HUMAN VACCINES & IMMUNOTHERAPEUTICS
2019, VOL. 15, NO. 10, 2328–2336
https://doi.org/10.1080/21645515.2019.1643676

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REVIEW

Does structurally-mature dengue virion matter in vaccine preparation in post-Dengvaxia era?

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ABSTRACT

The unexpectedly low vaccine efficacy of Dengvaxia®, developed by Sanofi Pasteur, and a higher risk of severe diseases after vaccination among dengue-naive children or children younger than 6 years old, have cast skepticism about the safety of dengue vaccination resulting in the suspension of school-based immunization programs in the Philippines. The absence of immune correlates of protection from dengue virus (DENV) infection hampers the development of other potential DENV vaccines. While tetravalent live-attenuated tetravalent vaccines (LATVs), which mimic natural infection by inducing both cellular and humoral immune responses, are still currently favored, developing a vaccine that provides a balanced immunity to all four DENV serotypes remains a challenge. With the recently advanced understanding of virion structure and B cell immune responses from naturally infected DENV patients, two points of view in developing a next-generation dengue vaccine emerged: one is to induce potent, type-specific neutralizing antibodies (NtAbs) recognizing quaternary structure-dependent epitopes by having four components of vaccine strains replicate equivalently; the other is to induce protective and broadly NtAbs against the four serotypes of DENV with a universal vaccine. This article reviews the studies related to these issues and the current knowledge gap that needs to be filled in.

ARTICLE HISTORY
Received 26 April 2019
Revised 6 June 2019
Accepted 5 July 2019

KEYWORDS
Dengue virus; dengue; virus-like particles; maturity; broadly neutralizing antibody

Introduction

Dengue is a mosquito-borne disease caused by any of the four genetically close-related but antigenically distinct virus serotypes of the genus *Flavivirus*.[1] An estimated 390 million dengue infections occur annually, and about 96 million people have clinically apparent disease.[2] Despite large efforts in implementing various mosquito control programs, dengue continues to be a growing public health problem due to many reasons, including climate change, mass and speed of public transportation, and population growth.[3] Besides vector control strategies, various vaccine approaches against dengue virus (DENV) have been developed over the last 50 years to prevent this infection. The first dengue vaccine, Dengvaxia®, licensed for use in a number of countries, is a live-attenuated chimeric yellow fever-DENV tetravalent dengue vaccine (CYD-TDV). However, an unexpectedly low vaccine efficacy among dengue naïve children or children younger than 6 years old was revealed in phase III clinical trials.[4,5] Follow-up studies also showed an elevated risk of severe dengue requiring hospitalization among seronegative individuals receiving the vaccination, thus suggesting that the vaccine mimics a primary DENV infection in naïve patients, who might be sensitized with increased vulnerability to antibody-dependent enhancement (ADE) after primary DENV infection.[6–8] As a result, this vaccine is now only recommended for DENV-immune individuals who are currently living in dengue-endemic countries.[9,10] Further, the safety concern of dengue vaccination among seronegative individuals has also resulted in the suspension of school-based immunization programs in the Philippines.[11]

Many reviews have discussed the difficulties of Dengvaxia® in inducing a balanced immunity against all four serotypes of DENV[12–20] and provided several guidelines in the development of next-generation dengue vaccines.[21–23] Hence, the development of a dengue vaccine capable of eliciting high levels of balanced immunity to all four DENV serotypes, which can persist for many years in the post-Dengvaxia era, has become imperative. However, the role of virion maturity of the vaccine strains comprising Dengvaxia® or other live-attenuated tetravalent (LATV) dengue vaccines in the induction of balanced immunity and vaccine protection remains poorly defined. This article focuses on the current knowledge on unbalanced B and T cell immunity from LATV immunization, broadly neutralizing antibodies (NtAbs) induced from natural DENV infection, and the role of structurally-mature or homogeneous virion particles in the future design of dengue vaccine in a post-Dengvaxia era.

