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Immune Correlates for Dengue Vaccine Development

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Abstract

Dengue virus is the leading cause of vector-borne viral disease with four serotypes in circulation. Vaccine development has been complicated by the potential for both protection and disease enhancement during heterologous infection. Secondary infection triggers cross-reactive immune memory responses which have varying functional and epitope specificities that determine protection or risk. Strongly neutralizing antibodies to quaternary epitopes may be especially important for virus neutralization. Cell-mediated immunity dominated by Th1 functions may also play an important role. Determining an immune correlate of protection or risk would be highly beneficial for vaccine development but is hampered by mechanistic uncertainties and assay limitations. Clinical efficacy trials and human infection models along with a systems approach may provide future opportunities to elucidate such correlates.

Keywords

Dengue; Dengue Vaccine; Correlate of Protection; Correlate of Risk; Antibody-dependent Enhancement; Humoral Immunity; Cell-mediated Immunity; Human Infection Model; Systems Immunology; Systems Vaccinology

Introduction

Dengue virus (DENV) is the leading cause of vector-borne viral disease globally with an estimated 390 million infections and 96 million symptomatic cases occurring annually [1]. Four antigenically distinct DENV serotypes (DENV-1 to -4) cause dengue. Infection with one serotype confers long lasting protection against the same serotype, but only short term protection against a different serotype [2]. DENV infection triggers an immune response that can result in protection or enhancement of subsequent infection, thus complicating the effort to develop dengue vaccines [3]. A variety of factors including viral characteristics, host immunity and genetics, and epidemiological context, along with the relative timing of these factors, play a role in ultimately protecting against or enhancing infection [4]. In order to

address this problem, vaccine developers have sought to induce simultaneous tetravalent immunity against all four DENV serotypes. However, these efforts have been hampered by an incomplete understanding of the relevant immune responses that contribute to protection or enhancement. A better understanding of these immune parameters is critically needed to inform ongoing dengue vaccine development efforts. The pursuit of dengue immune correlates is further complicated by the limitations of the assays themselves as highlighted by the lack of a standardized neutralization assay making inter-study comparisons difficult [5, 6]. This article will review current knowledge about humoral and cell-mediated immune responses in protection and enhancement of dengue, and prospects to define immune correlates of protection or risk for vaccine development.

Evidence of Immune-mediated Protection or Risk in Natural Infection

Studies of natural DENV infection have shown primary infection typically results in less severe disease than secondary infection [7, 8]. Primary infection confers long lasting protection against the infecting serotype. Paradoxically, the immune response to the primary infection can result in enhancement of subsequent heterologous infection. This potential for enhancement can vary over time. Epidemiological studies have indicated temporary protection occurs against a heterologous serotype with the duration of that protection estimated to vary from two months to three years [2, 9–12]. After 2–3 years, there appears to be a higher likelihood of symptomatic disease, supporting the notion of immune enhancement [9, 10]. This situation is mirrored in infants who appear to have temporary protection by passively acquired maternal antibody during the first few months of life followed by several months of increased risk of disease as maternal antibodies decline to presumably non-protective levels [13, 14]. This disease pattern in infants implies that IgG plays an important role in disease enhancement supporting the theory of antibody-dependent enhancement (ADE) in which non-neutralizing antibodies complexed with DENV facilitate viral entry into host mononuclear cells via Fc receptors [15, 16].

Relatively few field studies have been done in which immune status has been measured prior to natural infection to indicate protection or enhancement. In a cohort study in Thailand, pre-existing neutralizing antibody levels were not consistently associated with lower risk of subsequent homotypic infection, in particular with DENV-2 infection [17]. However, in cluster studies in Thailand, lower neutralizing antibody levels to DENV-1, -2 and -4 did appear to be associated with higher risk of homotypic infection when the neutralizing antibodies were measured within two weeks before infection [18]. Interestingly, the titer thresholds that were associated with increased risk seemed to be much higher for DENV-2 than DENV-1 or -4, although differences in subject ages among serotypes may have affected these thresholds [18]. A cohort study in Sri Lanka demonstrated that the baseline serotype-specific neutralizing antibody profile but not necessarily titer levels was associated with the risk of symptomatic secondary infection suggesting the importance of prior infection history rather than neutralizing titer levels in disease risk [19]. Cell-mediated immunity studies from a Thai cohort found that pre-existing IFN-gamma secreting memory T cells were associated with lower severity of secondary infection [20, 21] suggesting a protective role for specific functional subsets of T cells.

