105. D. W. Vaughn, Invited commentary: Dengue lessons from Cuba. *Am. J. Epidemiol.* **152**, 800–803 (2000).
106. D. H. Libraty, L. P. Acosta, V. Tallo, E. Segubre-Mercado, A. Bautista, J. A. Potts, R. G. Jarman, I.-K. Yoon, R. V. Gibbons, J. D. Brion, R. Z. Capeding, A prospective nested case-control study of Dengue in infants: Rethinking and refining the antibody-dependent enhancement dengue hemorrhagic fever model. *PLOS Med.* **6**, e1000171 (2009).
107. H. Clapham, D. A. T. Cummings, A. Nisalak, S. Kalayanarooj, B. Thaisomboonsuk, C. Klungthong, S. Fernandez, A. Srikiatkhachorn, L. R. Macareo, J. Lessler, J. Reiser, I.-K. Yoon, Epidemiology of infant dengue cases illuminates serotype-specificity in the interaction between immunity and disease, and changes in transmission dynamics. *PLoS Negl. Trop. Dis.* **9**, e0004262 (2015).
108. J. R. Fried, R. V. Gibbons, S. Kalayanarooj, S. J. Thomas, A. Srikiatkhachorn, I.-K. Yoon, R. G. Jarman, S. Green, A. L. Rothman, D. A. T. Cummings, Serotype-specific differences in the risk of dengue hemorrhagic fever: An analysis of data collected in Bangkok, Thailand from 1994 to 2006. *PLoS Negl. Trop. Dis.* **4**, e617 (2010).
109. K.-M. Soo, B. Khalid, S.-M. Ching, H.-Y. Chee, Meta-analysis of dengue severity during infection by different dengue virus serotypes in primary and secondary infections. *PLOS ONE* **11**, e0154760 (2016).
110. S. B. Halstead, S. N. Cohen, Dengue hemorrhagic fever at 60 years: Early evolution of concepts of causation and treatment. *Microbiol. Mol. Biol. Rev.* **79**, 281–291 (2015).
111. R. Aguas, I. Dorigatti, L. Coudeville, C. Luxemburger, N. M. Ferguson, Cross-serotype interactions and disease outcome prediction of dengue infections in Vietnam. *Sci. Rep.* **9**, 9395 (2019).
112. D. R. Glasner, H. Puerta-Guardo, P. R. Beatty, E. Harris, The good, the bad, and the shocking: The multiple roles of dengue virus nonstructural protein 1 in protection and pathogenesis. *Annu. Rev. .Virol.* **5**, 227–253 (2018).
113. J. J. Schlesinger, M. W. Brandriss, E. E. Walsh, Protection of mice against dengue 2 virus encephalitis by immunization with the dengue 2 virus non-structural glycoprotein NS1. *J. Gen. Virol.* **68** (Pt 3), 853–857 (1987).
114. S. B. Biering, D. L. Akey, M. P. Wong, W. C. Brown, N. T. N. Lo, H. Puerta-Guardo, F. T. G. de Sousa, C. Wang, J. R. Konwerski, D. A. Espinosa, N. J. Bockhaus, D. R. Glasner, J. Li, S. F. Blanc, E. Y. Juan, S. J. Elledge, M. J. Mina, P. R. Beatty, J. L. Smith, E. Harris, Structural basis for antibody inhibition of flavivirus NS1-triggered endothelial dysfunction. *Science* **371**, 194–200 (2021).
115. N. Modhiran, H. Song, L. Liu, C. Bletchly, L. Brillault, A. A. Amarilla, X. Xu, J. Qi, Y. Chai, S. T. M. Cheung, R. Traves, Y. X. Setoh, S. Bibby, C. A. P. Scott, M. E. Freney, N. D. Newton, A. A. Khromykh, K. J. Chappell, D. A. Muller, K. J. Stacey, M. J. Landsberg, Y. Shi, G. F. Gao, P. R. Young, D. Watterson, A broadly protective antibody that targets the flavivirus NS1 protein. *Science* **371**, 190–194 (2021).
116. D. H. Libraty, T. P. Endy, H.-S. H. Houng, S. Green, S. Kalayanarooj, S. Suntayakorn, W. Chansiriwongs, D. W. Vaughn, A. Nisalak, F. A. Ennis, A. L. Rothman, Differing influences of virus burden and immune activation on disease severity in secondary dengue-3 virus infections. *J Infect Dis* **185**, 1213–1221 (2002).