Current status of LATV dengue vaccine development

The majority of dengue infections are asymptomatic or present as flu-like symptoms known as "classical" dengue fever (DF); however, some may progress to severe disease, including dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS).[24]

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A new classification developed by the World Health Organization (WHO) in 2009 further divides dengue cases into two classifications: dengue with or without warning signs and severe dengue.[25] Due to the disease burden and its public health importance, the development of an effective dengue vaccine has been considered as a top priority. Since live-attenuated vaccines (LAV) against other flaviviruses such as yellow fever and Japanese encephalitis viruses have been proven to be highly effective, a LATV against DENV is favored and three of them are at the forefront. The world's first licensed dengue vaccine, Dengvaxia®, was developed by Sanofi Pasteur and is a recombinant tetravalent vaccine based on the capsid and non-structural (NS) proteins of highly immunogenic and safely attenuated yellow fever virus (YFV) 17D vaccine strain backbone, which has been in use since the 1930s.[18] The premembrane (prM) and envelope (E) protein genes of each of the four DENV serotypes replaced that of YFV, and were expressed as a chimeric yellow fever-DENV vaccine. The other leading LATV dengue vaccine candidates were respectively developed by the US National Institutes of Health (NIH) and Butantan Institute, and the Centers for Disease Control and Prevention (US-CDC).[26] The NIH/Butantan vaccine (LATV Δ30 with two formulations, TV003 and TV005) consists of DENV-1, −3 and −4 strains that were attenuated by deleting 30 amino acids at the 3′ untranslated region and a chimeric DENV-2 that was produced by substituting the prM and E protein genes of the attenuated DENV-4 with those of DENV-2.[27] The US-CDC vaccine (TDV, formerly DENVax), initially licensed to Inviragen and recently acquired by Takeda, is a mixture of an attenuated DENV-2 strain and three recombinant viruses with prM and E genes from DENV-1, −3, and −4 on a DENV-2 genome backbone.[28] Both NIH/Butantan and US-CDC/Takeda vaccines are still the subject of intensive clinical trials, while the Dengvaxia® vaccine already completed phase III clinical trials and is licensed in a number of countries including Brazil, Mexico, El Salvador, Paraguay, the Philippines and recently in countries within the European Union and the USA. However, Dengvaxia® is only licensed for use among individuals aged 9 to 45 years (with different age ranges in some countries). Importantly, the WHO recommends vaccination only to individuals who have had a previously documented dengue infection confirmed either by a diagnostic test or by a medical history of dengue illness.[20]

Dengue virions are heterogeneous and dynamic

Dengue virions exist as a heterogeneous population.[29] During virus replication, the newly synthesized immature DENV particle is assembled in the endoplasmic reticulum at neutral pH and then translocated through the trans-Golgi network (TGN) inside the low-pH secretory vesicles. The pr portion of prM protein, positioned to cover the fusion loop (FL) peptide at the distal end of each E protein, prevents premature fusion during the maturation process.[30,31] For the virion to become fully infectious, the pr molecule is cleaved by the TGN-resident furin-like protease and released during virion particle egress to become the membrane (M) protein containing mature virion exposed to neutral pH in the extracellular milieu. However, the pr/M cleavage by the furin-like protease within these low-pH secretory vesicles is inefficient due to the variations in the level of furin expression among different cell types. The computational prediction also indicated the presence of sub-optimal amino acid motif surrounding the pr and M junction region cleavable by furin-like protease.[32,33] For these reasons, dengue virions released from infected cells consist of different degrees of maturation, and can be characterized as heterogeneous and mosaic particles with pr portion of prM proteins that are uncleaved (immature DENV), incompletely cleaved (partially mature DENV) or completely cleaved (mature DENV).[34,35] The exact composition of these structurally heterogeneous DENV virions in human and mosquito during natural infection is unknown, although a recent publication suggested that it could be mostly mature in humans.[36] Notably, this heterogeneity of virion particles could be influenced by DENV serotypes, genotypes or strains, passage history of the virus or the target cell types supporting DENV replication.[37,38]

Furthermore, studies have established that the organization of E protein on the surface of DENV particles is flexible and dynamic under different temperatures and pH environments, although E proteins are tightly packed as 90 antiparallel homodimers forming an icosahedral symmetry.[39,40] This phenomenon, termed virus 'breathing', allows antibodies to bind to transiently exposed cryptic epitopes and neutralize the virus.[29] However, this dynamic behavior that creates a "bumpy" conformation of E protein on the surface of virion particles is not a common feature to all DENVs and is influenced by temperature, duration of incubation and different strains of DENV.[41] Different E protein mutations can also have profound effects on virus 'breathing' and exposure of antibody epitopes.[42]

Currently, the precise maturation state of the viral particles expressed by all dengue LAV candidates is undefined. Monitoring and controlling the maturation state during the production of LAV is also particularly difficult. Table 1 summarizes the strains of the three foremost dengue LATVs. DENV-1 strain 16007 (isolated in Thailand in 1964) is currently included as a vaccine antigen and belongs to an extinct genotype with mutations most likely derived from cell culture passage. So far, no study has been performed to investigate the virion structure heterogeneity of LATV dengue vaccine strain and its association with vaccine efficacy yet.