Some epidemiological studies have indicated post-secondary infections (i.e., third or greater sequential infections) are rarely severe [22] and are more likely to be subclinical [23], suggesting repeated exposures to DENV result in cross-protective immunity. The protective immunity induced by sequential exposures and the process by which this immunity is elicited are not well understood. However, heterologous infection occurring sequentially over time is likely to elicit a different immune response from tetravalent vaccination. Sequential heterologous immunization has, in fact, been suggested as an alternative to simultaneous tetravalent administration [24]. Understanding the molecular targets and the functional characteristics of the immune effectors would be invaluable in the identification of immune correlates and development of effective vaccines.

Evidence of Immune-mediated Protection or Risk in Vaccine Studies

Although a great deal of progress has been made in the pursuit of dengue vaccines over the past several years, CYD-TDV, sponsored by Sanofi Pasteur, remains the only vaccine candidate that has undergone clinical efficacy trials [25–29]. Two additional live attenuated tetravalent dengue vaccine candidates, TDV [30], sponsored by Takeda, and TV005 [31, 32], developed by the U.S. National Institutes of Health, are now close to entering clinical efficacy trials while other vaccine candidates are in various stages of early clinical and preclinical development using a variety of strategies including inactivated whole virus, recombinant constructs, DNA, virus vectors, and virus-like particles (VLPs) [33–36].

CYD-TDV is a live attenuated tetravalent chimeric vaccine consisting of the yellow fever virus 17D backbone with DENV pre-membrane (prM) and envelope (E) proteins from each of the four DENV serotypes [25]. CYD-TDV administered in three doses over 12 months was evaluated in a phase IIb clinical trial in Thailand and phase III trials in Asia and Latin America demonstrating good efficacy against DENV-3 and -4, marginal efficacy against DENV-1, poor efficacy against DENV-2, and generally lower efficacy among flavivirus-naïve than primed subjects [26–28]. In addition, vaccinated children 2–5 years of age had an almost eight-fold increased relative risk of dengue hospitalization during the third year of the Asian phase III trial [29]. The vaccine, nevertheless, appeared to have higher overall efficacy against severe disease compared to all symptomatic infections. The poor efficacy shown against DENV-2 was unexpected since immunogenicity as measured by plaque reduction neutralization tests was robust to all four serotypes. Clearly, homotypic neutralizing titer levels after vaccination were insufficient to predict risk of homologous infection. The lower efficacy in flavivirus-naïve subjects was also notable given the possibility that CYD-TDV's protective effect in primed subjects may have been driven by boosting of pre-existing immune memory. Unfortunately, direct measurement of pre-vaccination immune status was only possible in a subset of subjects for which baseline blood samples were available. A multivariate model applied to five different CYD-TDV phase II trials, however, showed neutralizing antibody response to vaccination was principally predicted by baseline neutralizing antibody titers [37]. A similar model applied to the phase III trials may be able to infer baseline primed status for more subjects and the impact of priming on protection or risk. In terms of cell-mediated immune parameters, peripheral blood mononuclear cells were not obtained in the CYD-TDV clinical efficacy trials

preventing direct assessment of the role of cell-mediated immunity in vaccine induced protection.

Correlates of Humoral Immunity in Dengue

DENV genome encodes 10 viral products including three structural proteins: envelope (E), pre-membrane/membrane (prM/M), and capsid (C); and seven non-structural proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. All structural proteins have been shown to be targets of antibodies in natural infection with most of the antibodies being specific to prM and E proteins [38]. Antibodies to non-structural proteins include those specific to NS1, NS3 and NS5 [39, 40].

