HHS Public Access

Author manuscript

Lancet Infect Dis. Author manuscript; available in PMC 2018 May 24.

Published in final edited form as:

Lancet Infect Dis. 2017 March ; 17(3): e88–e100. doi:10.1016/S1473-3099(16)30473-X.

Dengue: Knowledge gaps, unmet needs and research priorities

Leah C. Katzelnick, Ph.D., MPH, **Josefina Coloma, Ph.D.**, and **Eva Harris, Ph.D.*** **[Full Professor]**

Division of Infectious Diseases and Vaccinology, School of Public Health, University of California, Berkeley, Berkeley, CA

Summary

Dengue virus (DENV) is a mosquito-borne pathogen that causes up to ~100 million dengue cases each year, placing a major public health, social and economic burden on numerous low- and middle-income countries (LMICs). Major advances by scientists, vaccine developers, and affected communities are revealing new insights and enabling novel interventions and approaches to dengue prevention and control. Such research has highlighted further questions about both the basic understanding of dengue and efforts to develop new tools. We discuss existing approaches to dengue diagnostics, disease prognosis, surveillance, and vector control in LMICs as well as potential consequences of vaccine introduction. We also summarize current knowledge and recent insights into dengue epidemiology, immunology, and pathogenesis, and their implications for understanding natural infection and current and future vaccines.

Introduction

Dengue is the most prevalent arboviral disease of humans, with 3.6 billion people living in areas at risk of transmission and an estimated 390 million dengue virus (DENV) infections and 96 million dengue cases annually.[1] Dengue is endemic to the tropical belt of Asia, Latin America, and the Pacific, circulates across Africa, and has recently caused local outbreaks in the United States and parts of Europe.[1–3] Dengue has expanded globally since the 1960s, driven by population growth, urbanization, increased travel, and insufficient vector control programs. Despite increased funding and advances in dengue research, dengue epidemics are intensifying in frequency, magnitude and geographic reach.[4] The burden of dengue globally is estimated at 15.8 disability-adjusted life years per 100,000 individuals, with major economic, social, and political impact.[5,6] Public health systems are strained by the relentless spread of DENV and other arboviruses, such as chikungunya and Zika (ZIKV) viruses, and discouraged by decades of failed vector control programs and lack of interventions.

However, never before has the level of resources and commitment from diverse researchers and stakeholders been as great, focused on increasing the basic knowledge, potential treatments and vaccines, and new vector control strategies, with the ultimate goal of

*Corresponding author.

Author contributions

All authors contributed to the design, literature review, writing, revision, figures, and tables of this review article.

conquering dengue. Here, we address existing tools and current needs for dengue diagnostics and surveillance in low- and middle-income countries (LMICs) and how these will be impacted by the introduction of dengue vaccines. We review current knowledge and research gaps in immunology/epidemiology in the context of natural infection and vaccines, as well as in dengue pathogenesis and new approaches to vector control. Many of these insights call into question existing paradigms in the dengue field and raise many new and exciting questions.

Case management

Dengue is caused by four DENV serotypes (DENV1-4), transmitted by the daytime-biting mosquitoes *Aedes aegypti* and *Ae. albopictus*. Dengue fever is characterized by debilitating symptoms including high fever, arthralgia, myalgia, anorexia, petechiae/rash, and retro-orbital pain. Because symptoms are non-specific and overlap with many other infections, such as chikungunya and Zika, laboratory diagnosis is required. Upon defervescence, during "the critical phase" (days 4–6 of illness), a fraction of patients (~500,000/year) develop Dengue Hemorrhagic Fever/Dengue Shock Syndrome (DHF/DSS), characterized by well-described clinical and hematological features that can facilitate clinical diagnosis of dengue even without laboratory confirmation: thrombocytopenia, hemorrhagic manifestations, liver damage, leukopenia, pleural effusion and other signs of vascular leakage, and hypovolemic shock, leading to organ failure.[7] In 2009, a new classification of disease severity was adopted by the WHO, with "severe dengue" encompassing additional manifestations of severe disease.[8] Mortality due to dengue can be greatly reduced by early diagnosis and timely and judicious fluid management; hence, laboratory diagnosis together with clinical acumen is critical.[8]

A combination of diagnostic methods that target different time periods post-onset of symptoms maximizes diagnostic sensitivity (Table 1); however, only early acute-phase diagnostics can impact clinical management. These include detection of viral RNA, virus, and NS1 protein. Variable quality of available assays and high cost are still limiting factors,[9] often placing them out of reach for national coverage. The IgM ELISA is widely used for dengue diagnosis, but in fact the timing of the samples (≥5 days) means that results are obtained too late for clinical decision-making. A major unmet need is affordable, easy-to-perform acute-phase dengue diagnostics. This is even more urgent given the recent, widespread introduction of ZIKV into the Americas, as conventional dengue serological and even NS1 diagnostic assays are cross-reactive with ZIKV infections, making the interpretation of serological diagnostics more complex in endemic areas. Neutralization tests can be performed to detect virus-specific neutralizing antibodies and to determine the etiology of flavivirus infection, but only after ~6 months post-infection.

Importantly, for diagnostics to have a true impact on disease outcome, systems need to close the loop between the clinic, the laboratory and the patient. Other than rapid diagnostic tests (RDTs), which are rarely used in the public sector in LMICs, most diagnostic information obtained at centralized laboratories is used to inform national surveillance systems, while clinicians and patients often do not receive timely feedback for decision-making that can affect case management and disease outcome. New information and communication

technology (ICT) tools are being developed that improve work and information flows around time-sensitive disease diagnosis and response as well as outbreak prevention. A major priority is integration of these tools into national data systems and involvement of all stakeholders from the beginning to ensure bi-directional flow of clinical and diagnostic information that benefits both surveillance systems and patients alike.

Prognostic tests to detect biomarkers associated with severe dengue to enable better triage and treatment of patients are sorely lacking in clinical settings. Clinical manifestations are currently the only data used for predicting dengue disease severity. Recent studies have revealed quantifiable molecules and immune signatures during the course of DENV infection; translating these potential biomarkers into practical clinical tests requires significant research and evaluation, especially in the context of vaccine rollout. Advances in technology that allow for multiple biomarker analysis with small sample volumes will enable implementation of these assays in the clinical context in LMICs and could aid clinical trials of vaccine or drug interventions.

Surveillance/outbreak response/sero-epidemiogical studies

Most dengue-endemic countries rely on syndromic dengue surveillance, with laboratory-based confirmation of a subset of cases. Laboratory-enhanced sentinel surveillance systems provide more precise information to public health authorities on time, location, serotype, and disease severity, thus enabling an earlier trigger for interventions to mitigate outbreaks. Functional laboratory-based surveillance systems will be critical for deployment of vaccine trials and for eventual vaccine roll-out. Serological assays that detect increases in DENV-specific antibodies are widely used by most public health systems for confirmation of disease and for differentiating primary from secondary DENV infections (Table 1). Sero-epidemiological studies can monitor the overall prevalence of DENV infection in a population and are usually based on IgG or Inhibition ELISA or Hemagglutination Inhibition, although more specific methods, such as neutralization tests, are available.

Critically, once DENV vaccines are widely distributed, serological assays will no longer distinguish immune responses due to DENV infection from vaccine-induced immunity. Furthermore, cross-reactivity with other flaviviruses with widespread circulation, such as ZIKV and West Nile virus (WNV), as well as national vaccination programs against flaviviral diseases such as yellow fever in Latin America and Japanese encephalitis in Asia, can confound interpretation of serological assays. Serological methods to distinguish flavivirus infection are urgently needed, particularly in secondary infections.

Crowd-sourcing of symptom data and community entomology efforts enable affected communities to gain ownership of control strategies and represent powerful sources of information for outbreak detection. ICTs and social networks can play an important role in disease, vector and outbreak reporting from the community to local and regional authorities. A handful of such technologies have been used successfully in pilot implementations and within studies, including using cell phone tower signals to track human movement for prediction of the most likely outbreak locations in Pakistan;[10] online and smart phone applications to report dengue mosquito breeding sites in Mexico[11], Nicaragua and

Colombia; and near real-time search-query data that estimate impending outbreaks globally (Google dengue-trends, DengueMap). While information gained through crowd-sourcing needs to be validated by laboratory confirmation of etiology, the power of these technologies together with the strength of communities mobilized through knowledge should be harnessed as integral aspects of national surveillance systems and for documenting the impact of vaccine introduction.

Vector control

Excellent vector control reviews have been written recently;[12] here, we review key points in the context of vaccination programs. To date, preventing or reducing DENV transmission has depended entirely on controlling the mosquito vectors. Even after vaccine deployment, vector control will continue to be part of control strategies to reduce disease risk and burden. The concept of integrated dengue management is now widely accepted.[13,14]

The recent chikungunya and Zika epidemics have placed vector control at the forefront of public health responses, but the same challenges that existed decades ago remain today. LMICs mostly focus their efforts on source reduction (elimination of vector breeding containers), environmental management, and larvicide abatement of water storage receptacles and use chemical fogging as an outbreak response. Chemicals in thermal fogs or ultra-low-volume aerosols target adult mosquitoes and only work at a very local level. They need to be repeatedly applied, which has led to the development of resistance as well as environmental and health impacts of unknown magnitude. The widely applied organophosphate larvicides such as temephos are also of questionable efficacy and pose potential health risks.[15,16] A recent large cluster randomized controlled trial demonstrated that temephos had no effect in reducing household entomological indices or incidence of DENV infection, and in fact was associated with increased dengue risk, possibly due to a false sense of protection and consequential lack of environmental management activities.[15]

Other approaches exist, such as biological control with organisms that prey upon the aquatic immature *Aedes*, like small crustaceans and larvivorous fish,[17] and biologically-derived insecticides, but more data are needed to understand cost-effectiveness of large-scale implementation. Trials of genetically modified mosquitoes as a population suppression strategy are currently ongoing that involve engineering a lethal gene into mosquitoes to ensure sterility following mating with wild mosquitoes.[18] A new population replacement approach is underway in several trial sites worldwide using mosquitoes infected with the wMel strain of the bacteria *Wolbachia* that shortens the mosquito lifespan and blocks virus development, thus reducing DENV transmission.[19] The scale-up potential, trade-off between the fitness cost of these variants and their virus-blocking ability, and impact of introducing modified organisms into the ecosystem need to be further evaluated.[20] Finally, community-based approaches that include entomological surveillance and evidence-based, active participation of affected populations in source reduction have been shown to result in reduced household *Aedes* entomological indices, DENV infection incidence, and dengue disease.[15,21] For vector control efforts to work, they need to involve community participation and be sustained year-to-year, during inter-epidemic periods, and especially in high-risk locations.

