

Dengue virus: host-pathogen interactions and emerging role of DNA vaccines

Abstract

Dengue virus infections are a major cause of mortality and morbidity in Southeast Asia, South and Central America with 24'000 deaths annually. Two factors are accountable for the severe outcomes of Dengue Hemorrhagic Fever (DHF); one is the virulence of the virus and second is the cross-reactivity of various dengue serotypes with the immune system of the host. Rapid rise in the levels of various cytokines, particularly Tumor Necrosis Factor-alpha (TNF- α), Interleukin-2 (IL-2), Interleukin-6 (IL-6), Interleukin-8 (IL-8) have a major role in inducing distinctive clinical presentations of DHF. These range from simple plasma leakage to hemorrhagic problems and even shock. Another hallmark of DHF is the presence of cross reactive primary antibodies which produce an intense immune response in secondary infection resulting in immune mediated pathology seen in DHF. There have been many attempts made in the past for the development of a suitable vaccine for dengue fever. Vaccination using plasmid DNA against dengue fever is an active area of research. In this review the role of different cells in the multiplication of dengue virus and viral interactions with the immune system have been discussed. Special emphasis is given to the nature of DNA vaccines in general developmental efforts of a dengue fever vaccine.

Keywords: Dengue virus, Dengue fever, Dengue hemorrhagic fever, Macrophages, Antibodies, Dengue vaccine

Volume 3 Issue 3 - 2016

Muhammad Atif,¹ Ummar Raheel,²
Muhammad Imran,¹ Hafiz Umar Arshad,³
Fayyaz ul Hassan Baloch,³ Sadia Irshad
Leghari,² Fayyaz Ahmad,¹ Zareen Fatima,⁴ Yu
Qi,^{1,5} Sobia Manzoor,³ Najam us Sahar Sadaf
Zaidi,³ Ahmed Bilal Waqar^{1,6,7}

¹Department of Medical Lab Sciences (DMLS), FHAS, ICBS, Lahore, Pakistan

²University Institute of Medical Lab Technology (UIMLT), FHAS, UOL, Lahore, Pakistan

³Department of Diet and Nutritional Sciences (DDNS), FHAS, ICBS, Lahore, Pakistan

⁴Atta-ur-Rehman School of Applied Biosciences, NUST, Islamabad, Pakistan

⁵Department of Radiological Sciences and Medical Imaging (DRSMI), FHAS, ICBS, Lahore, Pakistan

⁶Department of Histology and Embryology, Institute of Basic and Translational Medicine, Xi'an Medical University, Xi'an, Shaanxi 710021, China

⁷Imperial Post Graduate Medical Institute, FHAS, ICBS, Lahore, Pakistan

Correspondence: Ahmed Bilal Waqar, Associate Professor/ Director, Post Graduate Medical Institute, Faculty of Health and Allied Sciences, Imperial College of Business Studies, 25 B Lower Mall, Lahore, Pakistan, Tel (92) 3349686443; Email drabwaqr@yahoo.com

Received: April 16, 2016 | **Published:** May 04, 2016

Abbreviations: DHF, Dengue Hemorrhagic Fever; DF, Dengue Fever; ADE, Antibody Dependent Enhancement; APCs, Antigen Presenting Cells; VEE, Venezuelan equine encephalitis

Introduction

Dengue Virus (DENV) is a positive sense RNA virus belonging to the family flaviviridae. Four serotypes of Dengue Virus (DEN1 to DEN-4) have been isolated till now.¹ Humans are infected by the bite of infected mosquito species known as Aedes Aegypti. A. Aegypti is the main vector of dengue virus transmission in humans and is prevalent in the tropical and subtropical parts of the world.