Table 1. Vaccine strains formulated within the three foremost dengue live-attenuated vaccines.

	DENV-1	DENV-2	DENV-3	DENV-4
Dengvaxia (by Sanofi Pasteur)[18]	PUO-359/TVP-1140	PUO-218	PaH881/88	1228 (TVP-980)
LATV Δ30 (by NIH/Butantan)[27]	Western Pacific	Tonga/74	Sleman/78	Dominica/814669/1981
TDV* (by US-CDC/Takeda)[28]	16007	16681, PDK-53	16562	1036

*formerly named DENVax

Virion heterogeneity as a way to evade antibody neutralization

The E protein is the major surface-exposed structural glycoprotein with an ectodomain comprising of three distinct domains – EDI, EDII, and EDIII, which are connected by flexible hinges that allow its rearrangement during virus assembly, maturation and infection.[43] As such, E protein is notably the major target of NtAbs.[44,45] However, the humoral immune response in humans who recovered from primary DENV infection is dominated by antibodies that recognize the prM or fusion-loop epitope (FLE) at the distal end of the EDII of the E protein.[37] These anti-prM and anti-FLE antibodies are highly cross-reactive with other DENV serotypes, but are poorly neutralizing and can potently promote ADE of infection.

ADE contributes an important role in the pathogenesis of severe dengue and is modulated by the concentration of antibodies. As posited by the concept of "multiple-hit" mechanism, neutralization occurs when the virion is bound by antibodies with a stoichiometry exceeding a required threshold; virion-antibody engagement below the threshold has the potential to enhance viral infection through cells bearing Fc-receptors.[46,47] This threshold of neutralization is also determined by the antibody affinity and the accessibility of epitopes on the virion. Detailed functional studies demonstrated that the concentration of antibodies to promote maximal enhancement of infection is about half the amount required for neutralization.[48] Although there is no robust serological assay that could accurately predict protection or the risk of ADE and severe disease, a pediatric cohort study in Nicaragua demonstrated that a narrow range of low antibody titers (1:21–1:80) predicts a high risk of severe dengue.[49] Furthermore, the maturation status of virion particles influences its susceptibility to neutralization or ADE of infection as shown in West Nile virus (WNV) and DENV.[37,50] Completely mature virions have diminished neutralization sensitivity to anti-FLE antibodies, with neutralization levels that fail to exceed 60–80% even at high concentration unless the virus breathes.[51] On the contrary, highly prM-containing immature particles, which better exposes the FLE epitopes under a trimetric organization of E protein, are preferentially recognized by anti-FLE antibodies.[52] Moreover, anti-prM antibodies do not bind to mature virions because the latter do not possess prM proteins, but when bound to partially-mature virions, they possess partial neutralization activities that plateau at 10–60% even at high antibody concentration.[37] Both anti-prM and anti-FLE antibodies can neutralize partially-mature virions but only at sub-optimal levels, which may predispose the individual to ADE of infection. The inefficient prM cleavage, particularly in DENVs, may have evolved as both an immune evasion and infection enhancement strategies, which are important for the virus to survive during its natural life cycle, leading to the production of poorly NtAbs targeting the prM protein and FLE.[53] Currently, there is poor understanding of the threshold of anti-prM or anti-FLE antibodies in dengue-experienced patients and their association with disease severity.[53,54] Finally, no investigations have been made to identify the level of anti-prM or FLE antibodies among seronegative individuals after receiving LATV dengue vaccine, and the potential of those antibodies in "sensitizing" them, thus increasing their risk for hospitalization due to an eventual dengue infection later on.