The surface of the viral particle is made up of prM and E proteins. The E protein binds cellular receptor(s) and mediates viral entry. The crystal structure of the E protein has been solved and is composed of three domains: envelope domains I, II and III (EDI, EDII, and EDIII, respectively). EDI forms the central part of the molecule and is connected to EDII and EDIII by flexible linkers [41]. EDII contains 13 highly conserved amino acids which form a fusion loop that mediates fusion of viral envelope to host cell membrane, a step necessary for the release of viral genome into the cytosol [42]. EDIII serves a critical function in binding host cell receptor(s). Differences in amino acid sequences in EDIII provide the basis for the antigenically distinct serotypes of DENV [43, 44]. These domains are connected to the transmembrane anchor at the C-terminus via a short stem region that is involved in the late stage of the fusion process [45, 46]. PrM/M protein functions as a chaperone for E protein by preventing exposure of the fusion loop that could lead to premature fusion [47]. PrM and E proteins on the viral surface undergo changes in association and arrangement during the maturation process. The surface of immature viral particles consists of 60 prM-E trimeric complexes arranged in a spike pattern with the prM protein interacting with the tip of the spike formed by the fusion loop [48]. In the trans-Golgi network, the surface E proteins rearrange to form 90 units of antiparallel dimers and the prM is cleaved by host protease furin [49]. The pr moiety of prM is released when the viral particle is discharged into the neutral pH environment outside the cell. The extent of prM cleavage varies among different flaviviruses. In DENV, this process is incomplete [50]. Electron microscopy studies have demonstrated that DENV consists of viral particles at various stages of maturity with varying areas of prM on the surface [51]. The presence of surface prM has important implications for the neutralizing and enhancing activity of immune sera [52, 53].

Early evaluations of antibody response focused on an important functional aspect of antibody, namely neutralizing activity. The finding that immune sera after primary infection contained serotype-specific neutralizing activity and that protective immunity to the infecting serotype persisted while cross-protective immunity was short-lived suggested that the envelope protein was the primary target for antibody neutralization [2]. Subsequent studies employing molecular techniques including western blots and recombinant protein antigens demonstrated that during and shortly after acute primary or acute secondary infection, the majority of antibody response to the E protein was actually serotype cross-reactive [38, 54]. The target of these antibodies was mapped to the fusion loop of EDII

which is highly conserved among serotypes. The neutralizing activity of immune sera from acute primary infections was low and cross-reactive indicating that the serotype cross-reactive, anti-fusion loop antibodies predominating during early response were non-neutralizing [38]. In contrast, serotype cross-reactive, anti-fusion loop antibodies induced during a secondary infection have been shown to contribute significantly to neutralizing activity of immune sera [24]. Serotype-specific antibodies against EDIII constituted only a small fraction of antibody response [24]. Studies employing mouse monoclonal antibodies and mutated EDIII demonstrated potent neutralizing activity of antibodies targeting EDIII [54]. The binding sites of neutralizing murine and human antibodies specific to EDIII have been mapped by yeast display techniques to two regions: (1) amino acids forming the lateral ridge of EDIII [55], and (2) a closely related site in the A strand of EDIII (figure 1) [56].