New guidelines, based on validated strategies and models and that expand the toolbox available to governments are urgently needed. The impact that vector control interventions have on health and disease burden needs be systematically investigated. Further, consensus on the timing and nature of a combination of vector control approaches with dengue vaccines should be pursued. Scaling up successful pilots and trials is the next frontier, and models informing this process are critical.[22] Despite the challenges, vector control will need to be included in any integrated strategy for dengue prevention.

Modeling dengue transmission

Modeling dengue transmission enables evaluation of benefits and trade-offs of different dengue control methods; this is increasingly important as interventions such as vaccine and new vector control strategies come closer to implementation (Table 2). Mathematical models have been developed to identify determinants of the oscillations of DENV serotypes in endemic settings, including the roles played by the mosquito vector and by enhancement from prior exposure to heterologous DENV strains.[23] Recent research using statistical models has shown that dengue epidemic size and disease severity may be affected by human movement, changes in population age structure, and regional climate variables;[24–29] further work is needed to better understand these effects and build predictive models for government preparedness on the local and regional level. Recent observations of homologous DENV re-infection,[30,31] viremic heterologous asymptomatic infections,[32] cross-reactivity with other flaviviruses,[33] and frequent DENV exposure[34,35] should be further studied in an epidemiological modeling framework to understand their role in DENV immunity and DENV transmission. In light of the different degrees of vaccine efficacy in previously DENV-naïve and DENV-exposed individuals,[36–38] models to estimate vaccine efficacy stratified by immune status as well as to explore effects of vaccination on DENV transmission are critical.[39] Such models should continue to be developed in concert with vaccination efforts and should account for heterogeneity in prior DENV exposure within countries and across regions.

The virus: serotypes, genotypes, and strains

DENV serotypes, genotypes, and clades can differ in intrinsic virulence and epidemic capacity.[34,40–43] A better understanding of the genetic basis for virulence and enhanced replication in mosquitoes and humans would improve our ability to predict epidemics that pose a greater risk of disease. A further question is whether epidemic force (the symptomatic:inapparent infection (S:I) ratio) affects the threshold of neutralizing antibodies or other immune correlates that protect against subsequent infection.[34] Prior immunity (determined by the serotype/genotype/clade/strain of the first infection) in relation to the second infecting strain can also affect infection outcome (inapparent vs. symptomatic) and disease severity at both the individual and population level.[31,44–47] Thus, a priority is estimating the impact of genetic variation on the breadth of the immune response induced by natural infection and vaccination[48] and the resulting protection against diverse DENV strains. Another recent discovery is that genetic differences alter the exposed epitopes on the virion and thus affect neutralization profiles.[49,50] Some such amino acid variants are seen in highly laboratory-adapted strains that are parent strains for current vaccines;[51] therefore,

how these substitutions affect the immunogenicity of vaccine strains compared with natural isolates needs to be investigated.

Clade replacement is a characteristic feature of DENV evolution.[52] The relative contribution of intrinsic fitness differences,[43,44,53] escape from neutralization,[54–56] cross-serotype enhancement,[50,57,58] or stochastic effects (e.g., climate, geography, genetic bottlenecks)[54,59] remain poorly understood. Estimating the contribution of these factors to DENV evolution could enable prediction of the strength and/or severity of epidemics. Tetravalent dengue vaccines induce imbalanced responses against DENV1-4 in some individuals[60,61] and different combinations of type-specific and cross-reactive neutralizing antibodies, raising the question of whether vaccination could create a level of population immunity that facilitates evolution of certain lineages that better escape host immunity.

Immune responses in the epidemiological context

Post-primary infections

The interval of time between first and second DENV infection modulates infection and disease outcome. The average period of cross-protection is 1.6–2 years against symptomatic DENV infection and 2.6 years against severe disease;[62–64] these observations are consistent with long-term follow-up data from vaccine trials.[36] Hypotheses include waning of cross-serotype neutralizing antibodies, epidemic force, increased risk of being exposed to a different serotype, or other immune mechanisms such as waning T cell protection.[34,62–65] In the absence of DENV re-exposure, neutralizing antibody titers decay rapidly in magnitude between 1–6 months, then decay gradually for up to a year after primary infection.[34,35,66,67] However, in endemic settings, after the initial decrease, the magnitude of neutralization titers remains relatively stable for years after primary infection, with some individuals exhibiting decay and others boosts.[34,35,67] Anti-DENV neutralizing antibody responses are also thought to become more type-specific for months and possibly years after primary infection.[68–70] However, many individuals have broad neutralization profiles one year post-infection in non-endemic settings[71,72] and after many years in endemic settings.[34,35,73] Comparing longitudinal patterns of neutralization titers in non-endemic compared with endemic settings is important for establishing the decay rate and cross-reactivity of neutralizing antibodies over time.

Post-secondary infections

Following a secondary infection with a different DENV serotype, the neutralizing antibody response becomes broadly neutralizing and is thought to reduce incidence of subsequent severe disease. Symptomatic third and fourth DENV infections do occur, but are rarely severe.[45,47,74] Understanding the properties of post-secondary immunity, for instance, the cross-reactive EDE epitope,[75] will improve understanding of what constitutes a protective multivalent response.

Homologous symptomatic re-infection

Type-specific neutralizing responses were thought to provide sterilizing immunity against homologous re-infection;[76,77] however, evidence now exists that homologous re-infection

can lead to symptomatic disease.[30,31] Study of symptomatic homologous infection will help us understand why type-specific protection fails, which may provide insight into cases of low vaccine efficacy.

Boosting

DENV re-exposures may "boost" neutralizing antibody titers in DENV-immune individuals, providing individuals longer protection against a subsequent symptomatic infection. Vaccine trials show boosted neutralizing antibody responses upon repeat vaccination (although not necessarily improvements in efficacy);[78] experimentally inoculated non-human primates challenged with the homologous strain a year later display boosted antibody responses;[79] and neutralization titers in children in endemic areas on average increase in magnitude between first and second infection.[34,35] It is critical to determine whether natural boosting in endemic areas modifies an individual's subsequent infection/disease risk and evaluate implications for vaccine deployment – does natural boosting improve long-term vaccine efficacy?

Immune correlates of protection and risk

Neutralizing antibodies

A critical metric of protection is the potency of neutralizing antibodies against future infecting strains; thus, neutralization assays are a key tool for decision-making by clinicians, epidemiologists, vaccine developers, and policy makers. However, current assays do not adequately capture the full complexity of the neutralizing antibody response, and results are highly variable across laboratories. Moreover, the results of recent clinical trials of Sanofi's tetravalent dengue vaccine evidenced that quality as well as quantity of neutralization titers need to be taken into account.[60]

Neutralization titers vary significantly across laboratories and between methods, with assay parameters such as virus preparation (maturation state, cell source), virus strain, complement, cell type, DC-SIGN expression, presence of EDTA, plasma vs. serum, percent plaque or immunofocus reduction, and method of measurement of infected cells influencing outcome.[80,81] The maturation state of the virus stock may alter how well the virus is neutralized, as for instance, antibodies that recognize the fusion loop or prM only bind to immature virions, where these epitopes are exposed.[81,82] DENV virions are now thought to be "mosaic", with some regions of the virus smooth and mature and others spiky and immature.[83] Even in the mature form, DENV particles "breathe", with E monomers sampling a range of structures between the mature and pre-fusion state.[51] Higher (e.g., febrile) temperatures and longer incubation periods enable antibodies access to otherwise concealed "cryptic" epitopes as the virions breathe, affecting neutralization titers.[51,84] Finally, the virus strain chosen can also impart variability, as some strains are better or worse neutralized than others.[73,85]

The cell type used as substrate for measuring neutralization remains a critical concern. The validated cell line for vaccine developers is Vero, derived from green monkey kidneys.[86] Neutralization assays conducted on Vero cells appear to detect only type-specific

neutralizing responses, while other cell lines, including those expressing DC-SIGN, can capture type-specific and cross-reactive neutralizing antibody responses.[81] Development of assays with biologically relevant substrates is paramount for better understanding of immune correlates and neutralization titers.

The variability in neutralization titers, and thus generalizability of what titer corresponds with protection under all assay conditions, has been a point of contention to date and has complicated studies of immune correlates in natural infections and vaccines. An optimal neutralization assay remains a holy grail for the dengue field. Although neutralization titers have long been treated as a correlate of protection against DENV, only recently have studies demonstrated a significant association between the quantity of cross-reactive pre-infection neutralizing antibody titers and reduced risk of symptomatic secondary infection.[34,87,88]

Aside from the quantity of neutralizing antibodies, more attention has focused recently on the quality: type-specificity versus serotype cross-reactivity and epitope repertoire. To date, the majority of antibodies generated after primary DENV infection have been found to be cross-reactive and weakly neutralizing.[89] Many bind around the fusion loop region on domain II (EDII); nonetheless, some antibodies that bind this region are potently neutralizing.[90,91] Following both primary and secondary infection, the most potently neutralizing antibodies are virion-specific and often bind across dimers, contacting up to three monomers simultaneously, thus throwing a ‘wrench’ into the DENV E protein machinery that successfully prevents fusion.[75,92–95] These antibodies bind multiple sites on the E protein, including regions of EDIII, the fusion loop, and the hinge of EDI/II.[93] New tools are becoming available to dissect the repertoire of polyclonal sera, using depletion methods and epitope-transplanted recombinant viruses;[49,96,97] applying these to better understand the antibody response and potential immune correlates in DENV natural infections and vaccines is an area of active research.