Human monoclonal antibodies from dengue patients as a gateway to understanding protection

Understanding B cell response and the induction of antibodies produced is crucial for human health and vaccine development. Different pathways to generate short- (SLPCs) and long-lived plasma cells (LLPCs) and memory B cells (MBCs), and how these different paths impact antibody diversity and

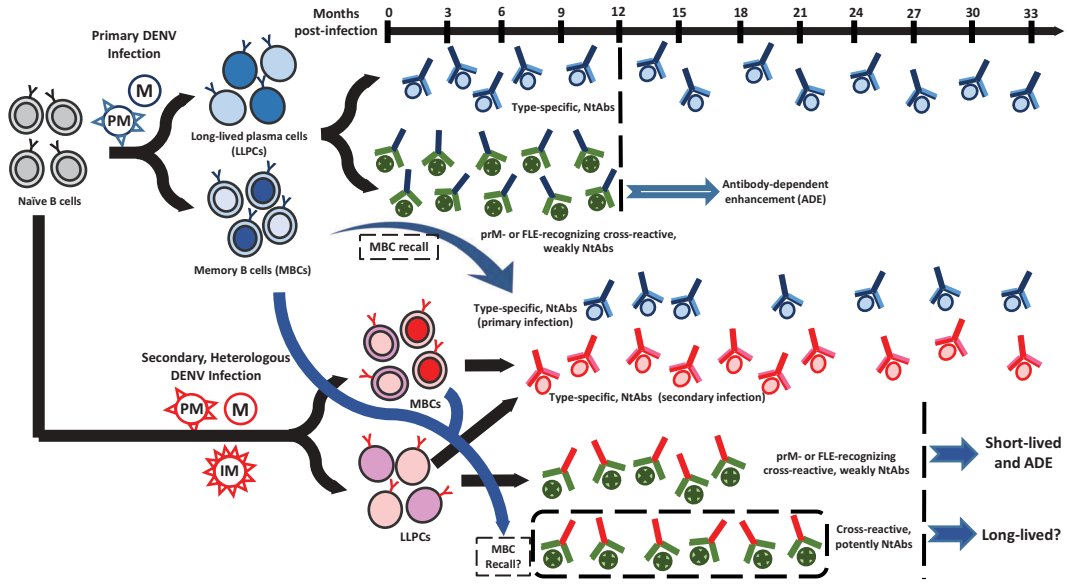

Figure 1. B cell activation and generation of DENV antibody repertoire after primary and secondary DENV infections.

affinity have been reviewed in detail.[55] Following the first DENV encounter (primary infection), humans develop MBCs and LLPCs that secrete polyclonal homotypic NtAbs against the infecting serotype, as well as short-lived, cross-reactive antibodies with low to moderate neutralizing activity to other serotypes (Figure 1).[56–58] Subsequent infection with a different serotype of DENV can lead to three types of antibody responses: induction of homotypic NtAbs specific to the newly infecting serotype, a memory recall and boost in the neutralizing antibody response to the prior infecting serotype,[59,60] and the production of heterotypic antibody capable of cross-neutralizing the remaining serotypes, even those that were not yet encountered by the individual.[13,61] Therefore, following infection with two different serotypes of DENV, an individual generally develops a broadly neutralizing antibody response sufficient to provide protection against all four serotypes of DENV based on epidemiological observations.[62–64]

Recent analysis of a panel of human monoclonal antibodies (MAbs) isolated from DENV-infected patients has identified a number of potently neutralizing MAbs, with 50% *in vitro* neutralization (Nt50) values at concentrations within the picomolar range.[65] Several of these antibodies bind to conformational-dependent epitopes on the quaternary structures of E protein present on the intact virions but not to monomeric recombinant E protein.[51,66–68] Some of them have been structurally characterized, including DENV-1-specific 1F4 and HM14c10 MAbs,[69,70] DENV-3-specific 5J7 MAb,[71] and DENV-2-specific 2D22 MAb.[72] The other types of MAbs bind to epitopes across the interface of two head-to-tail E monomers, making up the E dimer epitope (EDE) as mapped by X-ray crystallography and cryo-electron microscopy (cryo-EM).[65,73] They occupy a highly conserved site where prM interacts with E protein as it passes through the trans-Golgi network, hence many of these EDE mAbs are broadly cross-reactive and can neutralize all four DENV serotypes. Unlike the anti-FLE antibodies, these EDE mAbs can potently neutralize both high and low prM-containing DENVs that are produced in insect and human monocyte-derived dendritic cells, respectively.[51] Interestingly, these antibodies have prophylactic and therapeutic activities in mouse models of DENV infection and disease, implying that quaternary structure-dependent epitopes are the main targets of protective and broadly NtAbs induced after secondary DENV infection in humans.[70,72]

