Proceedings of the 2nd International Conference on Modern Medicine and Global Health
DOI: 10.54254/2753-8818/15/20240502

Dengue virus and vaccines: A review

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Abstract. Dengue disease is a significant global health issue with nearly 400 million infections per year. Therefore, it is vital to developed a universal dengue vaccine. However, developing such a vaccine faces major challenges including risk of ADE, lack of predictive animal models, and insufficient immunological correlates of protection. First licensed dengue vaccine Dengvaxia showed increased risk of severe dengue likely due to ADE from imbalanced immunity against the four dengue serotypes. This review summarizes current dengue vaccine candidates like Dengvaxia, TAK-003, and TV003/TV005. It highlights the mechanisms and implications of ADE in vaccine-induced immune responses. Potential innovative solutions are discussed including focusing on highly neutralizing epitopes like envelope domain III and leveraging new vaccine platforms such as virus-like particles and mRNA vaccines. These approaches may improve the quality of neutralizing antibodies over cross-reactive enhancement-prone responses. However, definitive immunological correlates for protection remain unknown. Continued research is urgently needed to enable universal dengue vaccine that provides long-lasting immunity against all serotypes without risk of ADE.

Keywords: Dengue Virus, Vaccine, Antibody-Dependent Enhancement.

1. Introduction

The dengue virus (DENV), which causes the viral infection dengue, is spread to people by mosquitoes of the Aedes spp. family. Although genetically diverse, the four DENV serotypes are nevertheless closely linked. Any of the 4 dengue virus serotypes can cause infection, which can lead to asymptomatic infection or clinical symptoms that range from a moderate, undifferentiated fever to severe dengue. Dengue virus is statistically the most prevalent arboviral illness in the world, with an estimated over 350 million infections and over 90 million symptomatic cases one year [1]. Dengue infections are particularly prevalent in some areas. The disease is currently prevalent in more than 100 countries, with Asia bearing the brunt of more than 70% of the global disease load [2]. None of the drugs that have been investigated as prospective dengue therapies have shown to reduce viremia, clinical symptoms, or consequences [3]. Therefore, it is important to develop effective vaccines against dengue virus, which can also help contain the spread of the disease. Among them, there are two Dengue vaccines in phase III and one vaccine called Dengvaxia in the market, all of which are live-attenuated vaccines. However, Dengvaxia has a huge defect: that is, it may induce antibody-dependent enhancement (ADE), making FDA only approved Dengvaxia for use [4].

ADE (Antibody-Dependent Enhancement) complicates the safe and universal Dengue vaccine. This process involves the IgG antibody structure, containing variable and constant portions that bind to

DOI: 10.54254/2753-8818/15/20240502

antigens and Fc receptors (FcR) on immune cells. If the virus is not neutralized during phagocytosis, it may escape and enter immune cells, causing replication and more severe infection. Dengue's four serotypes present a challenge; a primary infection may lead to antibodies that partially protect against a secondary infection, but not enough to prevent ADE. This results in the potential for more severe illnesses in a reinfection scenario. In vaccines, a "primary-like" silent infection could cause a more severe "secondary-like" illness upon true infection. The unbalanced immune response to different serotypes, as seen in vaccines like Dengvaxia and others in phase 3 trials, can induce ADE, further illustrating why an effective Dengue vaccine is hard to develop [4]. This paper introduces the current dengue vaccines under development or in the market, highlights, antibody-dependent enhancement (ADE), the major problems faced by researchers in this field, and focuses on the novel pathways scientists have used to try to avoid ADE.

2. Current vaccines

2.1. Dengvaxia

As a live attenuated tetravalent dengue vaccine, Dengvaxia consists of chimeras consisting of the prM and E genes from each of the DENV 1-4 combined with the yellow fever 17D backbone providing the nonstructural genes. The four vaccine virus chimeras were made individually with recombinant DNA technology and combined together to form a single formulation. Dengvaxia is manufactured in Vero cells and undergoes extensive testing during the production process to ensure genetic stability, appropriate viral morphology, restricted replication in mosquito vectors, and lack of potential to cause neurovirulence. The lyophilized vaccine is reconstituted in a saline diluent prior to administration as a 0.5 ml subcutaneous injection.