Certain monoclonal antibodies have been observed to bind to whole virion but not to the purified ectodomain of E protein suggesting that they recognize quaternary structures on the viral particle surface (figure 1) [57]. Cryogenic electron microscopy (Cryo-EM) and binding studies using virus-like particles presenting complex surface antigen structures have identified another type of antibody that binds complex quaternary epitopes made up of more than one molecule of envelope protein. Human monoclonal antibodies with serotype-specific neutralizing activity were shown to bind three adjacent E protein regions [58, 59]. Half of the epitope was on EDIII and the other half on EDI and EDI-EDII hinge of a neighboring E protein. A recent study demonstrated that human monoclonal antibodies with broadly neutralizing activity bind across the surface of adjacent E protein molecules that form a dimer (figure 1) [53, 60]. These E dimer-dependent epitope (EDE) antibodies can be divided into two groups based on the epitopes they recognize: (1) EDE1 antibodies that recognize the fusion loop, b-strand, and ij loop of EDII on one side, and EDI and EDIII on the other side of the dimer; and (2) EDE2 antibodies that recognize similar EDII structures as do EDE1 antibodies but interact with the 150 amino acid loop of EDI and glycan chain at N153 on the other side of the dimer. While EDE antibodies recognize the fusion loop in a conformational-dependent manner, another group of antibodies recognizes the linear fusion loop epitope (FLE) formed by the amino acid Tryp 101 and nearby residues. EDE antibodies exhibited broadly neutralizing activity against all four virus serotypes, and neutralized virus derived from either a mosquito cell line or human dendritic cells. In contrast, linear FLE antibodies showed neutralizing activity against mosquito cell-derived virus with a higher prM content but exhibited poor neutralizing activity against dendritic cell-derived virus with low prM content [53].

A major target of the humoral antibody response is prM protein. Although antibodies to prM are detectable during acute primary infection, the response is more rapid and stronger during acute secondary infection [38]. Antibodies to prM are cross-reactive among serotypes and have been shown to be weakly neutralizing. Importantly, these antibodies exhibited infection enhancing activity *in vitro* particularly in Fc receptor expressing cells [52], although one recent study showed no difference in anti-prM antibody levels between mild and severe disease [61]. Nevertheless, antibodies to prM have been implicated in ADE during secondary infection.

NS1 is a major viral product that elicits an antibody response particularly during secondary infection [38]. NS1 proteins from different serotypes differ significantly in amino acid sequences, and both cross-reactive and serotype-specific antibodies are elicited [62]. Considering the high amounts of NS1 in circulation during infection, it is likely that anti-NS1 antibodies form antigen-antibody complexes particularly during secondary infection [38]. In addition, NS1 has been shown to both activate and inhibit complement depending on the context [63, 64]. NS1 is produced as a membrane-associated and soluble molecule; the soluble form is able to bind glycoaminoglycans and deposit on the surface of many cell types [65]. It is conceivable that anti-NS1 antibodies bind to NS1 on the host cell surface and promote cell injury by complement activation. Alternatively, a recent study has demonstrated a permeability enhancing effect of NS1 on endothelium [66]. Consistent with this finding, immunization against NS1 has been shown to be protective against severe dengue in mice [66–68].

Mechanisms of antibody-mediated neutralization

Antibodies can neutralize viruses by multiple mechanisms. Antibodies against EDIII may inhibit viral binding to cellular receptors. The stoichiometric requirement for neutralization likely depends on a number of factors including the affinity of the antibodies and the accessibility of target epitopes. Some neutralizing antibodies have been shown to bind epitopes which are predicted to be buried but are accessible during conformational changes with rearrangement of E protein at higher temperatures *in vivo* [56]. The number of binding sites that need to be occupied to prevent infection has been estimated to be between 10–50% for serotype-specific antibody binding to EDIII of DENV-2 [69]. Another important mechanism of neutralization is prevention of viral membrane fusion by binding of the fusion loop or the internal surface of envelope dimers, thus preventing the rearrangement of E proteins into trimers which is required for viral membrane fusion [60, 70]. Other mechanisms that antibodies use to clear virus include various immune effector mechanisms such as complement-mediated lysis of virus and virus-infected cells, and antibody-dependent cell-mediated cytotoxicity (ADCC) [64, 71]. These mechanisms require expression of viral antigens on the infected cell surface. However, NS1 may be displayed on the cell surface of uninfected cells through attachment of soluble NS1 protein rendering these cells targets for cytolysis by complement or natural killer cells.