Primary infection with DENV causes an acute febrile illness known as dengue fever (DF) whereas secondary infection with dengue virus may sometime leads to fatal dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS).² Dengue virus infection is affecting every population of the world with 50-100 million cases of DF and 250,000 to 500,000 cases of DHF/DSS annually. The disease is prevalent in more than 100 countries in Africa, America, the Eastern Mediterranean, Southeast Asia and the Western Pacific. But Southeast Asia and the Western Pacific are most serious victims. About 2500 million people that are 2/5 of the world's population are now in danger from dengue infection. Still no antiviral therapy or vaccine is available to cure the disease. Nevertheless, appropriate symptomatic treatment has been successful to decrease the rate of mortality by DHF.²

The classical DF is self-limiting but fatal febrile illness which results from the primary dengue virus infection. Dengue virus is generally cleared by the immune system within seven days after infection. Classical sign and symptoms of DF include fever, headache, myalgia and retro-orbital pain.³

DHF or DSS is a life-threatening disease which results from the secondary infection in which a different serotype of dengue virus is involved. It can have lethal outcomes due to antibody dependent enhancement (ADE) and can result in the death of the patients.⁴ The four classical signs and symptoms of DHF include fever, hemorrhagic manifestations, thrombocytopenia (platelets counts of < 100,000 cells/mm³) and plasma leakage. DHF is further classified into four grades (Grade I to IV). DHF grades III and IV show extensive plasma leakage in various body cavities including pleura, pericardium and peritoneal cavities resulting in DSS.⁴

DHF has been found to occur upon subsequent infection with serotypes other than the one responsible for the primary infection.⁵ The immunity against one particular serotype of dengue virus is long lasting whereas upon subsequent infection with other serotypes causes only partial immunity and leading to further complications.⁶ A comprehensive understanding of the whole pathological process of the DF & DHF is still incomplete and further research is required. Better understanding of dengue virus pathogenesis will allow us to devise better strategies for the prevention and the treatment of DF &

DHF. This review will help scientists to better understand the immune interactions of dengue virus and help them devise a tetravalent DNA vaccine against dengue virus infection.

Role of dendritic Cells in DF

Dendritic cells (DC's) are potent and professional antigen presenting cells (APCs) which are involved in establishing the primary immune response against the dengue virus. DC's helps in the active multiplication of DENV as proven by the ability of DENV to enter cultured human DC's and produce virus particles.⁷ DENV-stimulated DC's expresses maturation markers such as B7-1, B7-2, Human leukocyte antigen- D related (HLA-DR), Cluster of differentiation molecule 11b (CD11b) and CD83.⁷

After infection with dengue virus, DC's migrate from peripheral tissues to the lymph nodes and activate CD4+ and CD8+ T lymphocytes.⁶ The infection of DC's by DENV induces production of TNF- α and Interferon- α (IFN- α).⁷

The production of TNF- α by DENV-infected DC's correlate with the clinical signs and symptoms showing the highest levels of TNF- α in patients at the time of plasma leakage. TNF- α induces the up-regulation of anti-apoptotic factors such as Bcl-xL that can protect DC from apoptosis.⁸ Hence, virus-induced production of TNF- α and IFN- α causes decreased apoptosis in DENV-infected DC in the late phase of virus infection.⁹ Cytokines produced in response to DC activation by DENV results in inflammation and shock as mentioned in (Figure 1).