117. V. Duong, S. Ly, P. Lorn Try, A. Tuiskunen, S. Ong, N. Chroeung, A. Lundkvist, I. Leparc-Goffart, V. Deubel, S. Vong, P. Buchy, Clinical and virological factors influencing the performance of a NS1 antigen-capture assay and potential use as a marker of dengue disease severity. *PLOS Negl. Trop. Dis.* **5**, e1244 (2011).
118. T. V. Ngoc, D. T. Ha, V. T. T. Hang, N. T. T. Kieu, P. R. Young, J. J. Farrar, C. P. Simmons, M. Wolbers, B. A. Wills, Kinetics of plasma viremia and soluble nonstructural protein 1 concentrations in dengue: Differential effects according to serotype and immune status. *J. Infect. Dis.* **203**, 1292–1300 (2011).
119. T. J. Braciale, Y. S. Hahn, Immunity to viruses. *Immunol. Rev.* **255**, 5–12 (2013).
120. C. M. Smith, N. S. Wilson, J. Waithman, J. A. Villadangos, F. R. Carbone, W. R. Heath, G. T. Belz, Cognate $CD4^+$ T cell licensing of dendritic cells in $CD8^+$T cell immunity. *Nat. Immunol.* **5**, 1143–1148 (2004).
121. R. V. Luckheeram, R. Zhou, A. D. Verma, B. Xia, $CD4^+$T cells: Differentiation and functions. *Clin. Dev. Immunol.* **2012**, 925135 (2012).
122. A. L. Rothman, Immunity to dengue virus: A tale of original antigenic sin and tropical cytokine storms. *Nat. Rev. Immunol.* **11**, 532–543 (2011).
123. A. L. St. John, A. P. S. Rathore, Adaptive immune responses to primary and secondary dengue virus infections. *Nat. Rev. Immunol.* **19**, 218–230 (2019).
124. J. Mongkolsapaya, W. Dejnirattisai, X.-N. Xu, S. Vasanawathana, N. Tangthawornchaikul, A. Chairunsri, S. Sawasdivorn, T. Duangchinda, T. Dong, S. Rowland-Jones, P.-T. Yenchitsomanus, A. McMichael, P. Malasit, G. Screaton, Original antigenic sin and apoptosis in the pathogenesis of dengue hemorrhagic fever. *Nat. Med.* **9**, 921–927 (2003).
125. A. Grifoni, H. Voic, S. K. Dhanda, C. K. Kidd, J. D. Brien, S. Buus, A. Stryhn, A. P. Durbin, S. Whitehead, S. A. Diehl, A. D. de Silva, A. Balmaseda, E. Harris, D. Weiskopf, A. Sette, T cell responses induced by attenuated flavivirus vaccination are specific and show limited cross-reactivity with other flavivirus species. *J. Virol.* **94**, e00089–e00020 (2020).
126. D. Weiskopf, M. A. Angelo, E. L. de Azeredo, J. Sidney, J. A. Greenbaum, A. N. Fernando, A. Broadwater, R. V. Kolla, A. D. de Silva, A. M. de Silva, K. A. Mattia, B. J. Doranz, H. M. Grey, S. Shresta, B. Peters, A. Sette, Comprehensive analysis of dengue virus-specific responses supports an HLA-linked protective role for $CD8^+$ T cells. *Proc. Natl. Acad. Sci. U.S.A.* **110**, E2046–E2053 (2013).
127. D. Weiskopf, D. J. Bangs, J. Sidney, R. V. Kolla, A. D. de Silva, A. M. de Silva, S. Crotty, B. Peters, A. Sette, Dengue virus infection elicits highly polarized CX3CR1+ cytotoxic $CD4^+$ T cells associated with protective immunity. *Proc. Natl. Acad. Sci. U.S.A.* **112**, E4256–E4263 (2015).
128. S. Hatch, T. P. Endy, S. Thomas, A. Mathew, J. Potts, P. Pazoles, D. H. Libraty, R. Gibbons, A. L. Rothman, Intracellular cytokine production by dengue virus-specific T cells correlates with subclinical secondary infection. *J Infect Dis* **203**, 1282–1291 (2011).
129. K. H. Ng, S. L. Zhang, H. C. Tan, S. S. Kwek, O. M. Sessions, C.-Y. Chan, I. D. Liu, C. K. Lee, P. A. Tambyah, E. E. Ooi, H.-K. Yap, Persistent dengue infection in an Immunosuppressed patient reveals the roles of humoral and cellular immune responses in virus clearance. *Cell Host Microbe* **26**, 601–605.e3 (2019).