Measuring Humoral Immune Correlates

Conceptually, techniques used in assessment of antibody response can be divided into two major categories: (1) binding assays which assess physical interaction between antibodies and antigens, and (2) functional assays which measure biological effects of antibodies including neutralization and enhancement of infection. The advantages of binding assays such as ELISA and western blot include the relative ease of performance compared to functional assays, the potential for high throughput, and the ability to identify molecular structures and epitopes recognized by the antibodies. However, binding ability does not necessarily correlate with biological function which can only be measured with functional assays such as neutralization tests.

Measuring protective antibody response in dengue is complicated by a number of factors at both biological and technical levels. Considering the emerging evidence that most antibodies that recognize quaternary epitopes are potentially neutralizing, assays should ideally use antigens with quaternary structures such as whole virus or VLPs. Even with these optimized antigens, the usual format of binding assays in which antigen is typically attached on a solid surface does not mimic *in vivo* binding dynamics characterized by dynamic rearrangement of E proteins on the viral particle surface, which can provide antibodies access to cryptic epitopes not exposed in a static condition [56, 72].

Functional assays can inform potentially relevant biological roles of antibodies. Neutralization potency is a result of the net effect of a mixture of antibodies targeting different antigens and epitopes with different neutralizing and enhancing capacities. Heterogeneity in DENV preparations in terms of the proportions of virus with varying amounts of viral surface prM poses a technical challenge since this could affect assay performance in detecting neutralization or enhancement.

Standardization of virus preparations to ascertain levels of prM and E protein would be an important step in improving reproducibility of these functional assays. Furthermore, the use of characterized cell lines with known and consistent expression of relevant molecules such as Fc receptors may be needed. The threshold for determining neutralizing activity should also be considered since low neutralizing antibodies have been shown to meet the 50% PRNT cut off used in many laboratories. Measurement of antibody response may include a composite of tests that combine binding assays using strategically designed viral antigens that display conformational and linear epitopes to measure the relative proportions of EDE and FLE antibodies, and an assay for antibodies to prM as a marker of non-neutralizing/potentially enhancing antibodies.

Correlates of Cell-mediated Immunity in Dengue

Enhanced cellular immune activation during secondary infection has been postulated to increase disease severity [73]. Studies comparing activation markers and the expansion of antigen-specific CD8⁺ T cells in dengue fever (DF) and dengue hemorrhagic fever (DHF) cases have demonstrated enhanced T cell activation and expansion in DHF patients [74, 75]. The expansion of antigen-specific T cells is characterized by activation of T cells that recognize serotype cross-reactive epitopes [75]. However, other studies did not find such an association [76, 77]. Although it has been assumed that the magnitude of T cell expansion will be more pronounced during secondary infection, a study comparing the frequencies and kinetics of antigen-specific T cells in primary and secondary infection demonstrated similar expansion of dengue-specific CD8⁺ T cells [78]. Functional analysis of these cross-reactive T cells demonstrated various patterns of response with regard to the types of cytokine production and cytolytic activity depending on stimulating variant peptides [78]. These findings suggest that disease severity may be related to the functional phenotypes of these cross-reactive T cells activated during both primary and secondary infection. The reported associations between certain HLA loci with disease severity support the role of T cell immunity in the pathogenesis of dengue [79]. However, some studies have failed to demonstrate a similar association. The interplay with other genetic loci encoding gene

products relevant to disease mechanisms such as other HLA molecules, Fc receptors, and cytokines likely modify the influence of certain HLA loci on disease severity [80–82].

Most of the studies of T cells have involved analysis of samples collected from DF and DHF patients during an acute infection. Such studies focused mainly on the markers associated with disease severity rather than protection from infection. Nevertheless, inferences can be made on the functional immune parameters that appear to be comparatively defective in more severe (i.e., DHF) cases. One study demonstrated that T cells from severe cases exhibited low cytolytic activity as determined by CD107 expression suggesting that cytolytic function may correlate with protection against severe disease [83]. Consistent with this notion, severe dengue can result in hemophagocytic syndrome which is found in severe viral infections in patients with genetic defects in cytolytic function [84, 85].