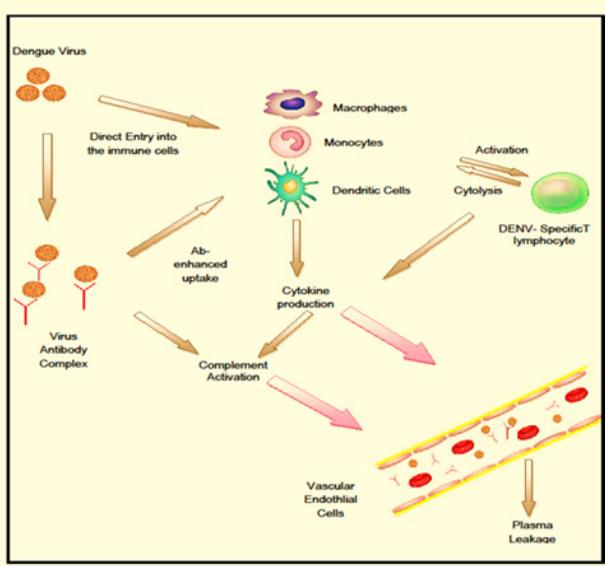


Figure 1 Pathway elaborates the immuno-pathogenesis of DENV; it can proceed in two ways, primary infection leads to direct entry of DENV into immune cells like Macrophages, Monocytes and Dendritic cells. This pathway can further activates DENV specific T cells, cause cytolysis, cytokines production, complement activation and finally leading to plasma leakage. In case of secondary infection antibody based enhancement occurs which leads to cytokines production and complement activation, high levels of cytokines and complement activation can damage vascular endothelial cells resulting in plasma leakage.

Role of cytokines in DF

The levels of TNF- α , IL-2, IL-6, IL-8, IL-10, IL-12 and IFN- γ have been found to be elevated in patients with DHF. Monocyte chemoattractant protein-1 (MCP-1) and IL-8 levels are also observed to be raised in the pleural effusion taken from DHF patients.¹⁰

TNF- α has been reported to be secreted by monocytes and endothelial cells infected by dengue virus. The activation of the immune effector cells and T lymphocytes leads to increased levels of soluble TNF receptors (TNFR175).¹¹ Upon activation virus specific T lymphocytes produce some cytokines namely IL-2, TNF- α , IFN- γ . IL-1 and IL-6 are produced by mast cells and basophils infected with dengue virus.¹² Raised levels of these cytokines cause increased vascular permeability in DHF/DSS patients.¹³ Cytokine production and cell inactivation seen in dengue virus infection can produce an anti-apoptotic effect during the last phase of the pathogenesis.¹⁴

Increasing levels of IFN- γ , IL-6, IL-8 can alter various cellular and body functions like raised Aspartate aminotransferase (AST)/Alanine aminotransferase (ALT), cell damage, thrombocytopenia, increased hematocrit, pleural effusion/ascites. These entire features are indicative of endothelial cell dysfunction. Increased levels of IFN- γ are seen in large number of DHF cases with plasma leakage.¹⁵ The level of IL-8 is significantly higher in DHF compared to DF and it also correlates with thrombocytopenia and raised ALT. In vitro studies of HepG2 cells infected with dengue virus have shown increased level of expression of those genes that are responsible for the secretion of pro-inflammatory cytokines such as IL-6 and IL-8.¹⁴ Increased levels of IL-8 have been associated with plasma leakage.¹⁵

Role of complement activation in DF

DHF displays the activation of complement cascade as a clinical manifestation. Levels of C5a, C3a and complement activation products are at their highest levels although the plasma leakage is markedly apparent (Table 1). Immune complexes tend to activate the complement proteins in DHF.¹⁶ Monocytes and endothelial cells infected by dengue virus activate complement via alternative and classical pathway. Nonstructural-1 (NS1) and pre-membrane (pM) proteins of dengue virus bind to clusterin (CLU). This association of clusterin with NS1 and pM may free C7, so helping in the formation of terminal complement complex (TCC).¹⁷

Table 1 Immune interactions of DENV

Immunological component	Immunological response to dengue virus
Dendritic Cells	TNF- α , IFN- α production Involved in Active Replication of Dengue Virus Decreased Apoptosis in Late Phase of Viral Infection Responsible for Inflammation and Shock due to Cytokine Production. Elevated Levels of TNF-A, IL-2, IL-6, IL-8, IL-10, IL-12 and IFN- Γ during Fever resulting in Liver Damage
Cytokines	Cell Damage Thrombocytopenia Platelet Destruction Pleural Effusion. Activated by Immune Complexes, Infected Monocytes & Endothelial Cells
Complement System	Increased Levels of Complement Proteins C5a And C3a During Plasma Leakage Cause of Oxidative Burst Principle Site of Viral Replication
Macrophages	Cause Production of IL-1, IL-6, IL-10, IL-12, IFN- α , IFN- β Blood Monocytes Main Target During Secondary Infection Production of Free Radicals During Infection.

Table Continued...