130. J. X. Y. Sim, E. S. Gan, H. C. Tan, M. M. Choy, H. M. Wong, B. H. Tan, T. Kee, Q. Y. Ho, S. Thangaraju, R. T. P. Lin, E. E. Ooi, J. G.-H. Low, Aviremic organ transplant dengue virus transmission—A case report. *Am. J. Transplant.* **21**, 1944–1947 (2021).
131. A. Grifoni, M. A. Angelo, B. Lopez, P. H. O'Rourke, J. Sidney, C. Cerpas, A. Balmaseda, C. G. T. Silveira, A. Maestri, P. R. Costa, A. P. Durbin, S. A. Diehl, E. Phillips, S. Mallal, A. D. de Silva, G. Nchinda, C. Nkenfou, M. H. Collins, A. M. de Silva, M. Q. Lim, P. A. Macary, F. Tatullo, T. Solomon, V. Satchidanandam, A. Desai, V. Ravi, J. Coloma, L. Turtle, L. Rivino, E. G. Kallas, B. Peters, E. Harris, A. Sette, D. Weiskopf, Global assessment of dengue virus-specific $CD4^+$ T cell responses in dengue-endemic areas. *Front. Immunol.* **8**, 1309 (2017).
132. L. Rivino, E. A. P. Kumaran, V. Jovanovic, K. Nadua, E. W. Teo, S. W. Pang, G. H. Teo, V. C. H. Gan, D. C. Lye, Y. S. Leo, B. J. Hanson, K. G. C. Smith, A. Bertoletti, D. M. Kemeny, P. A. MacAry, Differential targeting of viral components by $CD4^+$ versus $CD8^+$ T lymphocytes in dengue virus infection. *J. Virol.* **87**, 2693–2706 (2013).
133. B. Guy, N. Nougarede, S. Begue, V. Sanchez, N. Souag, M. Carre, L. Chambonneau, D. N. Morrisson, D. Shaw, M. Qiao, R. Dumas, J. Lang, R. Forrat, Cell-mediated immunity induced by chimeric tetravalent dengue vaccine in naive or flavivirus-primed subjects. *Vaccine* **26**, 5712–5721 (2008).
134. V. Tricou, R. Gottardo, M. A. Egan, F. Clement, G. Leroux-Roels, X. Sáez-Llorens, A. Borkowski, D. Wallace, H. J. Dean, Characterization of the cell-mediated immune response to Takeda's live-attenuated tetravalent dengue vaccine in adolescents participating in a phase 2 randomized controlled trial conducted in a dengue-endemic setting. *Vaccine* **40**, 1143–1151 (2022).
135. A. T. Waickman, K. Victor, T. Li, K. Hatch, W. Rutvisuttinunt, C. Medin, B. Gabriel, R. G. Jarman, H. Friberg, J. R. Currier, Dissecting the heterogeneity of DENV vaccine-elicited cellular immunity using single-cell RNA sequencing and metabolic profiling. *Nat. Commun.* **10**, 3666 (2019).
136. D. Weiskopf, M. A. Angelo, D. J. Bangs, J. Sidney, S. Paul, B. Peters, A. D. de Silva, J. C. Lindow, S. A. Diehl, S. Whitehead, A. Durbin, B. Kirkpatrick, A. Sette, The human $CD8^+$ T cell responses induced by a live attenuated tetravalent dengue vaccine are directed against highly conserved epitopes. *J. Virol.* **89**, 120–128 (2015).
137. D. Weiskopf, C. Cerpas, M. A. Angelo, D. J. Bangs, J. Sidney, S. Paul, B. Peters, F. P. Sanches, C. G. T. Silvera, P. R. Costa, E. G. Kallas, L. Gresh, A. D. de Silva, A. Balmaseda, E. Harris, A. Sette, Human $CD8^+$ T-cell responses against the 4 dengue virus serotypes are associated with distinct patterns of protein targets. *J. Infect. Dis.* **212**, 1743–1751 (2015).
138. C. Phetsouphanh, J. J. Zaunders, A. D. Kelleher, Detecting antigen-specific t cell responses: From bulk populations to single cells. *Int. J. Mol. Sci.* **16**, 18878–18893 (2015).
139. A. T. Tan, J. M. E. Lim, N. le Bert, K. Kunasegaran, A. Chia, M. D. C. Qui, N. Tan, W. N. Chia, R. de Alwis, D. Ying, J. X. Y. Sim, E. E. Ooi, L. F. Wang, M. I. C. Chen, B. E. Young, L. Y. Hsu, J. G. H. Low, D. C. Lye, A. Bertoletti, Rapid measurement of SARS-CoV-2 spike T cells in

