

Exploring Host Immune Modulation for the development of Effective Dengue Vaccine

ABSTRACT

The absence of a vaccine that is effective against the dengue virus (DENV) puts more than half of the world's population at risk of developing the disease. Although the symptoms of a primary infection are usually moderate, infections with a different serotype later on can have serious consequences, such as dengue shock syndrome (DSS) or dengue hemorrhagic fever (DHF). There are four different serotypes of the virus (DENV-1 to DENV-4), and the severity of the sickness is greatly influenced by antibody-dependent enhancement (ADE), in which non-neutralizing antibodies encourage viral entry and reproduction. This makes developing vaccines more difficult. A possible tactic is host modulation, which entails modifying immunological pathways to elicit a regulated protective response. To develop vaccines that protect against all serotypes with fewer side effects, researchers are focusing on cytokine signaling, T-cell polarization, and innate immunity. Furthermore, precise control over host-pathogen interactions is made possible by sophisticated adjuvants, delivery methods, and systems biology approaches. The difficulties in developing a dengue vaccine are examined in this paper, which also emphasizes host modulation as a possible remedy for safer, more potent vaccines.

Keywords - Dengue, Host modulation, Immune Responses, Vaccine development

I. Introduction:

One of the most important mosquito-borne diseases affecting people, especially in tropical and subtropical areas, is dengue virus (DENV), which belongs to the Flavivirus family. The complex immunopathology of the four serotypes of the dengue virus, which can produce severe consequences including dengue hemorrhagic fever and dengue shock syndrome, makes it an ongoing public health concern. The complexities of host-pathogen interactions and the phenomenon of antibody-dependent enhancement (ADE), where pre-existing immunity can worsen disease upon subsequent infection, are major obstacles to the development of an effective and universally safe dengue vaccine, which has remained elusive despite global efforts.

The idea of host modulation has recently come to light as a potential solution to the problems associated with dengue vaccine development. Host modulation is an alternative to direct virus targeting that seeks to augment protective responses or attenuate detrimental immunopathological processes by influencing the immunological milieu. To achieve this goal, we must learn how DENV manipulates host genetic factors, immune signaling pathways, and cellular responses during infection. With this knowledge, we can create vaccines that are more targeted and effective.

The changing role of host modulation in the development of dengue vaccines is examined in this review. Our objective is to demonstrate how this strategy provides a supplementary pathway to conventional vaccine efforts by investigating the mechanisms that can be used to positively adjust the human immune system. Enhancing immunogenicity, lowering side effects, and opening the door to more extensive and durable protection against all dengue serotypes are all possible outcomes of incorporating host-directed insights into vaccine development.

II. Dengue virus:

One member of the flavivirus family with a single positive strand is the Dengue Virus (DENV). The virus primarily infects mammals through female *Aedes aegypti* and *Aedes albopictus* mosquitoes, which are vectors for the virus [1]. The Latin term "flavus," meaning "yellow," has its origins in the disease jaundice, which is a common symptom of infection with the prototypical Yellow fever virus. Hence, the name flavivirus. There are 56 different species of flavivirus [2]. Filterable agents were first demonstrated to cause human infections by the yellow fever virus and DENV, respectively,[3]. In birds, domestic animals, and humans, they produce a variety of harmful symptoms. [2]. Several significant human pathogens, including Yellow fever virus (YFV), DENV, JEV, and TBEV, are members of more than half of the flavivirus that have been linked to human disease [4].

DENV infections seldom result in mortality and typically present with symptoms similar to those of the common flu. Dengue fever (DF), a common self-limited illness, and severe dengue (DHF/DSS), which occurs in uncommon occurrences, are the two primary categories into which the World Health Organization (WHO) divides DENV infections [5]. Along with a fever of 40 °C, the former can cause severe headache, eye pain, joint and muscle pain, nausea, and vomiting [6]. The latter is divided into two categories: dengue shock syndrome (DSS) and dengue haemorrhagic fever (DHF). Patients with DHF have uncontrollable bleeding when their blood platelet counts drop to abnormally low levels [7]. Four antigenically diverse DENV types (DENV-1 to DENV-4) that are prevalent in urban areas are the primary cause of dengue fever, and each one elicits a unique immune response [8]. Although there is at least 65% genetic commonality across the four DENV serotypes, each serotype may differ by more than 6%. [9].

Encased in an icosahedral capsid, the dengue virion is a 50 nm-sized contained particle [10]. In the genome, a single open reading frame is flanked by the 5' and 3' untranslated regions (UTR) [11]. Three structural proteins (capsid (C), precursor membrane (prM), and glycosylated envelope (E)) and seven non-structural proteins are encoded by the opening frame [12]. The attachment, entrance, assembly, and secretion processes of viral infection all depend on three structural proteins. The DENV genome is physically protected by the nucleocapsid protein, which is in charge of enveloping it. The prM protein forms a compound with the commonly synthesized E protein to stabilize its pH-sensitive epitopes [13]. Scientists view the E protein as a great target for vaccines and antiviral medication candidates since it plays a role in viral invasion.

The genome of dengue fever is around 10.6 kb in length and is composed of an open reading frame with genes that encode both structural and non-structural proteins. It is single-stranded and uses positive-sense RNA. The incubation period after an initial infection with DENV is typically between four and seven days. Dendritic cells near the bite start the viral replication process, which also involves infecting macrophages and lymphocytes before entering the bloodstream. An essential component in eliciting an immunological response is dendritic cells (DCs), which are cells that deliver antigens [14].

Alongside structural proteins, non-structural proteins (NPs) play a crucial role in immune evasion, enzymatic functions of the virus, and its replication process [15]. Among these, NS1, NS3, and NS5 play crucial roles as immunogenic proteins, capable of eliciting robust humoral or cellular immune responses in the fight against viral infections. NS1 is a glycoprotein derived from the E protein and plays a crucial role in the RNA replication process of the virus. The infected host cell releases a soluble NS1 hexamer made up of three groups of homologous dimers. Due to its accumulation in the serum, NS1 serves as a biomarker for diagnosing dengue fever [16][17][18]. The NS1 protein obtains antigenic epitopes linked to type I and type II major histocompatibility complex (MHC), triggering a T cell immune response against the virus [19].