Antibody-dependent enhancement (ADE)

A major concern for dengue vaccines is that they might induce enhancing DENV antibodies that could increase risk of severe disease in vaccinated individuals. ADE is posited to occur when antibodies to a previous DENV infection recognize but do not neutralize a subsequent infection with a different serotype, and the resulting immune complexes facilitate virus entry into target Fcγ receptor-bearing cells, leading to higher viral load and activation of T cells that secrete TNF-α and other vasoactive cytokines. Passively transferred heterotypic antibodies increase viremia levels in rhesus monkeys[98,99] and induce lethal vascular leak syndrome with an otherwise sub-lethal dose of DENV in interferon receptor-knockout mice.[100,101] In humans, studies of the incidence of severe dengue in infants demonstrate a strong correlation between peak incidence of DHF/DSS in infants, age of the infants, decay of maternal neutralizing antibodies, persistence of anti-DENV maternal IgG antibodies, and fold-enhancement by neat antisera on Fcγ receptor-bearing cells.[102–107] Fold-enhancement titers measured on primary human monocytes can also distinguish between asymptomatic and severe DENV2 infections in children.[108] Heterotypic immunity is a risk factor for severe dengue, consistent with the hypothesis that enhancing antibodies could contribute to severe disease.[40] The initial observation of higher rates of hospitalized dengue cases in young

vaccinees compared with controls in Year 3 follow-up data of recent clinical trials has raised concerns about vaccine-induced ADE of infection.[36] However, to date, the association between enhancement titer and disease severity has not been proven.[105,109,110] Further, there is still controversy as to which cell substrate to use for the *in vitro* enhancement assay.[110,111] A research priority is establishing whether disease severity is mediated by the degree of ADE. Possibly, enhancing antibodies in the absence of sufficient neutralizing antibodies are contributing factors, but symptomatic infections only progress to severe disease in the context of other host and virus-related determinants.

B cells

The B cell repertoire following DENV infection is composed of long-lived plasma cells (LLPCs) and memory B cells (MBCs). After primary infection, individuals are thought to develop a type-specific neutralizing response over time in non-endemic regions.[68–70] One hypothesis is that this occurs in the absence of re-infection, whereby high-affinity MBCs naturally replenish the LLPC population.[112] An alternative hypothesis is that LLPCs are long-lived, but not life-long, and the LLPC-derived serum neutralization titer decays over time in a pathogen-specific way.[113–115] A critical priority is to determine whether maintenance of long-lived type-specific neutralizing antibodies requires re-exposure to DENV. Following secondary infection, potent cross-reactive neutralizing antibodies have been identified,[75,116] but the origin of these B cells remains poorly understood. Identifying these antibodies in post-secondary infection peripheral blood mononuclear cells (PBMCs) and then tracing them back to identify their lineage in samples taken after prior infection(s) is a major priority. Another outstanding question is whether DENV in humans resembles WNV in mice, where LLPCs expressed the highest-affinity receptors and MBCs had lower-affinity receptors but recognized a wider range of epitopes.[117]

T cells

The importance of T cells in protection against and pathogenesis of severe dengue remains an active area of research. Cross-reactive T cells have long been postulated to play a role in dengue immunopathogenesis, but have also recently been shown to be protective as well.[118,119] In general, $CD8^+$ T cells contribute to the antiviral response by directly killing infected cells and secreting IFN-γ and TNF-α. Recent research indicates that $CD8^+$ T cells may protect against secondary DENV infection in mice and humans.[120–122] Because $CD8^+$ T cells primarily target non-structural proteins,[123] vaccines that do not include DENV non-structural proteins may miss an important immune mechanism for preventing severe disease.[124] In contrast, tetravalent vaccination that includes non-structural proteins from multiple DENV types induces a multifunctional $CD8^+$ T cell response that is directed toward epitopes conserved among serotypes, potentially providing $CD8^+$ T cell-mediated control of infection.[119]

$CD4^+$ T cells indirectly control DENV infection by facilitating B and $CD8^+$ T cell activation and memory, as well as secreting inflammatory cytokines.[65] Certain $CD4^+$ T cell responses are thought to be a signature for DHF pathogenesis;[125] however, this relationship has not been tested to predict disease outcome. In mouse models, memory $CD4^+$ T cells mediate protection in secondary cases.[126] Anti-DENV $CD4^+$ T cells in humans may also directly

control DENV infection by killing infected cells.[127] $CD4^+$ T cell epitopes identified to date focus on structural and non-structural proteins, with additional characterization ongoing in human populations.[65] The role of memory $CD4^+$ T cells induced by natural infection and vaccination in stimulating protective antibody-mediated immunity and robust $CD8^+$ T cell responses during secondary infection in humans is an important area of research.

Innate immunity

Interferons constitute a powerful antiviral response, and DENV has evolved numerous mechanisms to evade the human innate immune response, targeting multiple stages in the interferon signaling pathway.[128] Gene expression studies of dengue cases of varying severity highlighted differences according to day of illness and revealed shock signatures – DSS cases had reduced interferon-stimulated genes and increased mitochondrial function.[129,130] Another notable observation was that DENV induces $CD14^+CD16^+$ monocytes, which can promote B cell differentiation into plasmablasts and antibody secretion.[131] Current efforts are focused on generating network models of DENV infection and correlating innate immune signatures with adaptive immune responses.

Other antibody effector functions

Few studies have examined other antibody attributes in human populations. Regarding antibody isotypes, IgG_1 and IgG_3 differed significantly between DF and DHF cases, while IgG_4 and IgA were more common in DSS cases.[132] Antibody-dependent cell-mediated cytotoxicity (ADCC) in pre-infection samples was associated with reduced viremia during secondary infection,[133] but others found ADCC activity in acute sera from DHF/DSS cases but not DF cases.[134] More research is needed to explore these potential additional immune correlates.

Pathogenesis

Host genetics, DENV immune history, infection sequence with particular DENV serotypes and strains, and viral genetics modulate the immune response and impact disease outcome. A dominant theory has been that immunopathogenic mechanisms result in a “cytokine storm” that leads to vascular leak and thus contributes to severe dengue disease in secondary infections.[118] However, severe disease does develop in some instances after primary DENV infection, and many patients with high viremia recover from DF without developing plasma leakage.[42]

Complement activation, which coincides with the timing of plasma leakage, can be triggered by DENV-antibody complexes.[135] A hypothesis based on molecular mimicry posits that some DENV-induced antibodies can cross-react with host proteins such as plasminogen, thrombin and platelets.[136–140] More research is needed to understand the potential role of these pathways in dengue pathogenesis.

DENV NS1 has been shown to contribute to vascular leak via both cytokine-dependent and -independent mechanisms. In one study, purified NS1 directly activated mouse macrophages and human PBMCs via Toll-like receptor 4 (TLR4), leading to induction and release of proinflammatory and vasoactive cytokines/chemokines. Both NS1-mediated activation of

PBMCs and NS1-induced permeability *in vitro* were inhibited by a TLR4 antagonist and by anti-TLR4 antibodies.[141] A parallel study showed that recombinant NS1 directly induced vascular leak and a lethal NS1-mediated disease in a mouse model;[142] these pathogenic effects were blocked by NS1-immune polyclonal mouse serum and anti-NS1 monoclonal antibodies, and mice immunized with NS1 from each of the four DENV serotypes were protected against lethal DENV2 challenge.[142] NS1 was also shown to induce hyperpermeability in human endothelial cell monolayers independent of cytokines via disruption of the glycocalyx layer that lines the endothelium.[143] These findings identify new potential targets for dengue therapeutics and support inclusion of NS1 in dengue vaccines.

Dengue vaccines

Of the dengue vaccines in development, live-attenuated vaccines (LAV) are the most advanced, with three candidates in Phase 2/3 (NIH TV-003/TV-005 and Takeda TDV vaccines) or Phase 4 (Sanofi Dengvaxia) trials. Other approaches in pre-clinical or early clinical development include non-replicating or single-replication vectored vaccines packaged in DENV structural proteins; purified, inactivated virus vaccines; subunit-based vaccines (e.g., EDIII or NS1 protein); and DNA-based vaccines.[144] Novel approaches being explored include “scaffolding” complex epitopes, such as the cross-neutralizing EDE epitope; bivalent vaccines that present two potent neutralizing type-specific epitopes simultaneously; and recombinant viruses on which epitopes recognized by enhancing antibodies are “masked”.[97,145,146]

Remaining knowledge gaps and research priorities in relation to dengue vaccines are discussed throughout and summarized in Table 2. In addition, some questions were raised by the first Phase 3 efficacy trials and long-term follow-up studies. The Year 3 follow-up data from the Sanofi Phase 3 clinical trial showed an increased risk of hospitalization cases in young vaccinated versus control individuals.[36] Further, combined data for all Phase 2b and 3 clinical trials demonstrated no significant protective effect of vaccination in naïve individuals under age 9.[36] In discussing these results, the developers posit, among other hypotheses, that primary vaccination in young individuals place them at risk of a severe infection sooner than the placebo group, which will eventually catch up over time.[147]

Whether young age or naïve status is the greater risk factor is a critical remaining question, as is the effect of potential roll-out in highly-endemic versus low-endemic settings or in areas with only one DENV serotype circulating. Another question is the quality of the immune response after primary vaccination in naïve individuals as compared to a primary natural DENV infection and the risk of subsequent severe infection.

To date, no published vaccine correlate of protection against dengue exists. Recent studies of natural DENV infections show a significant association between the quantity of cross-reactive pre-infection neutralizing antibody titers and reduced risk of symptomatic secondary infection.[34,87,88] However, neutralizing antibody titers may be a “correlate of protection” but not a “mechanistic correlate of protection”.[148] Establishing both mechanistic and nonmechanistic correlates of protection will require examining a suite of measures of the immune response in relation to disease outcome for each vaccine. Collecting and

maintaining sample banks or biorepositories for continued monitoring of vaccines and research is of critical importance for identifying DENV correlates of protection.