Immunological component	Immunological response to dengue virus
Antibodies	Neutralization in Primary Infection
	Antibody Dependent Enhancement (ADE) During Secondary Infection
	IgG in Cross Protective Immunity
	Cause Endothelial Cell Dysfunction
	Cause Fibrinolysis by Antibodies Produced Against Plasminogen

In severe dengue infection large amounts of C3a have been detected, enlightening its role in dengue pathogenesis. C3a is one of the many important anaphylatoxins produced as a result of complement activation which in turn can disrupt the vasculature. C3a recruits macrophages, monocytes and dendritic cells and augment the effect of other pro inflammatory cytokines including IL-6, TNF- α and Stromal cell derived factor-1 (SDF-1). Although the precise mechanism of activation of complement anaphylatoxins has not been fully understood but the activation of C3aR enhances cytokine expression through AKT phosphorylation as well as MAP kinase activation. C3aR is expressed on major intermediaries of the immune system, fascinatingly not naïve T-cells. C5aR also activates a lot of downstream signaling pathways which includes Phospholipase D (PLD), Phospholipase C (PLC), Phosphoinositol -3 Kinase (PI3K- γ), rapidly accelerated fibro sarcoma (RAF) and Wiskott-Aldrich syndrome protein (WASP).¹⁸

Role of Macrophages in DF

Macrophages derived from monocytes are the principle cells of DENV replication and play an important role in the innate immunity.¹⁹ As a result of primary infection, only about 1-2% of blood monocytes and macrophages are infected, furthermore, monocyte-derived dendritic cells (DCs) are also infected.^{20,21} Blood monocytes and macrophages are the main target cells being infected during secondary infection via antibody dependent enhancement (ADE) mechanism.²² Dengue virus enters the macrophages through a virus receptor or Fc-receptor as an immune complex. There are certain receptors reported that recognize the constant region of IgG, Fc γ R1 and Fc γ R2 are known to participate in this process.²³ It has been also reported that there are no Fc receptors for Dengue serotype-2 in primary cultures of human macrophages.²⁴ Reyes et al also showed that the heat shock protein 90 (HSP90) and HSP70 act as a receptor complex in human cell lines and in macrophages.

It is reported that the macrophages not only destroy the virus infected cells but also damage non infected bystander cells.²⁵ Dengue virus infected macrophages present DENV antigens to B cells in vitro and which lead to their clonal expansion. Cytokines that are produced by the macrophages are tumor necrosis factor (TNF), IL-1, IL-6, IL-10, IL-12, IL-15, IL-18, interferon-alpha (IFN- α), IFN- β , transforming growth factor-beta (TGF β) and chemokines like IL-8, macrophage inflammatory protein (MIP)-1a, MCP-1 etc.²⁶ Several cytokines are specific for DENV and are included in cytotoxic pathway and the suppressor pathways. Ray et al.²⁷ proposed that there is a role of macrophages in the production of free radicals during DENV infection, and there is an alteration in antioxidant status in the patients with acute dengue infection which may restrict virus replication.²⁷

Thus macrophages not only ingest, digest and eliminate dengue virus from the body but also serve as a host cell for replication of DENV and variation in the immune response depends upon various factors that include the virulence of the virus.

Role of Antibodies in DF

Antibodies are pivotal in dengue virus pathogenesis as they have dual role of virus neutralization as well as disease enhancement.

Antibody dependent enhancement (ADE)

The antibodies produced in the primary infection act as non-neutralizing antibodies in secondary infection with a different serotype thus causing ADE of dengue virus infection.²⁸ Fc portion of the immunoglobulin (Ig) is involved in the binding of virus-antibody complexes via Fc- γ receptor. This in turn leads to ADE of viral infection as a result of allowance of viral entry into the cells expressing Fc- γ receptor via the receptor. Since the cells expressing Fc- γ receptor are usually macrophages or DCs, in case of DENV, viral replication is promoted. This ADE can be inhibited by pretreatment with appropriate blocking monoclonal antibodies. In addition to enhancement of infection through Fc- γ receptor linkage, increased infectivity is also observed when the antibodies interact with molecules such as β -2 microglobulin, CD15 or CD33.²⁹