The mosquito bite is the entry point for the dengue virus into the host body.. The illness's progression is linked to humoral, cellular, and innate host immune responses, and the more severe clinical indications appear after the virus has been quickly removed from the host organism [20]. According to some hypotheses, plasma leakage is mediated by endothelial cell activation brought on by monocytes, T-cells, the complement system, and other inflammatory chemicals. Changes in megakaryocytopoiesis, which show up as infection of human hematopoietic cells and impaired progenitor cell proliferation, may be linked to thrombocytopenia. Significant bleeding may result from platelet malfunction, injury, or depletion [21,22].

Table: Types of Dengue Virus Diseases

Stages	Features	Clinical Notes
Undifferentiated Fever	It is challenging to differentiate this illness from other viral diseases, and it is typically a primary infection with occasional first or secondary infections [20].	frequently goes undiagnosed
Dengue Fever (DV)	infection, either primary or secondary. GI symptoms, myalgia, joint pain, metallic taste, retrobulbar headache, and high grade biphasic fever (three days to one week) [23, 24].	50–82% of people had a rash, which includes "white islands in a sea of red" and early facial flushing followed by maculopapular or morbilliform rash [25].
Dengue Hemorrhagic Fever(DHF)	Usually a secondary infection, it can also occur in babies who have antibodies from their mothers. Hepatomegaly, positive tourniquet test, hemorrhagic symptoms, and fever (two days to one week) [20].	Thrombocytopenia (less than 100,000/cu mm) in the lab There are three stages: fever, convalescence, and plasma leakage (shock danger) [26].
Dengue shock syndrome (DSS)	DHF + restlessness, cyanosis, chilly, clammy skin, narrow pulse pressure (< 20 mmHg), and an unstable pulse [27].	High risk of death from DIC, shock, and multiorgan damage; supportive care can hasten recovery [28].

III. Concept of host modulation:

Host modulation in dengue virus infection involves strategies designed to manage or modify the host's immune response to lessen disease severity, rather than concentrating exclusively on eradicating the virus. The main cause of dengue's severe symptoms, such as dengue hemorrhagic fever and dengue shock syndrome, is an overreaction or poorly controlled immune response that leads to tissue destruction, inflammation, and vascular leakage. The deliberate targeting of different immune system components is referred to as host modulation. This includes the regulation of humoral immune responses, the prevention of antibody-dependent enhancement (ADE), the enhancement of protective T-cell responses, and the regulation of metabolic pathways that the virus exploits. The objective of host modulation is to establish a well-regulated immune response that efficiently manages the virus while reducing harm to the body, presenting a hopeful strategy for therapeutic interventions and vaccine advancement against dengue.

IV. Dengue virus life cycle:

A simplified model of the viral protein translation process and the DENV genomic RNA. The viral RNA molecule is translated into a single polyprotein after viral cell entrance (discussed below) and nucleocapsid uncoating [29]. The bidirectional movement of the polyprotein across the endoplasmic reticulum (ER) membrane is guided by its signal- and stop-transfer sequences throughout this process. Proteases from viruses and cells co-translation the polyprotein, breaking it down into three structural proteins (C, prM, and E) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5). Glycosylation of the E protein occurs at amino acid residues 67 and 153 to guarantee correct protein folding [30, 31]. At positions 7, 31, and 52 in prM and 130 and 207 in NS1, there are additional possible sites for N-linked glycosylation [32]. Upon completion of translation and folding, the NS proteins initiate viral genome replication [29]. The C protein then forms a nucleocapsid by packaging the freshly produced RNA. Protein heterodimers of prM and E face off in the endoplasmic reticulum (ER) lumen. The next step is for the prM/E heterodimers to form trimers. It is

thought that these oligomeric interactions promote virion budding by inducing a bent surface lattice [33]. How this is timed to coincide with the engulfment of the nucleocapsid is unknown because no specific interactions between C and prM/E proteins have been discovered thus far. Cells of the mononuclear phagocyte lineage [monocytes (MO), macrophages (MØ), and dendritic cells (DCs)], which include the skin-resident Langerhans cells, are the primary targets of DENV infection during natural infection [34]. The virus must either connect to a common cell-surface molecule or use many receptors to mediate infection because DENV-permissive cells are diverse. In the last decade, several possible receptors and/or attachment factors have been identified, suggesting that DENV can enter cells through a range of molecules.

4.1 Role of cell surface receptors in the entry of dengue virus

It became evident that the numerous host receptors known to be connected to the viral entry—such as the mannose receptor (MR) present in monocytes, macrophages, and the mouse embryonic fibroblast cell line 3T3; heparan sulfate, a form of glycosaminoglycan (GAG) that acts as the receptor in epithelial cell lines like Vero and CHO K1—all played a part in DENV entry once the roles of heat-shock proteins, HSP70, and HSP90 were identified; and the lipopolysaccharide (LPS)-CD14 complex—were suspected of being receptors of immune cells, such as the monocyte and macrophages [35].

A c-type lectin, referred to as Dendritic cell-intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN), facilitates the invasion of dendritic cells by the virus. A chaperone known as GRP78 is used by DENV to infect human hepatocyte cells, along with the receptors and cells that have been extensively investigated. The AXL protein helps DENV enter human primary astrocytes and epithelial cells like A549, Vero, and human primary epithelial cells [36]. For the same rationale, we also looked into a handful of additional molecules (proteins with reported molecular weights of 65kDa, 44kDa, 74kDa, etc.) [35]. Furthermore, it is known that Fcγ receptors enable an intriguing phenomenon called Antibody Dependent Enhancement, wherein DENV infection is enhanced by heterologous secondary infection in the presence of sub-neutralizing antibodies.