Conclusion

We have reviewed progress in diagnostics, clinical research, epidemiology, entomology, virology, immunology, pathogenesis, and vaccine development, as well as the results of multiple high-quality clinical studies and field sites, in the context of understanding natural DENV infections and current and future vaccination efforts. In addition, we have highlighted knowledge gaps and the urgent need for translational research. With the recent licensure of the first dengue vaccine and several others entering Phase 3 trials, as well as the current Zika pandemic, researchers, funders, vaccine developers and public health professionals have the responsibility to join efforts to focus research on key questions that will ensure that populations living in flavivirus-affected areas have access to cost-effective and accurate diagnostics, responsive surveillance systems, and the safest and most effective treatments and vaccines possible.

Acknowledgments

The authors thank Henry Puerta Guardo and Paulina Andrade for contributions to Figure 1. We apologize to those authors whose articles we were not able to specifically cite owing to space constraints.

Role of the funding source

Funding support was from the National Institutes of Health, National Institute for Allergy and Infectious Diseases grant P01 AI106695 (EH).

Conflict of interest

Dr. Katzelnick, Dr. Coloma, and Dr. Harris report grants from the National Institutes of Health; Dr. Coloma, and Dr. Harris report grants from the Bill and Melinda Gates Foundation, the Carlos Slim Health Institute, and the UBS Optimus Foundation, during the conduct of the study; Dr. Harris reports personal fees from Sanofi Pasteur Scientific Advisory Board, and a grant from Takeda for laboratory analysis of vaccinee samples, outside the submitted work.

References

1. Bhatt S, Gething PW, Brady OJ, et al. The global distribution and burden of dengue. Nature. 2013; 496:504–7. [PubMed: 23563266]
2. Radke EG, Gregory CJ, Kintziger KW, et al. Dengue outbreak in Key West, Florida, USA, 2009. Emerg Infect Dis. 2012; 18:135–7. [PubMed: 22257471]
3. Schaffner F, Mathis A. Dengue and dengue vectors in the WHO European region: past, present, and scenarios for the future. Lancet Infect Dis. 2014; 14:1271–80. [PubMed: 25172160]
4. WHO. Global strategy for dengue prevention and control. Geneva: 2012.
5. Shepard DS, Undurraga EA, Betancourt-Cravioto M, et al. Approaches to refining estimates of global burden and economics of dengue. PLoS Negl Trop Dis. 2014; 8:e3306. [PubMed: 25412506]
6. Murray CJL, Barber RM, Foreman KJ, et al. Global, regional, and national disability-adjusted life years (DALYs) for 306 diseases and injuries and healthy life expectancy (HALE) for 188 countries, 1990-2013 quantifying the epidemiological transition. Lancet. 2015:1990–2013.
7. Dengue haemorrhagic fever: diagnosis, treatment, prevention and control. 2nd. Geneva: 1997.
8. WHO/TDR. Dengue: guidelines for diagnosis, treatment, prevention and control. Geneva: 2009.

9. Hunsperger EA, Yoksan S, Buchy P, et al. Evaluation of commercially available diagnostic tests for the detection of dengue virus NS1 antigen and anti-dengue virus IgM antibody. PLoS Negl Trop Dis. 2014; 8doi: 10.1371/journal.pntd.0003171
10. Wesolowski A, Qureshi T, Boni MF, et al. Impact of human mobility on the emergence of dengue epidemics in Pakistan. Proc Natl Acad Sci U S A. 2015; 112:11887–92. [PubMed: 26351662]
11. Lozano-Fuentes S, Wedyan F, Hernandez-Garcia E, et al. Cell phone-based system (Chaak) for surveillance of immatures of dengue virus mosquito vectors. J Med Entomol. 2013; 50:879–89. [PubMed: 23926788]
12. Achee NL, Gould F, Perkins TA, et al. A Critical Assessment of Vector Control for Dengue Prevention. PLoS Negl Trop Dis. 2015; 9:e0003655. [PubMed: 25951103]
13. State of the art in the prevention and control of dengue in the Americas. Washington DC: 2014.
14. Gubler DJ. The partnership for dengue control - a new global alliance for the prevention and control of dengue. Vaccine. 2015; 33:1233. [PubMed: 25597939]
15. Andersson N, Nava-Aguilera E, Arosteguí J, et al. Evidence based community mobilization for dengue prevention in Nicaragua and Mexico (Camino Verde, the Green Way): cluster randomized controlled trial. BMJ. 2015; 351:h3267. [PubMed: 26156323]
16. George L, Lenhart A, Toledo J, et al. Community-effectiveness of temephos for dengue vector control: a systematic literature review. PLoS Negl Trop Dis. 2015; 9:e0004006. [PubMed: 26371470]
17. Hurst TP, Kay BH, Brown MD, Ryan PA. Melanotaenia duboulayi Influence oviposition site selection by Culex annulirostris (Diptera: Culicidae) and Aedes notoscriptus (Diptera: Culicidae) but not Culex quinquefasciatus (Diptera: Culicidae). Environ Entomol. 2010; 39:545–51. [PubMed: 20388286]
18. Winskill P, Carvalho DO, Capurro ML, Alphey L, Donnelly CA, McKemey AR. Dispersal of engineered male Aedes aegypti mosquitoes. PLoS Negl Trop Dis. 2015; 9:1–18.
19. Hoffmann AA, Montgomery BL, Popovici J, et al. Successful establishment of Wolbachia in Aedes populations to suppress dengue transmission. Nature. 2011; 476:454–7. [PubMed: 21866160]
20. Nguyen TH, Le Nguyen H, Nguyen TY, et al. Field evaluation of the establishment potential of wMelPop Wolbachia in Australia and Vietnam for dengue control. Parasit Vectors. 2015; 8:563. [PubMed: 26510523]
21. Arunachalam N, Tyagi BK, Samuel M, et al. Community-based control of Aedes aegypti by adoption of eco-health methods in Chennai City, India. Pathog Glob Health. 2012; 106:488–96. [PubMed: 23318241]
22. Ferguson NM, Kien DTH, Clapham H, et al. Modeling the impact on virus transmission of Wolbachia-mediated blocking of dengue virus infection of Aedes aegypti. Sci Transl Med. 2015; 7:279ra37.
23. Perkins, TA., Reiner, RC., Rodriguez-Barraquer, I., Smith, DL., Scott, TW., Cummings, DAT. A review of transmission models of dengue: a quantitative and qualitative analysis of model features. In: Gubler, DJ.Ooi, EE.Vasudevan, SG., Farrar, J., editors. Dengue and dengue hemorrhagic fever. 2nd. CAB International; 2014. p. 99-114.
24. Reiner RC, Stoddard ST, Scott TW. Socially structured human movement shapes dengue transmission despite the diffusive effect of mosquito dispersal. Epidemics. 2014; 6:30–6. [PubMed: 24593919]
25. Rabaa MA, Simmons CP, Fox A, et al. Dengue virus in sub-tropical northern and central Viet Nam: population immunity and climate shape patterns of viral invasion and maintenance. PLoS Negl Trop Dis. 2013; 7:e2581. [PubMed: 24340118]
26. van Panhuis WG, Choisy M, Xiong X, et al. Region-wide synchrony and traveling waves of dengue across eight countries in Southeast Asia. Proc Natl Acad Sci. 2015:201501375.
27. Cummings DAT, Iamsirithaworn S, Lessler JT, et al. The impact of the demographic transition on dengue in Thailand: insights from a statistical analysis and mathematical modeling. PLoS Med. 2009; 6:e1000139. [PubMed: 19721696]
28. Rodríguez-Barraquer I, Buathong R, Iamsirithaworn S, et al. Revisiting Rayong: shifting seroprofiles of dengue in Thailand and their implications for transmission and control. Am J Epidemiol. 2014; 179:353–60. [PubMed: 24197388]

29. Perkins TA, Garcia AJ, Paz-Soldan VA, et al. Theory and data for simulating fine-scale human movement in an urban environment. J R Soc Interface. 2014; 11:20140642–20140642. [PubMed: 25142528]
30. Waggoner JJ, Balmaseda A, Gresh L, et al. Homotypic dengue virus re-infections in Nicaraguan children. J Infect Dis. 2016
31. Forshey BM, Reiner RC, Olkowski S, et al. Incomplete protection against dengue virus type 2 re-infection in Peru. PLoS Negl Trop Dis. 2016; 10:e0004398. [PubMed: 26848841]
32. Duong V, Lambrechts L, Paul RE, et al. Asymptomatic humans transmit dengue virus to mosquitoes. Proc Natl Acad Sci. 2015; 112:14688–93. [PubMed: 26553981]
33. Fox A, Whitehead S, Anders KL, et al. Investigation of dengue and Japanese encephalitis virus transmission in Hanam, Viet Nam. Am J Trop Med Hyg. 2014; 90:892–6. [PubMed: 24615123]
34. Katzelnick LC, Montoya M, Gresh L, Balmaseda A, Harris E. Neutralizing antibody titers against dengue virus correlate with protection from symptomatic infection in a longitudinal cohort. Proc Natl Acad Sci. 2016:201522136.
35. Clapham HE, Rodriguez-Barraquer I, Azman AS, et al. Dengue virus (DENV) neutralizing antibody kinetics in children after symptomatic primary and postprimary DENV infection. J Infect Dis. 2016; 213:1428–35. [PubMed: 26704615]
36. Hadinegoro SR, Arredondo-García JL, Capeding MR, et al. Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. N Engl J Med. 2015:150727090428004.
37. Capeding MR, Tran NH, Hadinegoro SRS, et al. 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. 2014; 6736:1–8.
38. Villar L, Dayan GH, Arredondo-García JL, et al. Efficacy of a tetravalent dengue vaccine in children in Latin America. N Engl J Med. 2015; 372:113–23. [PubMed: 25365753]
39. Coudeville L, Baurin N, Vergu E. Estimation of parameters related to vaccine efficacy and dengue transmission from two large phase III studies. Vaccine. 2015:1–9.
40. Nisalak A, Endy TP, Nimmannitya S, et al. Serotype-specific dengue virus circulation and dengue disease in Bangkok, Thailand from 1973 to 1999. Am J Trop Med Hyg. 2003; 68:191–202. [PubMed: 12641411]
41. Morrison AC, Minnick SL, Rocha C, et al. Epidemiology of dengue virus in iquitos, Peru 1999 to 2005: interepidemic and epidemic patterns of transmission. PLoS Negl Trop Dis. 2010; 4:e670. [PubMed: 20454609]
42. Balmaseda A, Hammond SN, Pérez L, et al. Serotype-specific differences in clinical manifestations of dengue. Am J Trop Med Hyg. 2006; 74:449–56. [PubMed: 16525106]
43. Ohainle, M., Harris, E. Dengue pathogenesis: viral factors. In: Gubler, DJ.Ooi, EE.Vasudevan, SG., Farrar, J., editors. Dengue and dengue hemorrhagic fever. 2nd. CAB International; 2014. p. 231-50.
44. OhAinle M, Balmaseda A, Macalalad AR, et al. Dynamics of dengue disease severity determined by the interplay between viral genetics and serotype-specific immunity. Sci Transl Med. 2011; 3:114ra128.
45. Bhoomiboonchoo P, Nisalak A, Chansatiporn N, et al. Sequential dengue virus infections detected in active and passive surveillance programs in Thailand, 1994–2010. BMC Public Health. 2015; 15:1–10. [PubMed: 25563658]
46. Kochel TJ, Watts DM, Halstead SB, et al. Effect of dengue-1 antibodies on American dengue-2 viral infection and dengue haemorrhagic fever. Lancet. 2002; 360:310–2. [PubMed: 12147378]
47. Olkowski S, Forshey BM, Morrison AC, et al. Reduced risk of disease during postsecondary dengue virus infections. J Infect Dis. 2013; 208:1026–33. [PubMed: 23776195]
48. Guirakhoo F, Pugachev K, Arroyo J, et al. 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. 2002; 298:146–59. [PubMed: 12093182]
49. VanBlargan LA, Mukherjee S, Dowd KA, Durbin AP, Whitehead SS, Pierson TC. The type-specific neutralizing antibody response elicited by a dengue vaccine candidate is focused on two amino acids of the envelope protein. PLoS Pathog. 2013; 9:e1003761. [PubMed: 24348242]