At present, the mechanism of generating these potent, broadly NtAbs during DENV infections is not well-understood.[74] It remains unclear whether they come from a low-affinity, cross-reactive antibody-producing MBCs induced by primary infection, and are later activated and affinity-matured by repeat infection to become high-affinity cross-reactive NtAbs, or if they come from a small population of MBCs induced by structurally mature particles, which expanded after secondary infection and are maintained as MBCs or LLPCs (Figure 1). Since not all NtAbs are equally protective, the quality and quantity of these antibodies are of paramount importance.[16] Studies to define how these quaternary epitope-recognizing NtAbs evolve over time and how different DENV strains with varying degrees of maturity affect the induction of such immune response should be prioritized in the design of the next-generation DENV vaccine.

Current dengue vaccine candidates developed with consideration of the antigen maturity

Variations in the arrangement and conformation of the E proteins among dengue virion particles may be associated with its role in shaping the antibody response to DENV infection. Although there were dengue vaccine studies that intended to avoid the induction of anti-FLE antibodies,[75,76] very few studies have compared immunogenicity based on the maturity of the viral antigen. Nevertheless, a chimeric DENV-1/2 LAV (cD1-4pm) with improved prM cleavage was previously evaluated.[77] The cD1-4pm was generated from the attenuated DENV-2 strain 16681-PDK53 genome backbone having a prM/E gene fragments from DENV-1 strain 03–0398. This chimeric virus is relatively mature with 12% of the prM protein remaining on the surface of viral particles. Mice immunized with cD1-4pm developed NtAbs predominantly against DENV-1 and only a minimal anti-DENV-2 NtAbs. On the other hand, monkeys immunized with cD1-4pm showed no viremia as compared to those immunized with the less mature, higher prM-possessing parental vaccine strain of DENV-1, although the geometric mean antibody titers showed similar levels between the two groups of animals.[77] More recently, a study demonstrated that a highly mature DENV-2 strain 16681-derived virus-like particles (mD2VLP) produced from mammalian COS-1 cells can generate higher cross-reactive NtAbs, and mice receiving passively transferred antibodies from immunized mice were protected against all four serotypes of DENV.[32] Further, cryo-EM reconstruction showed that these mD2VLP particles possess a T = 1 icosahedral symmetry with a groove located within the E protein dimers near the 2-fold vertices and exposed highly overlapping, cryptic neutralizing epitopes.[32] The results highlighted the potential of these "epitope-resurfaced" mature-form D2VLPs in inducing quaternary structure-recognizing broadly cross-reactive NtAbs. Covalently locked E protein dimers through cross-linking of the inter-subunit disulfide bonds is another dengue vaccine design that can also present resurfaced conformational EDE epitopes and with reduced exposure of the immunodominant FLE.[78]

Unbalanced B cell immunity of LATV dengue vaccine

Ideally, a tetravalent dengue vaccine should induce a balanced B and T cell-mediated immunity against all four DENV serotypes while maintaining high structural maturity so that it is less capable of inducing anti-prM or anti-FLE antibodies. In order to confer robust B cell immunity, this vaccine should present quaternary structure-dependent epitopes that are unique to each serotype to act as the main targets of protective NtAbs. Nonetheless, unbalanced B cell immunity was observed in the clinical trials of LATV dengue vaccines. DENV-naïve subjects who received CYD-TDV developed high levels of type-specific NtAbs to DENV-4, whereas cross-

reactive NtAbs dominated the DENV-1, DENV-2, and DENV-3 responses.[79] This was further supported by the earlier studies which showed that the magnitude of neutralizing antibody response against DENV-4 was higher than that from the other serotypes after the first dose in seronegative subjects.[80,81] Similarly, the US-CDC/Takeda vaccine induced potent DENV-1 and −2 quaternary epitope-specific NtAb responses; however, DENV-3 epitope-specific antibody response was not observed.[82] A study among vaccinees who received sequential monovalent NIH/Butantan vaccines suggested that cross-reactive anti-E antibodies significantly contributed to the heterologous neutralizing activities against both exposed and unexposed DENV serotypes.[83] However, no further study on NIH/Butantan tetravalent dengue vaccine can be found whether neutralization antibodies against four serotypes of DENV are contributed by either serotype-specific or by cross-reactive epitopes.