whole blood from vaccinated and naturally infected individuals. *J. Clin. Invest.* **131**, e152379 (2021).

140. S. Kalimuddin, Y. F. Z. Chan, O. M. Sessions, K. R. Chan, E. Z. Ong, J. G. Low, A. Bertoletti, E. E. Ooi, An experimental medicine decipher of a minimum correlate of cellular immunity: Study protocol for a double-blind randomized controlled trial. *Front. Immunol.* **14**, 1135979 (2023).
141. L. W. Alexander, R. Ben-Shachar, L. C. Katzelnick, G. Kuan, A. Balmaseda, E. Harris, M. Boots, Boosting can explain patterns of fluctuations of ratios of inapparent to symptomatic dengue virus infections. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2013941118 (2021).
142. S. Collie, J. Champion, H. Moultrie, L. G. Bekker, G. Gray, Effectiveness of BNT162b2 vaccine against Omicron variant in South Africa. *N. Engl. J. Med.* **386**, 494–496 (2022).
143. R. Keeton, M. B. Tincho, A. Ngomti, R. Baguma, N. Benede, A. Suzuki, K. Khan, S. Cele, M. Bernstein, F. Karim, S. V. Madzorera, T. Moyo-Gwete, M. Mennen, S. Skelem, M. Adriaanse, D. Mutithu, O. Aremu, C. Stek, E. du Bruyn, M. A. Van Der Mescht, Z. de Beer, T. R. de Villiers, A. Bodenstein, G. van den Berg, A. Mendes, A. Strydom, M. Venter, J. Giandhari, Y. Naidoo, S. Pillay, H. Tegally, A. Grifoni, D. Weiskopf, A. Sette, R. J. Wilkinson, T. de Oliveira, L.-G. Bekker, G. Gray, V. Ueckermann, T. Rossouw, M. T. Boswell, J. N. Bhiman, P. L. Moore, A. Sigal, N. A. B. Ntusi, W. A. Burgers, C. Riou, T cell responses to SARS-CoV-2 spike cross-recognize Omicron. *Nature* **603**, 488–492 (2022).
144. F. C. M. Kirsebom, N. Andrews, J. Stowe, S. Toffa, R. Sachdeva, E. Gallagher, N. Groves, A.-M. O'Connell, M. Chand, M. Ramsay, J. L. Bernal, COVID-19 vaccine effectiveness against the omicron (BA.2) variant in England. *Lancet Infect. Dis.* **22**, 931–933 (2022).
145. J. Liu, A. Chandrashekar, D. Sellers, J. Barrett, C. Jacob-Dolan, M. Lifton, K. McMahan, M. Sciacca, H. VanWyk, C. Wu, J. Yu, A. R. Y. Collier, D. H. Barouch, Vaccines elicit highly conserved cellular immunity to SARS-CoV-2 Omicron. *Nature* **603**, 493–496 (2022).
146. V. Naranbhai, A. Nathan, C. Kaseke, C. Berrios, A. Khatri, S. Choi, M. A. Getz, R. Tano-Menka, O. Ofoman, A. Gayton, F. Senjobe, Z. Zhao, K. J. St Denis, E. C. Lam, M. Carrington, W. F. Garcia-Beltran, A. B. Balazs, B. D. Walker, A. J. Iafrate, G. D. Gaiha, T cell reactivity to the SARS-CoV-2 Omicron variant is preserved in most but not all individuals. *Cell* **185**, 1041–1051.e6 (2022).