V. Host modulation response in dengue infection:

5.1 Innate Immune Response

The generation of interferons (IFNs) serves as the initial barrier against DENV invasion. Infected interstitial DCs begin producing type-I IFNs within hours after DENV infection following a mosquito bite. In vivo and in vitro protection against DENV infection has been demonstrated to depend on both type I (α , β) and type II (γ) IFNs [37]. It seems that eradicating DENV infection relies on early activation of natural killer (NK) cells, which are the primary producers of IFN γ .

When a virus binds to pathogen-recognition receptors (PRRs) on myeloid cells, such as C-type lectins and toll-like receptors (TLRs) [38], IFN production begins. It has been revealed that some lectins, including DC-SIGN, MR, and CLEC5, as well as Toll-like receptors 3 and 7, are involved in the initiation of an innate response in response to DENV infection [39]. To trigger the production of IFN, activated PRRs transmit their signal via several transcription factors.

5.2 Humoral Immune Response or Antibody Response

About six days after being bitten by a mosquito carrying DENV, a person's humoral immunity begins to develop. The first things that antibodies aim for when responding to a virus are its surface glycoproteins, specifically its E and prM [40].

B cells react after innate immunity and constantly create B cell receptors (BCRs) that further develop into virus-specific antibodies following a primary infection (1°) or vaccination. IgM antibody levels peak two weeks after the start and then decline to an undetectable level two to three months later. IgG antibody levels, on the other hand, start low and then steadily rise for about a week. Consequently, the levels might be found months or even years after the patient has recovered and provide long-term protection against a particular DENV serotype [41].

5.3 Antibody-Mediated Neutralization of Infection

The multiple "hit" phenomenon is how infection is neutralized. This implies that the number of antibodies docked to the virion must surpass a specific threshold for the virus to become inactivated. Only a small portion of the available epitopes must bind in the case of powerfully neutralizing antibodies to neutralize them. Neutralizing antibodies prevent the virus from attaching to its native cell surface receptor and/or prevent further steps in the DENV entry process, according to in vitro research [42]. However, since virus-immune complexes can be internalized by cells that express Fc receptors (FcR), like macrophages and dendritic cells, preventing a virus from binding to a biological receptor would not eliminate the infection on its own.

The accessibility of epitopes plays a crucial role in determining if these regions on the antigen can engage with antibodies, thereby initiating an immune response. Secondly, the affinity and avidity of antibodies together influence the number of antibodies that attach to an antigen at a specific concentration. Third, the antibody titer plays a crucial role in antibody neutralization, as it indicates the levels of antibodies present in the patient's serum [6].

5.4 Antibody-Dependent Enhancement in Dengue

Research on antibody-dependent enhancement (ADE) is crucial for the development of DENV vaccines. After a secondary heterotypic DENV infection, the disease severity tends to rise, and an increase in serum viremia is a common symptom. One putative mechanism that could explain this is ADE.

Both the observation that infants born to mothers with dengue antibodies are at a higher risk of severe disease in the first year, when the levels of maternal antibodies are waning, and the strong association between secondary infection with a heterotypic serotype and severe disease suggest that the adaptive humoral immune response is a key player in the immunopathogenesis of dengue [43]. The term "ADE" describes how these preexisting heterologous antibodies boost viral uptake into cells that have Fcγ receptors, especially monocytes and macrophages, rather than neutralizing the current infecting serotype. The subsequent surge in viral replication and the number of infected cells has been associated with serious illness; viraemias in DHF are 10-100 times greater than in dengue fever.

Epidemiological studies have shown that infants born to dengue-immune mothers are far more likely to have severe disease if they have either a primary infection or a subsequent infection with a heterologous serotype. Antibodies are thought to particularly target virus particles to cells that carry FcR, including monocytes, macrophages, and dendritic cells. Since these cells are the virus's natural targets, they are permissive for DENV infection, increasing the virus burden and ultimately exacerbating the condition. According to research using E-specific antibodies, these antibodies may improve the effectiveness of virus attachment to the cell surface and make it easier for virions to enter through FcR-mediated endocytosis when virion opsonization takes place at an occupancy that is below the threshold needed for virus neutralization [44].

We still don't know the molecular process that causes ADE. An increased number of infected cells and an influence on the amount of virus particles produced per infected cell may result from the uptake of DENV-immune complexes via FcR-mediated entry. Recent research has shown that when DENV-immune complexes infect THP-1 cells, the production of IL-12, IFN-γ, TNF-α, and NO is downregulated, while IL-6 and IL-10 are upregulated. This suggests that FcR-mediated entry suppresses the antiviral immune response, which in turn promotes virus particle production. In the 45th, aside from E antibodies, prM antibodies have also been linked to the ADE phenomenon. Surprisingly, we have proven that prM antibodies can make completely immature particles that aren't nearly as infectious as wild-type virus [46].

5.5 Cellular Immune Response

The first cells that come into contact with DENV are the dermal cells, dendritic cells, and Langerhans cells that are present in the bite zone. The arrangement of DCs within the tissue ensures that they do not overlook any interaction with the virus [47]. There is a clear relationship between the migration of antigen-presenting cells and the host's adaptive responses and inflammation. Signals from dendritic cells, including TNF-α, attract natural killer cells, which play a vital role in limiting DENV replication and pathogenesis during the initial phases of infection through the production of interferon. Tissue-resident macrophages play a crucial role in recruiting neutrophils to the primary site of infection by secreting IL-8, TNF-α, and IFN-β. Neutrophils produce antiviral factors like TNF-α and defensins [48].

TLR-3 recognizes the dsRNA intermediates of DENV, which phosphorylates and activates the interferon regulatory factors IRF-3 and IRF-7 through the activation of TRIF.

The involvement of B cells also points to a relationship with T cells' function in the body's defence against DENV infection. CD4⁺ T cells primarily focus on the capsid as well as the NS2A/B, 3, 5, and E proteins, whereas CD8⁺ T cells concentrate on the capsid along with the NS3, 4A/B, and 5 proteins [49]. The responses lead to the secretion of perforins and granzymes by CD8⁺ T cytotoxic cells, while CD4⁺ T cells engage in Th1 effector functions, releasing IFN- γ and TNF- α .