Unbalanced B cell immunity could be further exacerbated when the LATV dengue vaccines are provided to persons with prior flavivirus exposure. The emergence of Zika virus (ZIKV) in DENV-endemic regions has raised the critical question of how the pre-existing ZIKV immunity modulates the immune response to and clinical outcomes of subsequent DENV vaccination and vice versa. Studies in human, mouse or monkey demonstrated that pre-existing anti-DENV antibodies can either contribute to cross-protection or mediate ADE, depending on the quality and quantity of antibodies generated (see reviews[84–87]). Few studies evaluating immune response of flavivirus vaccines in human or mice pre-exposed with JEV or DENV suggested no impact on the induction of virus-specific neutralizing antibody response; however, cross-reactive antibody response was observed.[88,89] At sub-neutralizing levels, these cross-reactive antibodies possess DENV infection-enhancement activity. In a small clinical trial of the NIH/Butantan LATV dengue vaccine, a single dose vaccination with TV003 among those with prior YFV or DENV exposure was associated with significantly higher neutralizing antibody titers to DENV-2 to −4, in particularly DENV-2, but not to DENV-1, than the flavivirus-naïve population.[90] Whether LATV dengue vaccine serves as "sensitizer" among flavivirus pre-exposed group and leads to severe disease as observed from Dengvaxia or not awaits the conclusions of phase III clinical trial.

(Un) balanced T cell immunity of LATV dengue vaccine

DENV infection induces potent innate immune responses, which in turn shape the adaptive immune responses, including not only B cell humoral responses with antibody production but also cellular responses mediated by $CD4^+$ and $CD8^+$ T cells.[74] The immunodominant epitopes recognized by $CD4^+$ and $CD8^+$ T cells have been mapped, with $CD8^+$ T cells preferentially targeting NS proteins, such as NS3, NS4b and NS5; and $CD4^+$ T cell responses toward structural proteins and NS1.[91–93] Both $CD4^+$ and $CD8^+$ T cell responses have been suggested to mediate protection in animal models or DENV-infected patients (see reviews[65,74,94,95]). Like B cell immunity, cellular immune responses against DENV can be also cross-reactive against multiple DENV serotypes due to the similarity of genomic sequences. Cross-reactive memory T cells recalled during secondary infection were observed with responses skewed towards a higher pro-inflammatory cytokine production and immunopathology in clinically severe patients.[74,91] The protective or pathogenic role of T cells in DENV infection remains controversial, which may be determined by the sequence of infection, the degree of cross-reactivity and the viral load.[74,96]

Although the key immunological elements required to produce an ideal dengue vaccine are currently unknown, induction of moderate and balanced $CD4^+$ and $CD8^+$ T lymphocyte-mediated immunity may improve the efficacy and safety. The inability to induce $CD8^+$ T cell response against DENV has been one of the explanations for the poor protective efficacy among DENV seronegative individuals who received Dengvaxia®, which contains the NS proteins of YFV.[18,97] Instead, a memory T cell recall to DENV NS3, similar to natural infection, was found in DENV-immune subjects and suggested that the persistent antigen-specific memory T cell response mediated the protection.[14,98,99] The two other LATV dengue vaccines, developed by US-CDC/Takeda and NIH/Butantan, are built on a DENV backbone rather than a YFV backbone and are hoped to generate better protective T cell responses than Dengvaxia. Immunization with two doses of US-CDC/Takeda TDV vaccine triggered both DENV-2 specific and cross-reactive $CD8^+$ T cell responses, which persisted for more than six months.[100] However, whether this cross-reactive $CD8^+$ T cell response is protective or pathogenic remains a question. On the other hand, vaccination with NIH/Butantan TV003 vaccine not only elicited protective NtAbs but also induced antigen-specific $CD8^+$ T cell response, which is focused on the highly conserved NS proteins.[101] Similar magnitude, frequency, and specificity of the vaccine-specific $CD4^+$ T cell response, compared to those humans naturally exposed to DENV, were also observed, suggesting a protective T cell response against severe disease.[102] It is important to note that none of those three LATV dengue vaccines, with at least one chimeric serotype, is capable of inducing type-specific T cell immunity. Whether cross-reactive T cell immune response confers immunopathology or protection as natural infection awaits the results of two currently ongoing phase III clinical trials for the NIH/Butantan and US-CDC/Takeda vaccines or the four-year safety follow-up of CYD-TDV.