50. Wang C, Katzelnick LC, Montoya M, Hue KDT, Simmons CP, Harris E. Evolutionarily successful asian 1 dengue virus 2 lineages contain one substitution in envelope that increases sensitivity to polyclonal antibody neutralization. J Infect Dis. 2016; 213:975–84. [PubMed: 26582957]
51. Dowd KA, DeMaso CR, Pierson TC. Genotypic differences in dengue virus neutralization are explained by a single amino acid mutation that modulates virus breathing. MBio. 2015; 6:e01559–15. [PubMed: 26530385]
52. Zhang C, Mammen MP, Chinnawirotpisan P, et al. Clade replacements in dengue virus serotypes 1 and 3 are associated with changing serotype prevalence. J Virol. 2005; 79:15123–30. [PubMed: 16306584]
53. Quiner CA, Parameswaran P, Ciota AT, et al. Increased replicative fitness of a dengue virus 2 clade in native mosquitoes: potential contribution to a clade replacement event in Nicaragua. J Virol. 2014; 88:13125–34. [PubMed: 25187539]
54. Adams B, Holmes EC, Zhang C, et al. Cross-protective immunity can account for the alternating epidemic pattern of dengue virus serotypes circulating in Bangkok. Proc Natl Acad Sci. 2006; 103:14234–9. [PubMed: 16966609]
55. Bennett SN, Holmes EC, Chirivella M, et al. Molecular evolution of dengue 2 virus in Puerto Rico: positive selection in the viral envelope accompanies clade reintroduction. J Gen Virol. 2006; 87:885–93. [PubMed: 16528038]
56. Twiddy SS, Woelk CH, Holmes EC. Phylogenetic evidence for adaptive evolution of dengue viruses in nature. J Gen Virol. 2002; 83:1679–89. [PubMed: 12075087]
57. Cummings DAT, Schwartz IB, Billings L, Shaw LB, Burke DS. Dynamic effects of antibody-dependent enhancement on the fitness of viruses. Proc Natl Acad Sci. 2005; 102:15259–64. [PubMed: 16217017]
58. Recker M, Blyuss KB, Simmons CP, et al. Immunological serotype interactions and their effect on the epidemiological pattern of dengue. Proc Biol Sci. 2009; 276:2541–8. [PubMed: 19369266]
59. Wearing HJ, Rohani P. Ecological and immunological determinants of dengue epidemics. Proc Natl Acad Sci. 2006; 103:11802–7. [PubMed: 16868086]
60. Sabchareon A, Wallace D, Sirivichayakul C, et al. Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: a randomised, controlled phase 2b trial. Lancet. 2012; 380:1559–67. [PubMed: 22975340]
61. Durbin AP, Kirkpatrick BD, Pierce KK, et al. A single dose of any of four different live attenuated tetravalent dengue vaccines is safe and immunogenic in flavivirus-naive adults: A randomized, double-blind clinical trial. J Infect Dis. 2013; 207:957–65. [PubMed: 23329850]
62. Anderson KB, Gibbons RV, Cummings DAT, et al. A shorter time interval between first and second dengue infections is associated with protection from clinical illness in a school-based cohort in Thailand. J Infect Dis. 2014; 209:360–8. [PubMed: 23964110]
63. Reich NG, Shrestha S, King AA, et al. Interactions between serotypes of dengue highlight epidemiological impact of cross-immunity. J R Soc Interface. 2013; 10:20130414. [PubMed: 23825116]
64. Montoya M, Gresh L, Mercado JC, et al. Symptomatic versus inapparent outcome in repeat dengue virus infections is influenced by the time interval between infections and study year. PLoS Negl Trop Dis. 2013; 7:e2357. [PubMed: 23951377]
65. Weiskopf D, Sette A. T-cell immunity to infection with dengue virus in humans. Front Immunol. 2014; 5:93. [PubMed: 24639680]
66. Corbett KS, Katzelnick L, Tissera H, Amerasinghe A, de Silva AD, de Silva AM. Preexisting neutralizing antibody responses distinguish clinically inapparent and apparent dengue virus infections in a Sri Lankan pediatric cohort. J Infect Dis. 2015; 211:590–9. [PubMed: 25336728]
67. Puschnik A, Lau L, Cromwell EA, Balmaseda A, Zompi S, Harris E. Correlation between dengue-specific neutralizing antibodies and serum avidity in primary and secondary dengue virus 3 natural infections in humans. PLoS Negl Trop Dis. 2013; 7:e2274. [PubMed: 23785536]
68. Halstead SB, Papaevangelou G. Transmission of dengue 1 and 2 viruses in Greece in 1928. Am J Trop Med Hyg. 1980; 29:635–7. [PubMed: 6996504]
69. Guzman MG, Alvarez M, Rodriguez-Roche R, et al. Neutralizing antibodies after infection with dengue 1 virus. Emerg Infect Dis. 2007; 13:282–6. [PubMed: 17479892]

70. Halstead SB, Grosz CR. Subclinical Japanese encephalitis. I. Infection of Americans with limited residence in Korea. Am J Hyg. 1962; 75:190–201. [PubMed: 13904014]
71. Hickey AC, Koster JA, Thalmann CM, et al. Serotype-specific host responses in rhesus macaques after primary dengue challenge. Am J Trop Med Hyg. 2013; 89:1043–57. [PubMed: 24062475]
72. Koraka P, Benton S, van Amerongen G, Stittelaar KJ, Osterhaus ADME. Characterization of humoral and cellular immune responses in cynomolgus macaques upon primary and subsequent heterologous infections with dengue viruses. Microbes Infect. 2007; 9:940–6. [PubMed: 17544804]
73. Katzelnick LC, Fonville JM, Gromowski GD, et al. Dengue viruses cluster antigenically but not as discrete serotypes. Science. 2015; 349:1338–43. [PubMed: 26383952]
74. Gibbons RV, Kalanarooj S, Jarman RG, et al. Analysis of repeat hospital admissions for dengue to estimate the frequency of third or fourth dengue infections resulting in admissions and dengue hemorrhagic fever, and serotype sequences. Am J Trop Med Hyg. 2007; 77:910–3. [PubMed: 17984352]
75. Rouvinski A, Guardado-Calvo P, Barba-Spaeth G, et al. Recognition determinants of broadly neutralizing human antibodies against dengue viruses. Nature. 2015; 520:109–13. [PubMed: 25581790]
76. Sabin AB. Research on dengue during World War II. Am J Trop Med Hyg. 1952; 1:30–50. [PubMed: 14903434]
77. Halstead S, Casals J. Studies on the immunization of monkeys against dengue I. Protection derived from single and sequential virus infections. Am J Trop Med Hyg. 1973; 22:365–74. [PubMed: 4196288]
78. Halstead SB. Identifying protective dengue vaccines: guide to mastering an empirical process. Vaccine. 2013; 31:4501–7. [PubMed: 23896423]
79. Bernardo L, Izquierdo A, Prado I, et al. Primary and secondary infections of Macaca fascicularis monkeys with Asian and American genotypes of dengue virus 2. Clin Vaccine Immunol. 2008; 15:439–46. [PubMed: 18094112]
80. Thomas SJ, Nisalak A, Anderson KB, et al. Dengue plaque reduction neutralization test (PRNT) in primary and secondary dengue virus infections: how alterations in assay conditions impact performance. Am J Trop Med Hyg. 2009; 81:825–33. [PubMed: 19861618]
81. Mukherjee S, Dowd KA, Manhart CJ, et al. Mechanism and significance of cell type-dependent neutralization of flaviviruses. J Virol. 2014; 88:7210–20. [PubMed: 24741083]
82. Pierson TC, Xu Q, Nelson S, et al. The stoichiometry of antibody-mediated neutralization and enhancement of West Nile virus infection. Cell Host Microbe. 2007; 1:135–45. [PubMed: 18005691]
83. Plevka P, Battisti AJ, Sheng J, Rossmann MG. Mechanism for maturation-related reorganization of flavivirus glycoproteins. J Struct Biol. 2014; 185:27–31. [PubMed: 24252771]
84. Austin SK, Dowd KA, Shrestha B, et al. Structural basis of differential neutralization of DENV-1 genotypes by an antibody that recognizes a cryptic epitope. PLoS Pathog. 2012; 8:e1002930. [PubMed: 23055922]
85. Brien JD, Austin SK, Sukupolvi-Petty S, et al. Genotype-specific neutralization and protection by antibodies against dengue virus type 3. J Virol. 2010; 84:10630–43. [PubMed: 20702644]
86. Timiryasova TM, Bonaparte MI, Luo P, Zedar R, Hu BT, Hildreth SW. Optimization and validation of a plaque reduction neutralization test for the detection of neutralizing antibodies to four serotypes of dengue virus used in support of dengue vaccine development. Am J Trop Med Hyg. 2013; 88:962–70. [PubMed: 23458954]
87. Endy TP, Nisalak A, Chunsuttitwat S, et al. Relationship of preexisting dengue virus (DV) neutralizing antibody levels to viremia and severity of disease in a prospective cohort study of DV infection in Thailand. J Infect Dis. 2004; 189:990–1000. [PubMed: 14999601]
88. Buddhari D, Aldstadt J, Endy TP, et al. Dengue virus neutralizing antibody levels associated with protection from infection in Thai cluster studies. PLoS Negl Trop Dis. 2014; 8:e3230. [PubMed: 25329173]