Clinical trials revealed that in dengue seronegative individuals, vaccination with Dengvaxia may lead to an increased risk of disease if they later experienced a natural dengue virus infection. The cause for this safety signal is thought to be that in seronegatives, Dengvaxia likely provides a transient period of heterotypic protection similar to a first natural infection. But as this cross-protection wanes over 2-3 years, subsequent natural infection acts essentially like a second dengue infection. And second infections after a long interval are known to carry a significantly higher risk of severe outcomes like plasma leakage and hemorrhage. This safety risk was highest in younger seronegative children around 2-5 years old. In contrast, Dengvaxia showed good efficacy against hospitalized and severe dengue in seropositive individuals with prior dengue immunity.

Overall efficacy of Dengvaxia against symptomatic dengue disease of any severity was relatively modest. It provided the highest level of protection against DENV4, followed by DENV3, DENV1 and DENV2 in descending order. Duration of protection appeared limited, declining significantly after the first 2 years post-vaccination. Issues like immunodominance of the DENV4 vaccine component may have impacted the quality and breadth of immunity produced. In seropositive individuals 9 years and older, Dengvaxia demonstrated good efficacy against hospitalized and severe dengue disease over the study periods. But the duration and safety of protection remains uncertain [5].

2.2. TAK-003 (DENVax)

DENVax is a recombinant, live attenuated tetravalent dengue vaccine candidate. It is comprised of chimeric viruses that contain the prM and E structural genes from wild-type DENV1, 3, and 4 inserted into the genetic backbone of the attenuated dengue serotype 2 PDK-53 virus. The PDK-53 virus was developed at Mahidol University by passaging a wild-type DEN-2 isolate in primary dog kidney cells. During this process, it accumulated mutations in the 5' untranslated region (UTR), as well as in the NS1, NS2A, NS3, and NS4A nonstructural genes that are associated with attenuation. The DENVax vaccine retains the three major attenuating mutations of PDK-53 located in the 5' UTR, NS1, and NS3 genes. The fourth component, DENVax-2, consists of the complete PDK-53 genome. Each of the DENVax strains expresses the surface antigens of the corresponding dengue serotype while sharing the attenuating mutations in NS genes that cripple replication. The vaccine is manufactured by growing the viruses in

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Vero cells followed by lyophilization. For vaccination, the freeze-dried vaccine is reconstituted and administered as three subcutaneous injections at 0, 6, and 12 months.

Preclinical studies in mice and non-human primates demonstrated the DENVax strains were highly attenuated compared to wild-type dengue viruses. They exhibited reduced replication efficiency, smaller plaque size, temperature sensitivity, and loss of neurovirulence. Early phase 1 human trials found the vaccine to be generally well tolerated with mostly mild, transient reactions like headache, malaise, and rash. Low levels of short-lived viremia were detected, mainly of the DENVax-2 component. Some doses and schedules elicited increased reactogenicity with dengue-like symptoms. This was related to the level of viremia and replication of certain strains, particularly DENVax-3. The 5' UTR attenuating mutation was prone to revert back to wild-type during passage in Vero cells. However, the stable NS1 and NS3 mutations maintained a sufficiently attenuated phenotype even with some reversion at the 5' UTR locus.

Preclinical immunogenicity studies showed DENVax induced neutralizing antibodies. Early phase 1 human trials demonstrated DENVax elicited neutralizing antibody responses against all four serotypes after one or two doses. Responses were lower for DENV-4 but improved with formulations containing higher concentrations of DENVax-3 and DENVax-4. The vaccine is currently being evaluated at two different dose levels using various administration schedules in both dengue endemic and non-endemic populations. Preliminary results indicate an acceptable safety profile. Further clinical testing will be needed to fully evaluate efficacy against dengue disease [6].