Conclusions

Ideally, a dengue vaccine should induce both potent humoral (NtAbs) and cellular (Th1 or cytotoxic T lymphocytes) protective immunity against the four serotypes of DENV. LAVs should be optimal in this respect. The main concern about Dengvaxia® is the increased risk of severe disease exposure to DENV infection in vaccinated children who were naïve to DENV.[103] The current most plausible hypothesis for the underlying mechanism is that the vaccine, acting as a "primary-like infection", initiates a first immune response to dengue in seronegative individuals; this predisposes them to a higher risk of severe disease upon encountering

a "secondary-like", wild-type dengue virus infection as explained by the ADE theory. However, the potential role of the lacking antigen-specific, protective $CD8^+$ T cell immunity could not be ignored. So far, the major immunological correlates of protection from infection or severe disease are still unknown.[104,105] In the future, antibody kinetics and the level of triggered cellular responses should be addressed in order to establish which profiles of responses are induced by vaccination and if such response is most likely to be protective or enhancing. To date, we do not know yet whether the second-generation dengue vaccines will exhibit a similar mechanism or not. As the protective immunity would be fundamentally different in both DENV-naïve and DENV-primed individuals, all dengue vaccine trials must be designed to obtain separate safety and efficacy data to prevent extrapolation of results from one group to the other.[6,106]

Future perspectives

DENVs are naturally produced as heterogeneous populations. Variations in prM and E sequences, virion maturation state, and virus breathing strongly affect epitope presentation and interactions with human antibodies, which lead to either virus neutralization or enhancement of infection. Most importantly, the presence of anti-DENV antibodies is not sufficient for neutralization and protection; instead, a stoichiometry is influenced by the heterogeneity of the virion surface. Given the well-established correlates of protection for Tick-borne encephalitis virus (TBEV), YFV and JEV vaccines based on the measurement of neutralizing antibody titers (1:10 respectively), it clearly indicates that the quality of the antibody response is an important element of protection provided by flavivirus vaccines.[107,108] Therefore, improving the quality as well as the quantity of anti-DENV NtAbs is a paramount objective in the development of the next-generation dengue vaccines.

Although a LATV dengue vaccine is currently preferable, it remains challenging to have all four component strains replicate equally with proper dosing and be able to induce balanced serotype-specific NtAbs. If an individual with repeated exposure to different serotypes of DENV generally develops a broad immune response sufficient to provide protection against all DENV serotypes, a strategy to induce such broadly NtAbs should be considered in developing the next-generation dengue vaccine.[13] A monovalent universal dengue vaccine capable of inducing broadly NtAbs or a heterologous prime-boost vaccination strategy in combination with LAV such as Dengvaxia® for immune-refocusing could possibly be an alternative solution.

Using system vaccinology to guide vaccine design by reverse engineering, vaccines that were previously difficult to make such as vaccines for human immunodeficiency virus (HIV) or respiratory syncytial virus (RSV), are now possible.[109] Although technically challenging, designing a dengue vaccine that presents quaternary structures and EDE epitopes to induce broadly cross-reactive NtAbs with rational aid from the current understanding of immune responses from natural DENV infection, is full of potentials.[110] VLP antigens, with repetitive proteins on the surfaces of antigens, allow "cross-linking" several receptors on the surface of B cells. VLPs could be the best candidate for dengue vaccine as they trigger the strongest and durable antibody responses, including LLPCs.[111] Yet the absence of immune correlates of protection from DENV infection hampers the development of other potential DENV vaccines. Currently, dengue LAVs are still favored because they induce both cellular and humoral immune responses mimicking those elicited by natural infection. We believe that it is imperative to apply the fundamental knowledge on memory B cell biology, broadly NtAbs, and virion structure biology to contemporary problematic infection to better guide vaccine design and future research and development.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This review was supported by the Ministry of Science and Technology Taiwan (MOST-107-2313-B005-038-MY3)

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