Researchers are starting to pay more attention to cellular immunity as a result of ADE, rather than humoral immunity. Cellular immunity relies on two subsets of T cells, the CD4⁺ and the CD8⁺. To prevent the spread of viruses, CD8⁺ T cells, which are also called cytotoxic T lymphocytes (CTLs), can directly destroy infected cells or assist in recruiting cytokines such as IFN- γ and TNF- α . Although CD8⁺ and B cells mediate the immune response, CD4⁺ T cells, also known as T helper cells, are in charge of enhancing it.. They do this by relaying signals to innate immune cells, which in turn generate inflammatory and antiviral cytokines, as well as promoting cytotoxicity and immunological memory [50].

The cellular response is initiated by a variety of proteins, including structural and non-structural, but DENV antibodies mainly target highly immunogenic structural protein epitopes. CD8⁺ T cells mainly attach to the serotype-specific immunodominant epitopes of DENV. While CTLs mostly detect non-structural proteins, including NS3, NS4b, and NS5, when infected with DENV1, DENV2, and DENV4, they detect both structural and non-structural proteins when infected with DENV3. Proteins NS1, C, and E, which are not structural proteins, contain antigen determinants that CD4⁺ T lymphocytes can identify [51].

Severe dengue has been shown to elicit larger T-cell responses in both breadth and magnitude. T cell proliferation in secondary dengue infections is characterized by poor avidity for the present virus serotype and high avidity for a putative prior serotype (referred to as the original antigenic sin). The T-cell response was shown to be the most NS3 protein-marked, with low CD107a (a marker of cell degranulation) and high cytokines predominating [52]. This implies that in severe dengue, high cytokine-producing cells predominate and that the limited cytotoxic capability of T cells fails to achieve early virus control. The excessive pro-inflammatory cytokines are responsible for the severe clinical phenotype of tissue destruction and plasma leakage.

5.6 Cytokine Storm

Extreme vascular permeability that results in vascular leakage is a hallmark of dengue hemorrhagic fever (DHF). Dengue Shock Syndrome (DSS), which is typified by reduced peripheral perfusion resulting in tissue destruction and the failure of several organs, may eventually follow DHF. Both DHF and DSS situations exhibit the "cytokine storm," which is defined as increased production of cytokines, primarily IL-1, IL-2, IL-10, CXCL-10, CCL-2, VEGF, TNF- α , IFN- α , and IFN- γ , which is not seen in mild/intermediate DF [53,54]. The imbalance between Th1 and Th2 cytokine responses is what causes the cytokine storm.

Endothelial dysfunction, thought to arise from an aberrant immunological response to the virus, is a defining feature of DHF/DSS pathogenesis. Evidence from mouse model systems supports the idea that cytokines contribute to enhanced vascular permeability [55]. A large number of researchers hold the view that "original antigenic sin" triggers the cytokine storm by activating a large number of low-avidity T cells that cross-react with one another. These low-avidity T cells not only fail to eliminate the virus adequately but also trigger a large immunological activation due to their poor degranulation, altered cytokine output, and cytolytic activity [56].

5.7 Host Factor DUSP5 Potentially Inhibits Dengue Virus

A vital part of cells, the cytoskeleton is necessary for several processes, such as signaling, cell division, and preserving an intact cell structure. The most prevalent cellular cytoskeletal protein is actin, which is mostly found in the cytoplasm and forms microfilaments as G-actin (monomeric globular actin) and F-actin (polymeric fibrous actin). At several phases of their life cycle, including viral adsorption, entry, replication, assembly, and release, viruses have been shown to adapt to use and modify the actin cytoskeleton of host cells. Our investigation demonstrated that both cell lines and patients infected with DENV had an upregulation of DUSP5 expression. There was a significant difference in the levels of viral replication and endothelial permeability caused by DENV when DUSP5 was overexpressed vs when it was depleted. Our research has also shown that DUSP5 blocks DENV cell entrance by reducing F-actin rearrangement through the ERK-MLCK-Myosin IIB

signaling axis negative regulation. Our results show that DUSP5 targets the actin cytoskeleton to inhibit virus infection, which was previously unknown [57].

VI. Current status of dengue vaccine development:

6.1 Live Attenuated Vaccine

6.1.1 Dengvaxia

Live attenuated vaccines include modified virus particles that are still infectious but have had their virulence reduced or eliminated. Due to their similarity to the virus, these vaccines can provide a collection of protective antigens that stimulate a stronger immune response. Development of vaccines for dengue, featuring three currently approved options: Dengvaxia, TAK-003, and TV003 [58]. To create Dengvaxia, a live attenuated tetravalent vaccination, Sanofi Pasteur used a chimeric technique.. This vaccine is also known as CYD-TDV. So far, the vaccine has received approval for use in over 20 countries around the globe. The vaccine is given in three doses spaced 6 months apart, specifically at 0, 6, and 12 months, to guarantee a robust immune response over the long term [8].

An attenuated strain of yellow fever virus (YFV) known as chimeric yellow fever dengue (CYD) serves as the basis for this vaccine [8]. To induce an immune response unique to DENV, researchers used recombinant DNA techniques to combine the nonstructural gene of CYD with the structural premembrane (prM) and envelope (E) genes of the four serotypes of DENV [8]. YFV induces particular cellular immunological responses, including CD4+ and CD8+ cells, with low cross-reactivity, in contrast to the primary immunogenic proteins of DENV. To that end, Dengvaxia is more effective at eliciting humoral than cellular immune responses.