2.3. TV003/ TV005

The NIAID dengue vaccine consists of four live attenuated recombinant dengue viruses (rDENV) representing each serotype. The DENV-2 and DENV-4 components are also chimeras with the structural genes of wild-type viruses inserted into an rDENV backbone. A key feature is that the vaccine contains the nonstructural proteins of wild-type DENV-1, -3, and -4, which is expected to elicit T cell immunity. The monovalent rDENVs were extensively tested clinically to select the optimal combination and doses. The final LATV formulation combines the four rDENVs as TV003 or TV005. In phase 1 studies, the NIAID LATV had a positive safety profile with transient low-level viremia and mild reactions like rash and myalgia. The 5' UTR attenuating mutation was prone to revert during passage in cells. However, the NS1 and NS3 mutations provided stable attenuation even with some reversion at the 5' UTR. Preclinical studies showed the rDENVs were highly attenuated with reduced replication and neurovirulence. One or two doses of TV003/TV005 induced neutralizing antibodies in 74-97% of flavivirus-naive recipients. A second dose given months later showed no evidence of breakthrough infection, indicating sterilizing immunity. The vaccine also elicited multifunctional T cell responses to conserved epitopes. A DENV-2 challenge study showed TV003 provided complete protection. The early clinical profile demonstrates promise for a well-tolerated, single-dose dengue vaccine with more balanced immunogenicity compared to Sanofi Pasteur's CYD vaccine. Further efficacy testing will be needed [7].

3. Challenges faced by researchers

Lack of Animal Models: for dengue has impeded preclinical development and testing of dengue vaccines. Because dengue virus only causes disease in humans, vaccines cannot be readily tested for efficacy in animal challenge models. Non-human primate models show limited fidelity to dengue disease pathogenesis in humans. The lack of a predictive animal model early in the development pipeline delays vaccine screening and necessitates large, time-intensive human trials to demonstrate efficacy. Having a suitable animal model would expedite vaccine development by enabling preclinical proof-of-concept studies and down selection of lead candidates. However, recapitulating the complex immune responses in humans continues to limit the utility of animal models for dengue [8].

3.1. The lack of definitive immune correlates of protection

Measuring neutralizing antibodies does not reliably indicate protection versus risk of enhancement. Unlike other vaccines, a threshold antibody titer that correlates with protection has not been defined for dengue. This complicates the immunological evaluation of vaccine candidates. Research is still needed

DOI: 10.54254/2753-8818/15/20240502

to better understand the type of antibody responses, B cell memory, and T cell responses that confer long-term protection against all serotypes without increasing susceptibility to enhanced disease upon subsequent infections. Defining these definitive correlates remains an important gap and barrier in successful dengue vaccine development. Reliable immunological markers that predict vaccine safety and efficacy would greatly accelerate vaccine design and testing [8].

3.2. The need to demonstrate long-term safety and efficacy

Long-term follow up is critical to evaluate dengue vaccine safety and efficacy. As evidenced by CYD-TDV, short-term clinical trials did not reveal the elevated risk of hospitalized dengue in seronegative vaccine recipients 2-5 years post-vaccination. This highlights the need for vaccine trials to actively follow participants for 3-5 years after vaccination. Efficacy measured at 1 year may not reflect longer term protection due to viral interference and waning heterotypic immunity over time. Safety signals may also emerge years later, once vaccine-induced antibody titers have waned. Evaluating long-term immunogenicity is also important to determine if booster doses will be required. Extensive long-term follow up presents logistical and financial challenges for dengue vaccine trials. Demonstrating sustained safety and efficacy over the long-term remains a key priority in this field [8].

3.3. ADE

Antibody-dependent enhancement (ADE) is a complex and intriguing biological phenomenon that refers to the somewhat counterintuitive situation where antibodies that are generated. In this puzzling scenario, what happens is that these antibodies bind with specificity to the surface of the virus particle, but unfortunately, they cannot fully neutralize or incapacitate it. Rather, this interaction facilitates the entry of the antibody-virus complex into the host's immune cells, particularly monocytes and macrophages, via Fc receptors. This sets the stage for increased viral replication within these host cells.

ADE is believed to be a critical factor that plays a significant role in dengue disease. It is a two-faced phenomenon that includes both intrinsic and extrinsic components. The sub-neutralizing, cross-reactive antibodies generated from the first dengue infection form complexes with the virus of the second infecting serotype. These complexes promote a more efficient viral uptake and replication in myeloid lineage cells like monocytes. This intrinsic form of ADE leads to elevated levels of circulating virus, or higher viremia. On the other hand, extrinsic ADE occurs when these same cross-reactive antibodies attach to immature, non-infectious viral particles, which then facilitates their entry into and maturation within monocytes. Combined, these forms of ADE contribute to a higher viral burden and a subsequent increase in disease severity.