Measuring Cell-mediated Immune Correlates

Most cell-mediated immunity assays are functional assays which require well preserved peripheral blood mononuclear cells. Variation in sample collection, cell preservation, and laboratory techniques can affect test performance and is an important obstacle in comparing results from different studies. Analysis of T cell functional profile in an individual prior to and during an acute infection could provide critical information about the type and magnitude of cell-mediated immune responses relevant to disease severity. Although it is possible that viral clearance and tissue injury may be mediated through different mechanisms, the more likely scenario is that outcome of an infection depends on the kinetics of viral replication relative to the activation of memory T cell response. As such, analysis of serial samples during an acute infection will be informative in defining this kinetics. An ideal assay should measure multiple functional aspects of cell-mediated immunity, particularly those aspects speculated to exert antiviral effects and those that may contribute to plasma leakage. These include markers for cytolytic function such as CD107 or perforin, and key cytokines including IFN-gamma, IL-2, and TNF-alpha. Elevated levels of IL-10 have been reported in severe dengue and the frequencies of T cells expressing this regulatory cytokine may be an important marker of disease severity. Multifunctional analysis of T cell immunity will furnish a large and complex dataset that may require analytical tools of systems biology to facilitate data analysis and interpretation [86, 87].

Future Opportunities

Definitive demonstration of vaccine-induced immune correlates of protection or risk will require reproducible measures of pre-infection immunity with documentation of infection or disease in vaccinated and unvaccinated individuals. In the absence of accurate animal models of dengue, this can be done only through large clinical trials with clinical endpoints or through dengue human infection models. Clinical efficacy trials have the advantage of identifying authentic natural infections that can lead to disease. However, the enormous resources required to conduct such studies limit the number that can be done. Even within these large trials, the actual number of infections, the nature, quality and volume of biological samples collected, and the number of collection time points are limited. Thus, the amount of data that can be generated and analyzed is also limited. Currently, only the phase

IIb and phase III trials of Sanofi Pasteur's CYD-TDV vaccine have samples and data available with associated clinical outcomes. However, as other vaccine candidates begin to enter phase III trials, the number and variety of these important reagents will increase, providing expanded opportunities to evaluate immune correlates. Nevertheless, just as immunity from naturally-acquired sequential infections may differ from vaccine-induced immunity, alternative vaccine designs may rely on mechanisms of immunity that vary substantially among vaccine candidates. Investigations of potential immune correlates need to take these differences into account.

Unlike large clinical efficacy trials, human infection models have the advantage of occurring in a controlled setting where the infecting virus strain, host immune status, and timing of infections can be manipulated [88]. Furthermore, a larger number and volume of biological samples can be collected at more frequent time points; and multiple vaccine candidates can be tested with relatively fewer resource requirements. A higher number of immune measures can, therefore, be assessed, with more data generated for analysis. More frequent collection time points also allow for empiric determination of the relevant times for measuring certain immune parameters. However, the experimental infecting virus strain, the timing of sequential infections, and clinical outcomes in human infection models do not reflect natural infections. Thus, immune parameters found to be relevant in human models may not necessarily be applicable to protection or risk in natural infections. This is especially the case for severe disease which cannot be systematically evaluated in human infection models for ethical reasons. Nonetheless, human models could play an important role in suggesting exploratory correlates which could be further validated using samples from clinical efficacy trials.