89. de Alwis R, Beltramello M, Messer WB, et al. In-depth analysis of the antibody response of individuals exposed to primary dengue virus infection. PLoS Negl Trop Dis. 2011; 5:e1188. [PubMed: 21713020]

90. Smith SA, de Alwis AR, Kose N, et al. The potent and broadly neutralizing human dengue virus-specific monoclonal antibody 1C19 reveals a unique cross-reactive epitope on the bc loop of domain II of the envelope protein. MBio. 2013; 4:e00873–13. [PubMed: 24255124]

91. Tsai W-Y, Durbin A, Tsai J-J, Hsieh S-C, Whitehead S, Wang W-K. Complexity of neutralizing antibodies against multiple dengue virus serotypes after heterotypic immunization and secondary infection revealed by in-depth analysis of cross-reactive antibodies. J Virol. 2015; 89:7348–62. [PubMed: 25972550]

92. Fibriansah G, Tan JL, Smith SA, et al. A highly potent human antibody neutralizes dengue virus serotype 3 by binding across three surface proteins. Nat Commun. 2015; 6:6341. [PubMed: 25698059]

93. Fibriansah G, Tan JL, Smith SA, et al. A potent anti-dengue human antibody preferentially recognizes the conformation of E protein monomers assembled on the virus surface. EMBO Mol Med. 2014; 6:358–71. [PubMed: 24421336]

94. Fibriansah G, Ibarra KD, Ng T, et al. Cryo-EM structure of an antibody that neutralizes dengue virus type 2 by locking E protein dimers. Science. 2015; 349:88–91. [PubMed: 26138979]

95. Teoh EP, Kukkaro P, Teo EW, et al. The structural basis for serotype-specific neutralization of dengue virus by a human antibody. Sci Transl Med. 2012; 4:139ra83.

96. de Alwis R, Smith SA, Olivarez NP, et al. Identification of human neutralizing antibodies that bind to complex epitopes on dengue virions. Proc Natl Acad Sci. 2012; 109:7439–44. [PubMed: 22499787]

97. Messer WB, Yount BL, Royal SR, et al. Functional transplant of a dengue virus serotype 3 (DENV3)-specific human monoclonal antibody epitope into DENV1. J Virol. 2016; 90:5090–7. [PubMed: 26962223]

98. Halstead SB. In vivo enhancement of dengue virus infection in rhesus monkeys by passively transferred antibody. J Infect Dis. 1979; 140:527–33. [PubMed: 117061]

99. Goncalvez AP, Engle RE, St Claire M, Purcell RH, Lai C-J. Monoclonal antibody-mediated enhancement of dengue virus infection in vitro and in vivo and strategies for prevention. Proc Natl Acad Sci U S A. 2007; 104:9422–7. [PubMed: 17517625]

100. Zellweger RM, Prestwood TR, Shresta S. Enhanced infection of liver sinusoidal endothelial cells in a mouse model of antibody-induced severe dengue disease. Cell Host Microbe. 2010; 7:128–39. [PubMed: 20153282]

101. Williams KL, Sukupolvi-Petty S, Beltramello M, et al. Therapeutic efficacy of antibodies lacking Fcγ receptor binding against lethal dengue virus infection is due to neutralizing potency and blocking of enhancing antibodies [corrected]. PLoS Pathog. 2013; 9:e1003157. [PubMed: 23459315]

102. Simmons CP, Chau TNB, Thuy TT, et al. Maternal antibody and viral factors in the pathogenesis of dengue virus in infants. J Infect Dis. 2007; 196:416–24. [PubMed: 17597456]

103. Chau TNB, Quyen NTH, Thuy TT, et al. Dengue in Vietnamese infants–results of infection-enhancement assays correlate with age-related disease epidemiology, and cellular immune responses correlate with disease severity. J Infect Dis. 2008; 198:516–24. [PubMed: 18598189]

104. Chau TNB, Hieu NT, Anders KL, et al. Dengue virus infections and maternal antibody decay in a prospective birth cohort study of Vietnamese infants. J Infect Dis. 2009; 200:1893–900. [PubMed: 19911991]

105. Kliks SC, Nimmanitya S, Nisalak A, Burke DS. Evidence that maternal dengue antibodies are important in the development of dengue hemorrhagic fever in infants. Am J Trop Med Hyg. 1988; 38:411–9. [PubMed: 3354774]

106. Clapham H, Cummings DAT, Nisalak A, et al. Epidemiology of infant dengue cases illuminates serotype-specificity in the interaction between immunity and disease, and changes in transmission dynamics. PLoS Negl Trop Dis. 2015; 9:e0004262. [PubMed: 26658730]

107. Hammond SN, Balmaseda A, Pérez L, et al. Differences in dengue severity in infants, children, and adults in a 3-year hospital-based study in Nicaragua. Am J Trop Med Hyg. 2005; 73:1063–70. [PubMed: 16354813]
108. Kliks SC, Nisalak A, Brandt WE, Wahl L, Burke DS. Antibody-dependent enhancement of dengue virus growth in human monocytes as a risk factor for dengue hemorrhagic fever. Am J Trop Med Hyg. 1989; 40:444–51. [PubMed: 2712199]
109. Libraty DH, Acosta LP, Tallo V, et al. A prospective nested case-control study of dengue in infants: rethinking and refining the antibody-dependent enhancement dengue hemorrhagic fever model. PLoS Med. 2009; 6:e1000171. [PubMed: 19859541]
110. Laoprasopwattana K, Libraty DH, Endy TP, et al. Dengue Virus (DV) enhancing antibody activity in preillness plasma does not predict subsequent disease severity or viremia in secondary DV infection. J Infect Dis. 2005; 192:510–9. [PubMed: 15995967]
111. Halstead SB. Dengue antibody-dependent enhancement: knowns and unknowns. Microbiol Spectr. 2014; 2:249–71.
112. Bernasconi NL, Traggiai E, Lanzavecchia A. Maintenance of serological memory by polyclonal activation of human memory B cells. Science. 2002; 298:2199–202. [PubMed: 12481138]
113. Amanna IJ, Slifka MK. Mechanisms that determine plasma cell lifespan and the duration of humoral immunity. Immunol Rev. 2010; 236:125–38. [PubMed: 20636813]
114. Amanna IJ, Carlson NE, Slifka MK. Duration of humoral immunity to common viral and vaccine antigens. N Engl J Med. 2007; 357:1903–15. [PubMed: 17989383]
115. Slifka MK. Vaccine-mediated immunity against dengue and the potential for long-term protection against disease. Front Immunol. 2014; 5:195. [PubMed: 24834067]
116. Dejnirattisai W, Wongwiwat W, Supasa S, et al. A new class of highly potent, broadly neutralizing antibodies isolated from viremic patients infected with dengue virus. Nat Immunol. 2015; 16:785.
117. Purtha WE, Tedder TF, Johnson S, Bhattacharya D, Diamond MS. Memory B cells, but not long-lived plasma cells, possess antigen specificities for viral escape mutants. J Exp Med. 2011; 208:2599–606. [PubMed: 22162833]
118. Rothman AL. Immunity to dengue virus: a tale of original antigenic sin and tropical cytokine storms. Nat Rev Immunol. 2011; 11:532–43. [PubMed: 21760609]
119. Weiskopf D, Angelo MA, Bangs DJ, et al. The Human CD8+ T Cell responses induced by a live attenuated tetravalent dengue vaccine are directed against highly conserved epitopes. J Virol. 2015; 89:120–8. [PubMed: 25320311]
120. Yauch LE, Zellweger RM, Kotturi MF, et al. A protective role for dengue virus-specific CD8+ T cells. J Immunol. 2009; 182:4865–73. [PubMed: 19342665]
121. Hatch S, Endy TP, Thomas S, et al. Intracellular cytokine production by dengue virus-specific T cells correlates with subclinical secondary infection. J Infect Dis. 2011; 203:1282–91. [PubMed: 21335561]
122. Weiskopf D, Angelo MA, de Azeredo EL, et al. Comprehensive analysis of dengue virus-specific responses supports an HLA-linked protective role for CD8+ T cells. Proc Natl Acad Sci. 2013; 110:E2046–53. [PubMed: 23580623]
123. Weiskopf D, Cerpas C, Angelo MA, et al. Human CD8+ T-cell responses against the 4 dengue virus serotypes are associated with distinct patterns of protein targets. J Infect Dis. 2015; 212:1743–51. [PubMed: 25980035]
124. Guy B, Nougarede N, Begue S, et al. Cell-mediated immunity induced by chimeric tetravalent dengue vaccine in naive or flavivirus-primed subjects. Vaccine. 2008; 26:5712–21. [PubMed: 18762226]
125. Mangada MM, Rothman AL. Altered cytokine responses of dengue-specific CD4+ T cells to heterologous serotypes. J Immunol. 2005; 175:2676–83. [PubMed: 16081844]
126. Yauch LE, Prestwood TR, May MM, et al. CD4+ T cells are not required for the induction of dengue virus-specific CD8+ T cell or antibody responses but contribute to protection after vaccination. J Immunol. 2010; 185:5405–16. [PubMed: 20870934]