Natural infection with the dengue virus produces an array of antibodies, many of which are highly cross-reactive but possess only weak neutralizing capabilities against serotypes other than the infecting one. A significant proportion, somewhere between 60 to 70%, of these antibodies are specifically directed against certain viral epitopes such as prM and the fusion loop. While these epitopes are immunodominant, the antibodies targeting them tend to be relatively inefficient at neutralization and instead actively potentiate ADE. On the contrary, only a minor fraction, estimated at about 5-10%, of the antibodies generated during a natural infection target the serotype-specific epitopes like the EDIII, which have the capability to induce potent neutralizing antibodies and consequently have a lower risk of causing ADE [9].

Most vaccine candidates, which include approaches like live attenuated or whole inactivated virus vaccines, unfortunately, generate a preponderance of cross-reactive, ADE-inducing antibodies. These candidates often fail to generate robust, serotype-specific neutralizing responses, which is the goal for any efficacious vaccine. Dengvaxia, which lacks NS proteins, has been found in seronegative individuals within a 2 to 5 years post-vaccination period. This underscores the issue of insufficient neutralization and the potential for ADE activity induced by the vaccine-generated antibodies upon encountering a subsequent infection. It is thought that viral interference between the four different vaccine components likely leads to an imbalanced immune response.

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In summary, while Dengvaxia may offer a degree of protection for seropositive individuals who have been previously exposed to the virus, it unfortunately failed to meet the pressing need for a universal vaccine capable of protecting the most vulnerable, seronegative populations most at risk. The path to developing such a universal dengue vaccine requires meticulous scientific research to optimize the neutralization capacity against all four distinct serotypes, while at the same time avoiding the induction of cross-reactive antibodies that could potentiate ADE. Innovative strategies such as immunization with EDIII-based subunit vaccines are currently showing promise in steering the antibody response toward serotype-specific, highly neutralizing epitopes. Detailed characterization and understanding of the dichotomy between neutralizing and infection-enhancing antibodies elicited by various vaccine candidates remain an area of high priority. Overcoming the complex hurdle of ADE continues to stand as a central challenge in the ongoing efforts to develop an effective dengue vaccine [4].

4. Potential solutions

4.1. EDIII

E and M proteins are incorporated into the viral envelope all the way across the bilayer. Together, these proteins form a wall that keeps the virus from getting into the host cells. Each E protein is a homodimer, consisting of two identical molecules with three distinct domains in each. The names of these domains are ED1, 2, and 3, respectively. Regarding EDIII, it not only activates host cell receptors for viral entry. On the surface of the virion, this domain is exposed and usable, serving as a tool for identifying host cell surface receptors. Importantly, the abundance of type- and subtype-specific conformation-dependent neutralizing epitopes and the lack of additional non-neutralizing and cross-reactive epitopes result in EDIII having only a very low inherent potential for creating cross-reactive antibodies that cause ADE. It also folds slowly and independently [10].

4.2. Other platforms

Recently, VLPs have emerged as promising vaccine. VLPs mimic the overall structure of native viruses but lack infectious genetic material. VLPs can stimulate robust immune responses like whole viruses, but are safer since they are non-infectious [11].

Several studies have constructed DENV VLPs using recombinant expression of the viral structural proteins capsid (C), prM/M, and E in different hosts. Three main types of DENV VLPs have been generated: 1) C protein-only VLPs (nucleocapsid-like particles); 2) prM/M+E VLPs; and 3) chimeric VLPs containing non-DENV sequences fused to DENV proteins. C protein VLPs can be readily produced in E. coli and have been shown to provide protection, likely via inducing DENV-specific T cell responses. Mammalian cells and yeast such as Pichia pastoris are better systems for generating prM/M+E VLPs, which elicit high DENV neutralizing antibody titers and protection comparable to inactivated DENV vaccines in mouse models. Chimeric VLPs fuse heterologous antigens like hepatitis B surface antigen to DENV E protein, forming divalent particles that could serve as multivalent vaccines [12].