6.1.2 TV003

Following the release of Dengvaxia, the National Institutes of Health (NIH) in the United States created TV003, another vaccine that employs the tetravalent live attenuated approach. Live attenuated tetravalent vaccination (LATV) and monovalent vaccinations are combined in TV003 [59]. The immunological response to the vaccine is balanced against each of the four DENV serotypes. In the first study that evaluated the infectiousness of several attenuated vaccine candidates, the National Institutes of Health (NIH) used mice, macaques, and mosquitoes to determine which eight monovalent vaccine components had the best immunogenicity and safety profile [59]. TV003 includes the four monovalent components of DENV1-4—'rDEN1D30,' 'rDEN2/4D30,' 'rDEN3D30/31,' and 'rDEN4D30'—that are attenuated recombinant dengue vaccines. Compared to Dengvaxia and TAK-003, TV003 elicits a more thorough DENV-specific cellular response because it contains the complete sequence of three distinct serotypes of DENV. In contrast to two doses of TAK-003 or three doses of Dengvaxia, TV003 can generate an antibody response in 90% of seronegative people with a single vaccination dose [60].

6.1.3 TAK-003

Takeda Pharmaceutical Company's recombinant tetravalent vaccine candidate TAK-003 (formerly DENVax) is another potential possibility. Although both the TAK-003 and CYD-TDV live attenuated vaccines are made utilizing chimeric techniques, the TAK-003 differs from CYD-TDV in that it uses an attenuated PDK-53 DENV2 strain for its genetic core and chimeric carrier [61]. Coding sequences of DENV1, DENV3, and DENV4, which encode prM and E proteins, were substituted with those of DENV2 PDK-53 to elicit immunological responses against the other three serotypes. With the PDK-52 backbone, the vaccine can simultaneously generate cellular and humoral responses that are unique to DENV, which is an additional advantage. As of December 1, 2021, Takeda had undertaken five phase III trials for this vaccine, the largest of which was DEN-301, also called TIDES [62]. The TIDES study included 20,099 children, ranging in age from 4 to 16, from 8 different countries, which is a large sample size compared to prior vaccine trials [62]. In addition, between 1.5 and 11-year-old children and adolescents in phase I and II studies conducted in Colombia and other dengue-prevalent countries, TAK-003 effectively elicited immune responses against all four dengue serotypes. With only one dose needed to induce humoral and cellular immune responses, TAK-003 protects children under

the age of nine, is safe for both seropositive and seronegative patients, and considerably lowers the risk of dengue fever-related hospitalizations compared to its attenuated chimeric predecessor.

6.2 Genetic Vaccines

Genetic vaccines, including DNA and mRNA types, harness segments of the virus's genetic material to elicit immune responses, contrasting with traditional vaccines that employ recombinant bacteria or viruses.

6.2.1 DNA Vaccine

DNA vaccines consist of viral genetic material presented in a plasmid format, which includes multiple virus-specific antigens encoded by various genes. Twenty years ago, researchers used immuno-stimulatory CpG DNA sequences in conjunction with a DNA vaccine that expressed DENV2 prM/E proteins to successfully establish immune protection against DENV2 [63]. However, the biggest obstacle facing DNA vaccines is still their lack of immunogenicity. A recent investigation enhanced the immunogenicity of DNA vaccines for DENV by incorporating antigens aimed at dendritic cells (DCs), which serve as the main antigen-presenting cells (APCs) within the immune system [64]. Given that DCs bridge innate and adaptive immunity, this vaccine has the potential to elicit both cellular and humoral immune responses.

6.2.2 mRNA Vaccine

There is also the mRNA vaccine, which is a kind of genetic vaccine. For greater efficacy, mRNA vaccines employ messenger RNAs (mRNAs) that code for viral proteins rather than a DNA plasmid. Researchers developed a "serotype-specific mRNA vaccine" in May 2021 that encodes the DENV1 prM and E proteins. The potential vaccine, like earlier mRNA vaccines, was shielded from the host's enzymes by being encased in lipid nanoparticles (mRNA-LNP) [65].

VII. Role of host modulation in enhancing dengue vaccine efficiency:

7.1 Regulating T Cell Responses

A more thorough understanding of the effector T cell response to DENV has been made possible by recent research employing multiparameter flow cytometry. As previously mentioned, the majority of research has been on type 1 cytokine-producing T cells (Th1/Tc1), and these investigations have demonstrated a significant amount of individual cell heterogeneity in cytokine production. Although polyfunctional T cells have been found to exhibit three or more effector functions, there are also sizable populations of cells that only express one or two of the functions that have been evaluated, such as cells that exclusively express pro-inflammatory cytokines (TNF and/or chemokines). It has been demonstrated that stimulation with the matching epitopes of distinct DENV serotypes changes the profile of cytokines generated, indicating that variant epitopes function as modified peptide ligands for certain T cells unique to DENV [66,67].

Immune responses to initial and secondary infections of distinct DENV serotypes will vary significantly, according to models of sequential infection with different serotypes: (a) the induction and levels of the memory T cell response will be increased more quickly; (b) the memory response will activate T cells primarily targeting non-structural proteins that are more highly conserved across the various DENV serotypes; and (c) the memory T cell response will exhibit a different effector profile due to differential activation by peptides from the second DENV serotype [68].

In alignment with the forecasts, variations have been observed in the expression of certain phenotypic markers, in the predominant epitopes targeted, and in the pattern of serotype cross-reactivity. Interestingly, there were no notable differences found in the kinetics of the response or the peak T cell frequencies throughout the acute infection. The studies focused solely on symptomatic DENV infections, and thus, the intrinsic incubation period before symptom onset remained undetermined.

7.1.1 Vaccination vs Natural Infection

All of the recent studies have focused on candidate live attenuated vaccines. The findings indicate that memory T cells specific to DENV, particularly polyfunctional Th1/Tc1 cells, are generated within 21 days following the vaccination of individuals who have not been previously exposed to flavivirus. When compared to vaccination using its separate components, the tetravalent formulation of the NIH/Butantan vaccine notably promoted T cell responses to peptides derived from the more conserved non-structural proteins [69].