Ideally, immune correlates should be shown to have a mechanistic role, especially when attempting to elucidate pathophysiology or vaccine design strategies. However, demonstration of an association with clinical endpoints may also be sufficient, especially if the intent is to support regulatory approval [89, 90]. Potential mechanistic correlates such as those involving complex quaternary epitopes are promising. However, given the complexity of the immune response to DENV infection and vaccination, and the myriad interactions among the different serotypes, pre-existing immunity and other contributory variables, determination of a single mechanistic or statistically-associated correlate of protection or risk may not necessarily be feasible. Recent approaches to assessing correlates in diseases such as yellow fever and influenza have incorporated systems analysis of a wide array of parameters [87, 91]. A systems approach has also been used to identify signatures to predict immunity induced by different classes of vaccines [92]. Systems analysis is made possible by new computational tools along with continuing advancement of high throughput techniques such as transcriptomics and proteomics [93]. A systems approach could potentially identify computationally-determined correlates, or lead to unexpected mechanistic markers which could be further explored. The large amount of data that should optimally feed into such analyses could be obtained more readily from human infection models in which samples and data are more accessible. At the same time, clinical efficacy trials with a larger number and variety of subjects could better yield data about certain other parameters such as host genetics. In either case, inclusion of such tests and analyses should be considered in the design of future studies.

Expert Commentary

Efforts and resources applied to dengue vaccine development have accelerated dramatically over the past decade. However, the issues which had concerned dengue researchers for decades have complicated current development efforts. The complex interactions among the four dengue virus serotypes, pre-existing immunity, host genetics, viral characteristics and numerous other factors that can lead to protection or disease enhancement during secondary heterologous infection have become real-world issues in Sanofi Pasteur's CYD-TDV phase III trials. Key elements of the humoral and cell-mediated immune responses that may be beneficial or detrimental continue to be elucidated. The potentially important role of quaternary envelope dimer-dependent epitope (EDE) is one such example. However, much uncertainty remains, and determining immune correlates of protection or risk will be a challenge. Nevertheless, the existence of vaccine trials with clinical outcomes to validate laboratory findings provides opportunities for progress which had not previously been available. In conjunction with ongoing development of dengue human infection models and other new tools, the dengue research community may be able to accelerate their pursuit of immune correlates in dengue.

Five-year View

With one dengue vaccine candidate already far advanced in phase III trials, two others close to initiating phase III trials, and several other candidates in phase I or II trials, a vast amount of clinically-relevant data will become available in the near future. This may be a mixed blessing as the information is likely to present a confusing picture of the mechanisms of protection and risk. The different vaccine candidates each have unique design strategies which will make the results of any future clinical trials difficult to compare among vaccines. Furthermore, one or more of the vaccine candidates will likely be licensed and introduced in certain endemic countries in the near future. Information from such vaccine introduction(s) will be difficult to interpret compared to data from clinical trials. In the meantime, dengue researchers investigating various immune mechanisms will continue to make further advances as they have already been doing. Within this mix, many opportunities will arise to link research and real-world outcomes greatly adding to our understanding of dengue immunity which will assist both vaccine development and disease management.

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Key Issues

- Dengue is caused by four antigenically distinct dengue virus (DENV) serotypes with secondary heterologous infection leading to more severe disease than primary infection.
- Six dengue vaccine candidates currently in active development have entered human clinical trials. The most advanced vaccine candidate, Sanofi Pasteur's CYD-TDV, has undergone phase IIb and phase III trials with good efficacy against two serotypes, marginal or poor efficacy against the other two serotypes, and increased relative risk of dengue hospitalization in vaccinated children 2–5 years of age.
- Cross-reactive memory B and T cells from primary infection are activated during secondary DENV infection and may contribute to immune enhancement.
- Humoral immune responses to structural proteins are dominated by antibodies to pre-membrane and envelope protein.
- Strongly neutralizing antibodies to complex quaternary epitopes may be important in humoral immunity.
- Cell-mediated immunity may be important in protection or enhancement depending on functional T cell subsets and epitope specificity.
- Data and samples with clinical outcomes from ongoing and upcoming phase III clinical trials and from dengue human infection models can be used to explore and validate potential immune correlates of protection or risk.
- A systems approach to data generation and analysis may be useful to determine complex computational correlates or suggest unexpected mechanistic correlates for further exploration.

Figure 1.

Binding sites of envelope protein-specific antibodies. Antibody binding sites were determined by binding characteristics to mutated recombinant proteins or virus-like particles with point mutations, or by cryogenic electron microscopy. Antibody binding sites are depicted as red shaded areas on envelope protein molecules.