The precise structure of prM/M+E VLPs resembles native, infectious DENV particles. Hence these VLPs present authentic, conformational epitopes from the DENV surface that elicit neutralizing and protective antibodies. The lack of infectious genome also enhances their safety relative to live attenuated or inactivated DENV vaccines. Yeast expression systems can produce prM/M+E VLPs more quickly and economically than mammalian cell culture. While C protein VLPs do not resemble whole DENV particles, they still confer protection via stimulating cellular immunity against DENV proteins.

Certain limitations still need to be overcome in DENV VLP generation, such as poor secretion yields for some constructs. Strategies like modifying ER retention signals in the E protein have improved secretion. The immunodominance and specificity of VLPs relative to different DENV serotypes also requires further evaluation. Overall, the published data clearly demonstrate the potential for DENV VLPs to serve as safe, effective, and inexpensive vaccine candidates. DENV VLPs have advantages over other vaccine approaches, including capability for rapid, scalable production (vs inactivated vaccines)

DOI: 10.54254/2753-8818/15/20240502

and inherent safety due to the lack of infectious genome (vs live attenuated vaccines). With further optimization and clinical development, DENV VLP vaccines could provide an important public health tool for controlling dengue worldwide [12].

4.3. mRNA vaccine

mRNA vaccines represent an innovative technology for preventing infectious diseases like dengue. They can stimulate potent immune responses by delivering mRNA encoding viral antigens into host cells. The mRNA is translated into protein antigens, which elicit protective antibody and cellular immune responses. mRNA vaccines have emerged as promising alternatives to conventional live, inactivated, or subunit vaccines.

Scientists designed a tetravalent modified mRNA vaccine against dengue using immunoinformatic approaches. Consensus sequences for NS1, prM, and EIII proteins from Pakistani dengue isolates were obtained. Favorable mRNA structure was confirmed by minimum free energy predictions. B-cell epitopes and T-cell epitopes were identified that showed high antigenicity, non-allergenicity and non-toxicity. Molecular docking revealed strong binding between the epitopes and MHC alleles. Normal mode analysis further supported structural stability of vaccine-receptor complexes [13].

Key advantages of mRNA vaccines include rapid, inexpensive and scalable manufacturing without requiring cell cultures or fermentation. The mRNA production process is cell-free and highly consistent. mRNA vaccines can be synthesized quickly, enabling rapid responses to emerging viral strains. They are non-infectious and non-integrating due to the lack of genomic material, improving their safety profile. mRNA vaccines demonstrate high potency at low doses and do not induce anti-vector immunity. The mRNA sequence can be readily engineered to optimize stability and immunogenicity. Overall, mRNA technology provides significant flexibility, versatility and control in vaccine design.

For dengue, mRNA vaccines offer the ability to focus immune responses on critical neutralizing B-cell and T-cell epitopes. This may help overcome challenges with balancing the tetravalent response. Modified nucleotides like N1-methylpseudouridine can boost translation and limit innate immune detection. Nanoparticle delivery systems protect the mRNA from degradation. In vivo studies in mouse models have shown promising efficacy of mRNA vaccines against flaviviruses like dengue and Zika. Given their many benefits, mRNA vaccines represent an innovative and disruptive technology for dengue vaccine development [13].

5. Conclusion

Dengue is a worldwide infectious disease which has no effective vaccines due to antibody-dependent enhancement. So far, these has been one licensed vaccine and several promising vaccines in important phases of clinical trials. For example, Dengvaxia showed good efficacy against hospitalized and severe dengue in seropositive individuals with prior dengue immunity. Preclinical studies demonstrated the DENVax strains were generally well tolerated. However, the current challenges cannot be ignored. In the absence of an animal model of dengue, the effectiveness of the vaccine cannot be tested in an animal challenge model. Low levels of neutralizing antibodies do not reliably reflect protective effects. There are no long-term follow-up data. Another concern is ADE. The paper proposes several possible ways to develop an effective universal vaccine against Dengue virus, including choosing other vaccine platforms, such as mRNA vaccine and virus-like particles vaccine, and other target antigens. However, relevant technologies are still under development, and whether these ways can function well requires further investigation and research.

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