Role of IFN- γ in ADE

Previously sensitized human T lymphocytes are responsible for producing IFN- γ after secondary antigenic stimulation.³⁰ It was demonstrated that DENV infection of dendritic cells and human monocytes is enhanced in the presence of IFN- γ produced by dengue virus stimulation of immune T cells. This led to the hypothesis that IFN- γ might play a major role in the pathogenesis of DHF/DSS through enhanced expression of Fc receptors on human monocytes. This phenomenon leads to high incidence of infectious DENV in the presence of anti-dengue virus antibodies.³¹ ADE can be avoided if a tetravalent vaccine elicits strongly neutralizing antibodies against all four serotypes of DENV.³²

Role of immunoglobulin G (IgG) in DENV infection and cross protective immunity

IgG class of antibodies are believed to be primary antibodies produced against DENV. The function of IgG antibodies in different viral diseases is dependent on their subclass types. In acute phase of viral infection, IgG3 levels increases much more rapidly than that of IgG1.³³ In case of DENV infection the phenomenon of cross protective immunity is also observed. Homotypic IgG antibodies are produced against a particular serotype of DENV which forms a memory pool. Moreover, some heterotypic IgG antibodies are also produced that will provide immunity against other serotypes as well.²⁸

Endothelial cells, plasminogen cross reactivity

Dengue patient sera (Abs) are cross reactive react with endothelial cells. The percentage of endothelial cells reactive with DHF/DSS patient's sera is higher than with the sera of dengue fever patient. Endothelial cell binding activity with neutralizing Abs is found to be inhibited by pre-treatment with dengue virus nonstructural protein 1 (NS1). The cross-reactivity of patient sera with endothelial cells may be due to antibodies produced against NS1 after DENV infection. Endothelial cell apoptosis induced by caspase dependent pathway in the presence of dengue patient sera is inhibited by NS1 pretreatment. Endothelial cell dysfunction is manifested in the case of generation of autoantibodies against them, resulting in pathogenesis of dengue virus infection.³⁴

DENV infection can lead to fibrinolysis as a result of antibodies produced against plasminogen. The cross reactive antibodies produced during DENV infection stimulate the conversion of plasminogen to

plasmin thus leading to hyper fibrinolysis.³⁵ The similarity of Dengue E glycoprotein with plasminogen induces cross-reactivity where antibodies produced against DENV E protein target plasminogen and cause fibrinolysis.³⁶

Vaccine strategies against DENV

There is an increasing demand for a global DENV vaccine which can provide protection against all four serotypes of dengue virus. For a successful vaccine against DENV it is vital that it must have properties of cross protection inducing long lasting immunity and should be cost effective so that it can be available to populations in low income regions of the world. Till now there is no FDA approved DENV vaccine or drug in the market, global presence of DENV and a lack of vaccine is a major threat to the mankind.

History of DNA vaccination

After discovering that DNA can be injected into the host in the form of a vaccine and activates both humoral and cellular arms of immune response, there have been tremendous efforts to precisely understand DNA Vaccination.³⁷ Cytotoxic T Lymphocytes and antibody responses were produced by the influenza DNA vaccine inoculated in mouse, which confirmed that DNA vaccine can successfully activate both cellular and humoral immune responses.³⁸

DNA vaccines against DENV

Dengue virus has 3 structural proteins, capsid (C), membrane associated protein (prM) and envelope protein (E). The largest and immunogenic DENV structural protein is E which has several neutralizing epitopes, that's why it is an important protein to be used in the construction of Dengue Vaccine. Among the nonstructural proteins of dengue virus, nonstructural protein 1 (NS1) is the most antigenic and can be used for vaccine development owing to its expression on the surface of infected cells.^{39,40,41} Previous approaches used for the development of Dengue Vaccine s include live-attenuated vaccines, inactivated vaccines, infection clone derived vaccines and nucleic acid vaccines, but none of them proved to be useful in combating dengue virus infection.^{42,43}