127. Weiskopf D, Bangs DJ, Sidney J, et al. Dengue virus infection elicits highly polarized CX3CR1^{+} cytotoxic CD4^{+} T cells associated with protective immunity. Proc Natl Acad Sci. 2015; 112:E4256–63. [PubMed: 26195744]
128. Pagni S, Fernandez-Sesma A. Evasion of the human innate immune system by dengue virus. Immunol Res. 2012; 54:152–9. [PubMed: 22569913]
129. Popper SJ, Gordon A, Liu M, Balmaseda A, Harris E, Relman DA. Temporal dynamics of the transcriptional response to dengue virus infection in Nicaraguan children. PLoS Negl Trop Dis. 2012; 6:e1966. [PubMed: 23285306]
130. Simmons CP, Popper S, Dolocek C, et al. Patterns of host genome-wide gene transcript abundance in the peripheral blood of patients with acute dengue hemorrhagic fever. J Infect Dis. 2007; 195:1097–107. [PubMed: 17357045]
131. Kwissa M, Nakaya HI, Onlamoon N, et al. Dengue virus infection induces expansion of a CD14+CD16 + monocyte population that stimulates plasmablast differentiation. Cell Host Microbe. 2014; 16:115–27. [PubMed: 24981333]
132. Koraka P, Suharti C, Setiati TE, et al. Kinetics of dengue virus-specific serum immunoglobulin classes and subclasses correlate with clinical outcome of infection. J Clin Microbiol. 2001; 39:4332–8. [PubMed: 11724841]
133. Laoprasopwattana K, Libraty DH, Endy TP, et al. Antibody-dependent cellular cytotoxicity mediated by plasma obtained before secondary dengue virus infections: potential involvement in early control of viral replication. J Infect Dis. 2007; 195:1108–16. [PubMed: 17357046]
134. García G, Arango M, Pérez a B, et al. Antibodies from patients with dengue viral infection mediate cellular cytotoxicity. J Clin Virol. 2006; 37:53–7. [PubMed: 16787760]
135. Avirutnan P, Punyadee N, Noisakran S, et al. Vascular leakage in severe dengue virus infections: a potential role for the nonstructural viral protein NS1 and complement. J Infect Dis. 2006; 193:1078–88. [PubMed: 16544248]
136. Markoff LJ, Innis BL, Houghten R, Henchal LS. Development of cross-reactive antibodies to plasminogen during the immune response to dengue virus infection. J Infect Dis. 1991; 164:294–301. [PubMed: 1856477]
137. Falconar AK. The dengue virus nonstructural-1 protein (NS1) generates antibodies to common epitopes on human blood clotting, integrin/adhesin proteins and binds to human endothelial cells: potential implications in haemorrhagic fever pathogenesis. Arch Virol. 1997; 142:897–916. [PubMed: 9191856]
138. Lin Y-S, Yeh T-M, Lin C-F, et al. Molecular mimicry between virus and host and its implications for dengue disease pathogenesis. Exp Biol Med (Maywood). 2011; 236:515–23. [PubMed: 21502191]
139. Chuang Y-C, Lin Y-S, Liu H-S, Yeh T-M. Molecular mimicry between dengue virus and coagulation factors induces antibodies to inhibit thrombin activity and enhance fibrinolysis. J Virol. 2014; 88:13759–68. [PubMed: 25231318]
140. Chuang Y-C, Lin Y-S, Liu H-S, Wang J-R, Yeh T-M. Antibodies against thrombin in dengue patients contain both anti-thrombotic and pro-fibrinolytic activities. Thromb Haemost. 2013; 110:358–65. [PubMed: 23740201]
141. Modhiran N, Watterson D, Muller DA, et al. Dengue virus NS1 protein activates cells via Toll-like receptor 4 and disrupts endothelial cell monolayer integrity. Sci Transl Med. 2015; 7:304ra142.
142. Beatty PR, Puerta-guardo H, Killingbeck SS, Glasner DR, Hopkins K, Harris E. Dengue virus NS1 triggers endothelial permeability and vascular leak that is prevented by NS1 vaccination. Sci Transl Med. 2015; 7:304ra141.
143. Puerta-Guardo H, Glasner DR, Harris E. Dengue Virus NS1 disrupts the endothelial glycocalyx, leading to hyperpermeability. PLOS Pathog. 2016; 12:e1005738. [PubMed: 27416066]
144. Vannice KS, Roehrig JT, Hombach J. Next generation dengue vaccines: A review of the preclinical development pipeline. Vaccine. 2015; 33:7091–9. [PubMed: 26424602]
145. Sampath S, Carrico C, Janes J, et al. Glycan masking of Plasmodium vivax Duffy Binding Protein for probing protein binding function and vaccine development. PLoS Pathog. 2013; 9:e1003420. [PubMed: 23853575]

146. McLellan JS, Correia BE, Chen M, et al. Design and characterization of epitope-scaffold immunogens that present the motavizumab epitope from respiratory syncytial virus. J Mol Biol. 2011; 409:853–66. [PubMed: 21549714]

147. Guy B, Jackson N. Dengue vaccine: hypotheses to understand CYD-TDV-induced protection. Nat Rev Microbiol. 2015; 14:45–54. [PubMed: 26639777]

148. Plotkin SA, Gilbert PB. Nomenclature for immune correlates of protection after vaccination. Clin Infect Dis. 2012; 54:1615–7. [PubMed: 22437237]

149. Teoh BT, Sam SS, Tan KK, et al. Early detection of dengue virus by use of reverse transcription-recombinase polymerase amplification. J Clin Microbiol. 2015; 53:830–7. [PubMed: 25568438]

150. John DV, Lin Y-S, Perng GC. Biomarkers of severe dengue disease - a review. J Biomed Sci. 2015; 22:83. [PubMed: 26462910]

Panel 1

Search strategy and selection criteria

We identified articles published in English and Spanish by searching PubMed for the section headings below (e.g. “dengue vector control”). These articles were reviewed for relevant references. Historical articles describing major discoveries, recent studies (last 10 years), and studies covering distinct topics were selected for review.

Panel 2

Major public health and research priorities

- Develop affordable, easy-to-perform acute-phase diagnostics and prognostics to enable timely evidence-based case management, including rapid feedback from central national laboratories to clinics to directly improve patient treatment.
- Promote laboratory-enhanced sentinel surveillance to enable early introduction of interventions to mitigate dengue outbreaks, which can be further supplemented with information from crowd-sourcing technologies that enable communities to be directly involved in control efforts.
- Generate new guidelines for vector control based on validated strategies proven to reduce DENV infection and disease, including community-based approaches for source reduction, that governments can use to imrpove control programs.
- Incorporate recent insights into the immune responses to natural DENV infection and vaccination, including the differences observed between DENV-naïve and DENV-exposed individuals in vaccine efficacy, to develop increasingly sophisticated models of dengue immunity.
- Understand the genetic basis for virulence, enhanced replication in mosquitoes and humans, immunogenicity, and neutralizing properties of genetically diverse DENV strains.
- Estimate decay rates of cross-reactive neutralizing antibodies following primary infection in both endemic and non-endemic settings.
- Understand why type-specific protection can fail and result in homologous reinfection, and further study if frequent DENV exposure maintains long-term protective immunity.
- Establish why post-secondary responses induce multivalent protective immunity, including identifying B cells producing potently neutralizing antibodies and identifying their lineage.
- Determine to what degree disease severity is mediated by antibody-dependent enhancement.
- Identify the role of memory $CD4^+$ T cells in stimulating protective antibody-mediated immunity and robust $CD8^+$ T cell responses during secondary infection.
- Investigate protective effector functions of anti-DENV antibodies.
- Further study the role of the NS1 protein in dengue pathogenesis to develop new targets for therapeutics and protective dengue vaccines.

- Clearly convey to public health practitioners in dengue-affected areas that naïve dengue status is likely a major risk factor for the increase in severe disease observed in young children in the Sanofi Phase 3 clinical trial so that public health practitioners can decide whether the vaccine should be used in low-endemic settings and in areas with only one DENV serotype circulating.