7.1.2 Dengue Vaccine Research and Development: The Role of Dengue T Cells

Finding correlates of vaccination efficacy is undoubtedly the area where evaluation of T cell responses to dengue vaccines would have the biggest influence. Testing for vaccines in various populations, regimens, or epidemiological settings might be accelerated by a trustworthy immunological correlate of vaccine-induced protective immunity. Finding correlations between T cell IFN- γ [70,71] production and protective immunity in human cohort studies and animal trials supports the possibility of identifying T cell responses linked to vaccination-induced protective immunity. There isn't much published data, though.

Measuring T cell responses could also be useful in determining how long vaccine-induced protective immunity lasts. Given the evidence that incomplete immunization raises the risk for more severe illness, this is likely to be especially important for dengue vaccines. The role of early T cell activation in the formation of long-term T cell and B cell memory has been well-understood, and this process has been effectively modulated in animal models using drugs like rapamycin. Licensed vaccines for different diseases have different pathogen-specific antibody and T cell durability estimates. Some of these vaccines have early response indicators found using comprehensive "systems vaccinology" approaches [72,73], but more research is needed to determine if these estimates can predict the response's longer-term durability.

Newer assays can disclose amazing detail on the interactions between these responses due to their single-cell resolution and ability to examine several T cell effector activities. Given the multivalent nature of dengue vaccines, the requirement to offer protective immunity against all four DENV serotypes, and the evidence linking more severe dengue disease to an inflammatory immune response, this capability is probably of particular interest in the case of dengue vaccines. It is heartening to see data from various research studies demonstrating that various tetravalent dengue vaccinations induce polyfunctional T cells [74]. Although comparable numbers of polyfunctional T cells are observed following a natural DENV infection, a situation that does not accurately reflect (i.e., tetravalent) protective immunity, it is uncertain whether the level of "polyfunctionality" reported is ideal.

7.2 Regulating Humoral Immune Response

7.2.1 B Cell Responses Against Dengue Virus

Much of the research on human antibody responses to DENV has so far been on MAb produced from memory B-cell pools and circulating serum antibodies, as mentioned above. Research is necessary to determine which subsets of B cells are activated by dengue and to determine the functional significance of antibodies produced by various B cell types. Follicular B-cells are the source of most antibodies that are activated by viruses; these responses are classical T-dependent. Additionally, T-dependent follicular (B-2) B-cells, which can develop into memory B-cells and long-lived plasma cells, are anticipated to play a key role in the primary antibody response to DENV. New research suggests that B-cell subsets that have received less attention, including those in the marginal zone as well as B1a and B1 b cells, offer viral protection [75]. To determine whether comparable reactions are critical parts of the reaction to DENV, further research is required. The recent observation that numerous human flavivirus antibodies recognize epitopes preserved on the intact virion, yet not on recombinant E protein, is quite intriguing. This suggests that such antibodies may be generated through the direct activation of B-cells by the multivalent virus particle, independent of T-cell assistance [76]. The infection caused by DENV also hampers type I interferon responses and diminishes antigen presentation by myeloid cells. These effects are likely to impact the quality of the adaptive immune response, including the production of antibodies. It is essential to allocate resources towards human studies and animal models to delineate the B-cell subsets that play a role in the response to DENV, focusing specifically on the distinctions in these responses between primary and secondary cases, as well as between severe and mild disease presentations.

7.2.1.1 Antibodies induced by DENVs

Upon infection with DENV, the immune system primarily targets two structural proteins, prM and E, as well as one non-structural protein, NS1. It is possible for the immune response to NS1 to cross-react with any of the serotypes of DENV. The activation of the Toll-like receptor-4 is the mechanism via which NS1 directly causes vascular hyperpermeability, according to studies [77], in Natural DENV infections can produce antibodies that can either protect or harm the host. In essence, the pathogenic antibodies are antibodies that promote the spread of disease by allowing non-neutralized and immature DENV to enter monocytes and macrophages. Studies have demonstrated that prM and the FL epitope (FLE) are highly immunodominant and generate antibodies that spread disease [78].

There is roughly sixty percent of the human antibody response that is directed toward the prM protein, according to the findings of an investigation into memory B cell responses in individuals who were infected with DENV [79]. These antibodies can identify the prM of all four serotypes of DENV. Even though anti-prM-antibodies only moderately inhibit the infectiousness of DENV, they are potent promoters of ADE. By opsonizing non-infectious immature virions and enabling their intracellular entry through the Fc γ receptor pathway, these antibodies can turn them into infectious agents.

The immature virions can grow once inside the cell, become naturally contagious, and spread to other cells. Similarly, anti-FLE antibodies, which make up 20–30% of the antibody response to DENV, are likewise cross-reactive and typically operate as strong ADE promoters rather than neutralizers [80]. Normally hidden within the mature virion, the conserved FLE of flaviviruses becomes exposed when the virus breathes. The presence of prM and FLE antibodies makes even partly or immature DENVs fully infectious, which increases the cellular viral burden due to ADE.

7.2.1.2 Design Of the Vaccine

Given the significant hurdles inherent to whole virus-based vaccinations, it is clear that other dengue vaccine techniques need to be investigated. Dengue vaccinations that work effectively focus on neutralizing epitopes (EDIII and quaternary epitopes like EDE) and avoid pathogenic ones (prM and FLE).

Scientists have recently developed a stable EDE, a new subunit DENV vaccine candidate. This has been accomplished by connecting the two E-monomers to create an E-dimer and securing this E-dimer through covalent disulfide linkages. Monoclonal antibodies specific to the quaternary epitopes of DENV were used to identify the stabilized EDE protein. Furthermore, the FLE was not present on the surface of these stabilized EDE, thereby preventing a considerable amount of cross-reactive antibodies to this FLE that are triggered by immunizations with E-monomers. This EDE design produced a greater degree of serotype-specific immune responses when compared to the E-monomers [81].