147. A. Tarke, J. Sidney, N. Methot, E. D. Yu, Y. Zhang, J. M. Dan, B. Goodwin, P. Rubiro, A. Sutherland, E. Wang, A. Frazier, S. I. Ramirez, S. A. Rawlings, D. M. Smith, R. da Silva Antunes, B. Peters, R. H. Scheuermann, D. Weiskopf, S. Crotty, A. Grifoni, A. Sette, Impact of SARS-CoV-2 variants on the total $CD4^+$ and $CD8^+$ T cell reactivity in infected or vaccinated individuals. *Cell Rep. Med.* **2**, 100355 (2021).
148. S. Flasche, M. Jit, I. Rodríguez-Barraquer, L. Coudeville, M. Recker, K. Koelle, G. Milne, T. J. Hladish, T. A. Perkins, D. A. T. Cummings, I. Dorigatti, D. J. Laydon, G. España, J. Kelso, I. Longini, J. Lourenco, C. A. B. Pearson, R. C. Reiner, L. Mier-y-Terán-Romero, K. Vannice, N. Ferguson, The long-term safety, public health impact, and cost-effectiveness of routine vaccination with a recombinant, live-attenuated dengue vaccine (Dengvaxia): A model comparison study. *PLOS Med.* **13**, e1002181 (2016).
149. S. Olkowski, B. M. Forshey, A. C. Morrison, C. Rocha, S. Vilcarromero, E. S. Halsey, T. J. Kochel, T. W. Scott, S. T. Stoddard, Reduced risk of disease during postsecondary dengue virus infections. *J. Infect. Dis.* **208**, 1026–1033 (2013).

Acknowledgments

Funding: E.E.O. receives salary support from the Singapore Translational Research Award, and S.K. receives salary support from the Transition Award and NMRC Research Training Fellowship, all awarded by the National Medical Research Council (NMRC) of Singapore. **Competing interests:** E.E.O. has served in various advisory capacities on dengue vaccines for Sanofi Pasteur and MSD. Both authors have served on the advisory boards on dengue vaccines and antiviral drugs for Takeda and Janssen, respectively.

Submitted 23 February 2023
Accepted 8 June 2023
Published 12 July 2023
10.1126/scitranslmed.adh3067

Science Translational Medicine

Insights into dengue immunity from vaccine trials

Eng Eong Ooi and Shirin Kalimuddin

Sci. Transl. Med., **15** (704), eadh3067.
DOI: 10.1126/scitranslmed.adh3067

Science Translational Medicine (ISSN) is published by the American Association for the Advancement of Science. 1200 New York Avenue NW, Washington, DC 20005. The title *Science Translational Medicine* is a registered trademark of AAAS.