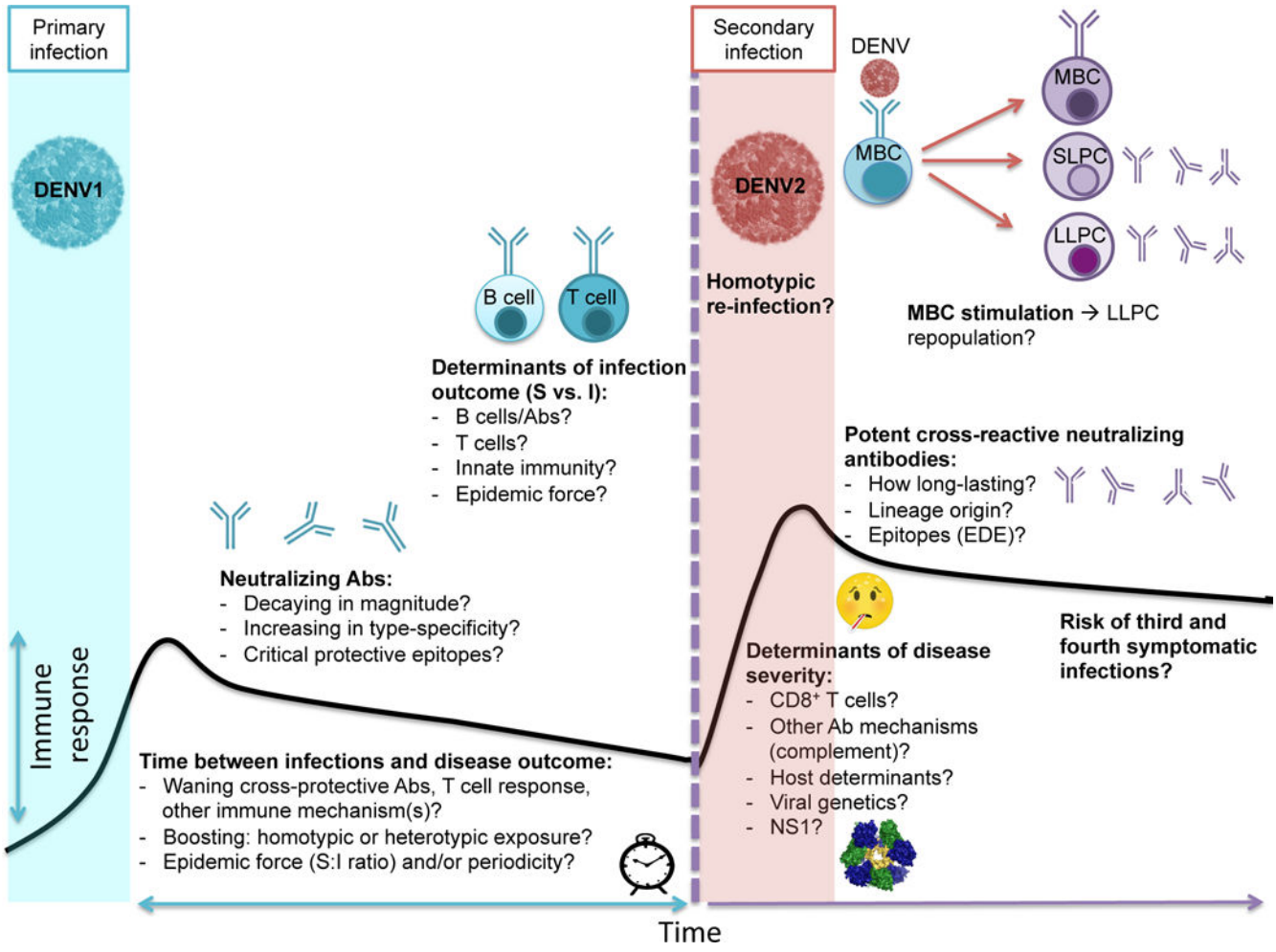

Figure 1. Key questions about the adaptive immune response following primary and secondary DENV infection

The black line represents the level of individual host immunity (y-axis) over time (x-axis). Abbreviations: Ab, antibody; S:I ratio, symptomatic to inapparent infection ratio; MBC, memory B cell; LLPC, long-lived plasma cell; SLPC, short-lived plasma cells.

Table 1

Tools for case management, surveillance, outbreak response, and sero-epidemiological studies.

Description	Application and future priorities
Case management tools: diagnostics using direct assays during the viremic phase (<5 days of illness)	
Viral RNA detection such as reverse transcriptase (RT)-PCR, real-time RT-PCR, as well as virus isolation, are highly sensitive but expensive.	Promising lower-cost molecular biological techniques are being developed[149] but require further validation to be commercialized.
Numerous commercial ELISA kits and RDTs for NS1 exist, but have a wide range of sensitivity depending on DENV serotype, primary/secondary infection, and commercial brand, and are too expensive for public sector use.	More affordable NS1 assays are needed, and new commercial assays need to continue to be evaluated by a panel of reference laboratories.[9]
NS1 is present early in infection, and anti-DENV IgM antibodies are detectable later in illness, making a combination test a promising tool for dengue diagnosis.	Current assays are of variable quality and are expensive; thus, they need to be further developed to be used affordably in LMICs.
Case management tools: prognostic tests for triage and treatment of severe dengue patients	
Clinical manifestations are currently the only tool for predicting severe dengue disease.	Promising biomarkers include: innate immunity gene expression profiles, specific cytokines, T and B cell activation markers, and complement activation,[150] but require development into validated assays.
Surveillance and outbreak response tools: serological assays and crowd-sourcing	
IgM capture ELISAs are widely available and are used by most public health systems; however, a single positive DENV-specific IgM is only a probable diagnosis.	IgM seroconversion in paired acute- and convalescent-phase (15–21 days post-onset) sera is required for confirmation of DENV infection, but paired samples are often not available.
Commercial IgM ELISAs and RDTs are available but of variable quality.	New commercial IgM assays should be evaluated by a panel of reference laboratories.
Changes in IgG titer and the IgG/IgM ratio are often detected byIgG capture ELISA to differentiate primary from secondary DENV infections; many kits are comercially available.	Some kits need to be better validated and standardized. For example, kits are comercially available to distiguish primary and secondary infections using a single IgG test during the acute phase; these should be interpreted with caution.
There have been some successes using crowd-sourcing technologies to monitor and control dengue outbreaks[10,11] (Google dengue-trends, DengueMap, DengueChat).	Crowd-sourcing technologies for illness reporting need to be validated by laboratory confirmation of etiology; these technologies should be paired with community entomology efforts and be used to enhance national surveillance.
Sero-epidemiological tools: more specific serological assays in post-convalescent phase or vaccinees	
DENV infection in a population can be measured using IgG or Inhibition ELISA or Hemagglutination Inhibition.	These methods are not as specific as neutralizing assays, but are easier to perform at large scale.
Neutralization assays are considered most specific and can differentiate certain flaviviruses, including DENV1-4 and ZIKV, in primary infections and >6 months post-infection.	In secondary DENV infections, cross-reactivity among the four serotypes impairs interpretation of neutralization titers in a single sample, although longitudinal annual samples have enabled reconstruction of DENV immune history.[41,64]

Table 2

Research priorities for modeling dengue transmission.

Issues	Research priorities
Geographic dissemination of DENV is attributable to ***human movement***, which functions by heterogeneous mixing.[24,29]	Develop models that accurately describe human movement and mosquito density to understand different levels of disease risk within cities.
Climate variables play a major role in region-wide epidemics.[25,26]	Incorporate climate variables to improve prediction of epidemics so that governments can prioritize dengue control efforts.
The ***demographic transition*** affects R_0, leaving more individuals DENV-naïve until older ages.[27,28]	Identify the role of population age structure on DENV transmission in additional settings.
Homologous re-infection has recently been shown to occur, and infected individuals can have high viremia, suggesting they may contribute to transmission.[30] ***Heterologous asymptomatic infections contribute to transmission***, as these individuals can have high viremia and have greater movement than sick individuals[32].	Incorporate homologous re-infection and heterologous inapparent infections into epidemiological models to more accurately estimate DENV exposure and transmission.
Frequent DENV exposure in high-transmission settings may modify immunity and transmission in DENV-immune individuals.[34,35]	Take into account frequent DENV re-exposure in maintaining long-term protection and attenuating transmission in the DENV-immune.
Worldwide, ***other flaviviruses*** circulate in dengue-endemic areas, e.g. Japanese encephalitis/JEV vaccination in Southeast Asia,[33] yellow fever vaccination and now ZIKV in the Americas.	Estimate changes in DENV transmission in populations also infected by related flaviviruses.

Table 3

Research priorities in the context of dengue vaccination roll-out and evaluation.

Issues	Research priorities
Some immune biomarkers may be ***predictive of disease severity***.[150]	Develop prognostic tests for research and clinical trials.
Dengue vaccine roll-out will make distinguishing previous DENV infection from ***vaccine-induced immunity*** difficult.	Develop serological methods to distinguish natural and vaccine-induced immunity.
Other flaviviruses such as Zika confound serological diagnostic methods.	Develop new serologic assays to differentiate acute infections with DENV from ZIKV and other flaviviruses
Crowd-sourcing technologies used by empowered ***communities*** have great potential for outbreak detection.	Validate and incorporate crowd-sourcing for surveillance systems and documenting the impact of vaccine introduction.
Vaccines may have ***different efficacy*** in previously DENV-naïve and -immune individuals.[36–38]	Stratify vaccine efficacy by immune status[39] and update/develop models with additional vaccine trial and follow-up data.
Dengue vaccines may ***protect against disease but not transmission***.	Use models to compare effects of full vs. partial protection against transmission following vaccination.
Vaccine-induced neutralizing antibody responses are often higher to ***vaccine parent strains*** than genetically distinct isolates.[48]	Study how immune responses induced by vaccination protect against diverse strains.
Amino acid substitutions in laboratory-adapted/vaccine parent strains can ***alter the exposed epitopes***.[51]	Estimate how substitutions in laboratory-adapted strains affect immunogenicity compared with immunogenicity of natural isolates.
Tetravalent dengue vaccines may induce ***unbalanced responses*** or different combinations of type-specific and cross-reactive antibodies against DENV1-4.[60,61]	Evaluate whether vaccination could increase the risk of DENV lineages evolving to escape host immunity.
Homologous re-infection can lead to symptomatic disease.[30,31]	Understand why type-specific protection fails, in order to provide potential insight into low vaccine efficacy.
Frequent DENV re-exposures may "***boost***" levels of neutralizing antibody titers.	Evaluate whether natural boosting improves immunity and long-term vaccine efficacy.
Neutralization titers are highly variable under distinct assay conditions.[80,81]	Define the most critical variables that modulate neutralization assays and identify neutralization tests that correlate with vaccine-induced protection.
New tools are available ***to dissect the repertoire of polyclonal sera***.[49,96,97]	Apply these new tools to assess the "quality" of the neutralizing antibody response following natural infection and vaccination.
CD8$^+$ T cells primarily target non-structural proteins.[119,123]	Determine the role of non-structural proteins in inducing protective CD8$^+$ T cells in vaccinees.
CD4$^+$ T cells facilitate B cell and CD8$^+$ T cell activation and memory.[119]	Determine the role of memory CD4$^+$ T cells in inducing protective natural and vaccine-induced immunity.
Mice immunized with ***NS1*** are protected against lethal DENV challenge and anti-NS1 antibodies prevent vascular leak and endothelial hyperpermeability.[142]	Investigate whether anti-NS1 immunity following dengue vaccination contributes to protection against endothelial hyperpermeability and severe disease.
Following CYD vaccination, young vacinees had ***higher risk of hospitalized dengue*** than controls in follow-up data of recent clinical trials.[36]	Determine whether the risk of hospitalized dengue in young vaccinated individuals is mediated by age and/or DENV-naïve status,[147] and whether risk is mediated in part by ADE.
Neutralizing antibody titers may not be the only ***correlate of protection***.[148]	Study diverse measures of the immune response in relation to disease outcome for each vaccine.
Improved correlate of protection measures will be ***developed over time***.	Establish biorepositories of vaccines samples for future research.