DSV4 (Dengue Subunit Vaccine Tetravalent), a virus-like particle (VLP) vaccine candidate, has lately shown very encouraging pre-clinical findings [82]. This rival uses the EDIII model. Domain EDIII produces strong, serotype-specific antibodies that neutralize viruses, in contrast to domains EDI and EDII, which mostly generate antibodies that are cross-reactive with flaviviruses and either strongly neutralize or do not neutralize at all. "A tetravalent vaccine candidate, known as 'four-in-one', integrates the EDIII domains from all four dengue virus (DENV) serotypes into a single open reading frame using flexible linkers. Upon assembly into virus-like particles (VLPs), DSV4 presents key neutralizing epitopes representative of each DENV serotype."

7.3 Modulating Innate Immunity by Multivalent Vaccination- Protection Against Dengue Challenge

To prevent the induction of partial immunity to one or more DENV serotypes, which could result in antibody-dependent enhancement infection upon subsequent DENV infection, vaccine candidates must concurrently protect against all four serotypes of dengue, as the immune response is crucial to both protection and pathogenesis. "Therefore, a comprehensive understanding of how innate immune responses shape and influence the adaptive immune system is essential for researchers developing dengue vaccines." The innate immune response that such vaccine candidates generate during the early stages of immunization has not been precisely defined, despite experiments on viral replication and antibody responses to NIH DENV vaccine strains in humans and rhesus macaques [83]. Finding immunological profiles in a DENV challenge model after vaccination and challenge is the primary objective of our investigation. Two randomized, double-blind, placebo-controlled trials, CIR299 and CIR300, were conducted to evaluate the protective efficacy of two dengue vaccine admixtures against the DENV2 virus and to investigate the influence of homotypic versus heterotypic antibodies in that protection. "In the CIR299 clinical trial, a group of participants (n = 18) received the live attenuated

tetravalent dengue vaccine formulation TV005—which includes rDEN1Δ30, rDEN2/4Δ30, rDEN3Δ30/31, and rDEN4Δ30—while another group (n = 18) was given a placebo”. Meanwhile, in the CIR300 trial, participants received an attenuated trivalent admixture (rDEN1Δ30, rDEN3Δ30/31, rDEN4Δ30, n = 15) or a placebo (CIR-300; n = 6) [84].

7.3.1 Discussion

We conducted a thorough analysis of the immunological profiles of samples taken from participants who were vaccinated with a placebo, trivalent, or tetravalent vaccine, and then subjected to the DENV challenge. Using a challenge paradigm, we aimed to determine how heterotypic protection and homotypic antibodies affected the DENV2 viral challenge's infectiousness. The trivalent and tetravalent admixtures differed solely in that the rDEN4Δ30 genome had the prM and E proteins of DENV2 NGC swapped out for those of DENV4. We identified key infection markers (IP-10, MCP-1, IL-1RA, and MIP-1β) by examining challenge-response immunological profiles. These markers demonstrate that the tetravalent vaccination outperformed the trivalent cocktail in protecting against the rDEN2Δ30 challenge virus. Differences in the immunological response after a challenge between tetravalent and trivalent admixtures suggest that the presence of DENV2-specific antibodies influences the challenge virus's capacity to elicit an immune response after a challenge [84].

As demonstrated by lower expression of IP-10, MCP-1, IL-1RA, and MIP-1β in viral challenge response profiles and a lower proportion of patients with viremia across each group, the tetravalent vaccine provides a higher level of protection against the rDEN2Δ30 challenge virus than the trivalent admixture. As a result, homotypic antibodies against DENV2 are much more protective than heterotypic antibodies generated by the rDEN1Δ30, rDEN3Δ30/31, and rDEN4Δ30 vaccine components. These results demonstrate that the rDEN2/4Δ30 component of the vaccination is essential for the protection of the DENV2 virus [85].

According to our findings, the tetravalent vaccination provides superior protection against challenge viruses as compared to the trivalent admixture. Important markers were IP-10, MCP-1, IL-1RA, and MIP-1β. Strong antiviral immune responses have been linked to these markers in the past.

VIII. Challenges and limitations

8.1 Preexisting Cross-Reactive Immunity

The primary obstacle in developing a vaccine for dengue lies in the intricate nature of the virus. Infection with one serotype leads to enduring immune protection against that particular serotype while providing temporary immune protection against the other three different serotypes. This may result in serious dengue during later infections, potentially influenced by ADE. This presents a significant challenge for the development of a dengue vaccine, as it requires a tetravalent formulation that provides balanced, long-term protection against all four DENV serotypes at the same time [86].

An important obstacle in the development of dengue vaccines is ADE, which has already been described. Infection with a single DENV serotype results in protection against that serotype for the long term and protection against all DENV serotypes for a short period of time. After this period ends, antibody titers drop to levels below neutralizing concentrations, which can make the disease worse when infected with a different type of virus. Multiple mouse investigations have provided evidence that heterotypic DENV infection plays a role in the ADE process. Virus titers and disease severity were both elevated in AG129 mice pre-infected with DENV2 after administration of heterotypic DENV immunological sera or DENV-specific monoclonal antibodies [87].

When maternal DENV-specific antibody titers reach sub-neutralizing, enhancing concentrations between 6 and 9 months of age, infants born to dengue-immune mothers are more likely to acquire severe dengue. In a mouse model with maternally acquired heterologous DENV antibodies, this clinical observation was experimentally verified [88].

Additionally, as was previously indicated, CYD-TDV vaccinees were suspected of having vaccine-induced ADE because they produced poorly neutralizing, enhancing antibodies, which raised the likelihood of hospitalization for vaccinated infants [89]. According to the study, people who had a limited range of preexisting anti-DENV antibody titers were more likely to develop severe dengue.

Adverse events may also occur after a series of DENV and Zika virus (ZIKV) infections. There is a lot of sequence similarity between the E proteins of DENV and ZIKV, and antibodies that target the EDI/II

ectodomains have shown cross-reactivity. Regardless of their neutralizing capacity, sub-neutralizing doses of antibodies that cross-react with DENV or ZIKV prM and E proteins have been found in several in vitro investigations to potentially increase viral infection [90].

8.2 In-Vivo Models to Evaluate the Protective Efficacy of Dengue Vaccines

The absence of a suitable animal model that can mimic and duplicate the pathophysiology, immunological reactions, and clinical progression of dengue infection as observed in humans has further impeded the development of dengue vaccine candidates. The use of mouse models has limited relevance due to the virus's specificity and tropism for key cell subsets, as well as the difficulty in clinical isolates of DENV have establishing infection and causing disease in the murine host [91]. Since DENV cannot block or evade mouse interferon signaling, immunologically competent mice are inherently resistant to the virus, unlike humans.

The absence of appropriate animal models that display symptoms analogous to those seen in humans suffering from dengue presents a significant challenge in the advancement of dengue vaccines. At present, the AG129 mice strain, lacking IFN $\alpha/\beta/\gamma$ receptors, stands out as the most appropriate mouse model for investigating dengue replication in vivo. This mouse strain has been shown to elicit DENV-specific antibodies and provide protection against both heterologous and homologous virus challenges [92]. In addition, creating humanized mice by engrafting human hematopoietic progenitors into immunocompromised strains like BALB/c-Rag2null ILR2 γ null (BRG) and NOD/scid/ILR2 γ null (NSG) may prove advantageous for advancing future dengue vaccine development and investigating pathogenesis.

Infection with DENV strains tailored to mice led to viremia, thrombocytopenia, neuropathological illness, and occasionally death in mice with different immunologically capable backgrounds. The poor IFN- γ signaling in AG129 mice suggests that they might not be an appropriate model to investigate the function of T cells in vaccine-induced protection [93]. Because the importance of T cells in immunity is being better understood and proven, this is a very crucial consideration [94,95]. Therefore, it could be better to use Type I IFN-deficient mice (A129 or IFNAR) that have full IFN γ signaling for this purpose. However, there have been very few reports of productive infection in these mice caused by DENV strains, perhaps because they are more resistant to DENV than their AG129 counterparts [96].

Researchers have also mentioned studying dengue using models of non-human primates (NHPs). Except for one study involving rhesus macaques infected with an exceptionally high inoculum dose, DENV infection in NHP causes asymptomatic transitory viremia [97]. Hence, NHPs are useful for studying the immunogenicity and safety of potential dengue vaccines, but they don't provide much in the way of protection efficacy assessments.

Additionally, the effectiveness of live attenuated tetravalent dengue vaccinations has been assessed using dengue human infection models (DHIM). A DHIM has been created by the NIH Laboratory of Infectious Diseases (LID) to evaluate the Tetravax TV003 candidate's ability to defend against DENV2 [98]. The challenge was conducted using DEN2 Δ 30, a modified strain of DENV2, which caused viremia, rash, and neutropenia in 30 patients in 100%, 80%, and 27% of cases, respectively. Comparing 21 recipients to the unvaccinated controls, it was discovered that the vaccine produced antibodies against all four dengue serotypes, protected them against challenge [98].

DHIM may help with many aspects of the clinical development of the vaccine. Researchers and vaccine developers might assess the candidates' safety and effectiveness in various vaccine recipients. Researchers and vaccine developers may be able to produce dengue-primed subjects for initial safety testing of vaccine candidates in carefully monitored, low-risk settings thanks to DHIM. DHIM can also be used to assess how dengue priming affects safety and the priming of various DENV strains. Decisions about early vaccine formulation, including adjuvant selection, dosage, antigen concentration, and antigen selection, may also benefit from the use of DHIM. Consequently, because of its strong predictive value, DHIM would be useful for vaccine development efforts [99].

8.3 Immune Correlates of Protection

So far, the immune correlates that protect DENV have yet to be determined. Historically, the effectiveness of vaccine candidates has depended on assessing neutralizing antibodies targeting a particular DENV serotype, as established by the plaque reduction neutralization test (PRNT). This is quantified as the reciprocal of the

antibody dilution that neutralizes 50% to 90% of viral infectivity [100]. A PRNT titer value of 10 or higher suggests the presence of neutralizing antibodies, indicating potential protection [100]. The World Health Organization has provided guidelines on the methodology for PRNT; however, not all laboratories adhere to these recommendations. This inconsistency complicates the comparison of PRNT titers across different studies, as the results can be affected by various factors such as the virus strain, virus preparation, mammalian cell line, presence of EDTA, the type of sample used (plasma versus serum), and the method employed for measuring infected cells [101,102].

Moreover, cells lacking Fcγ receptors, like BHK-21, Vero, and LLC-MK2, are usually the ones used for PRNT. Because these receptors are crucial for DENV replication and antibody-mediated entrance into target cells [103,104], the PRNT assay cannot be used to determine the true impact of DENV antibodies on protection vs pathogenesis [105]. The makeup of the vaccine candidate determines whether antibodies generated during natural DENV infection or immunization are of distinct species. To develop and assess vaccine efficacy, it would be helpful to conduct a comprehensive characterization of the antibody repertoire produced during DENV infection [106].

IX. Conclusion

The molecular complexity of the dengue virus and the inherent unpredictability of the host immune response have posed challenges to the quest for a vaccine that is both safe and broadly protective. Modern approaches to improving vaccine efficacy have moved away from focusing solely on the virus and toward learning about and influencing the host immune landscape. An essential strategy in this regard is host modulation, which aims to shape the immune system to react more efficiently without inducing unwanted side effects like antibody-dependent enhancement.

Scientists are trying to develop vaccines that not only protect against all four dengue serotypes but also have a good safety record by guiding immune responses with specific adjuvants, immune-regulatory components, or delivery mechanisms. The balance between immunogenicity and immunopathology, which has proven to be a recurring problem in the development of dengue vaccines, can be more precisely controlled with this approach.

Further research into host-pathogen interactions, the discovery of immunological correlates of protection, and the incorporation of host modulation methods into clinical vaccine design are all necessary for the future advancement of this discipline. Research and innovation in host modulation have the potential to revolutionize dengue prevention by creating vaccinations that are more effective and can respond to the unique immunological profiles of people at risk.

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