A candidate DNA vaccine expressing DENV-1 PrM and enveloped proteins was developed and used to immunize monkeys.⁴⁴ Virus neutralizing antibodies were produced in the recipient monkeys as a result of candidate vaccine and provide partial protection after challenging the monkeys with dengue virus infection. Both Intradermal and intramuscular route of DNA vaccine administration were employed; it was observed that intradermal immunization produces less immunogenic response than intramuscular immunization in rhesus monkeys. In another trial Konishi et al.⁴⁴ PrM and E genes of Guinea C strain of DENV-2 produced neutralizing antibodies and anamnestic response in immunized mice.⁴⁵

Route of administration of DNA vaccines

Route of administration of a vaccine into the body has a vital role in its immunogenicity. Recent studies of DNA vaccines showed the use of Intramuscular and Intradermal inoculation by needle or a more convenient Biojector® device without involving a needle achieve a limited success in comparison with electroporation.⁴⁶ The question remains though whether electroporation strategy of vaccine delivery is conducive for large public health immunization campaigns. Many resource-poor areas would likely have problems with any vaccine that depends on an expensive technically advanced delivery system.⁴⁷ (Figure 2).

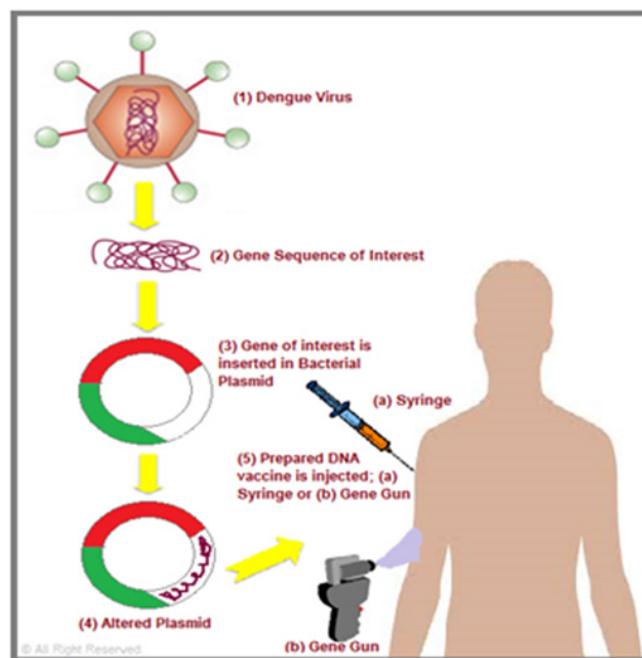


Figure 2 How DNA vaccine are produced and injected.

Different approaches used in DNA vaccines production against DENV

The antibody response is naturally produced against DENV structural (C, E and PrM) and non-structural proteins (NS1, NS3, NS4B and NS5). Mainly E protein has several epitopes which are targeted by anti-dengue antibodies. Recent studies show that PrM gene is also encoded in some DNA vaccines since it is required for proper processing and attachment of E protein.^{48,49} Plasmid encoding E, prM and two NS proteins in DNA vaccines were tested in various animals' models with no significant interference from competing monovalent components.⁴⁶ When smaller animals mainly mice were studied for the neutralizing antibody responses, DNA plasmid constructs containing 80% of the E gene (E80) and 100% of the E gene including the prM gene (ME100) showed best results in mice.⁵⁰

Another research conducted enhanced gene delivery and immunogenicity by formulating DNA vaccine in combination with viral vector. Vaccine had both Adenovirus vector along with multivalent construct as CAdVax-Den12 and CAdVax-Den34 each expressing prM and E proteins. In one of the study a tetravalent vaccine in association with adenovirus vector (CAdVax-DenTV) was tested against DENV1-4 serotypes in rhesus macaques which resulted in production of elevated levels of anti-DENV neutralizing antibodies. One of the drawbacks of this vaccine was the low immune stimulation against DENV2 otherwise the level of neutralizing antibodies was consistent against all other serotypes. In some individuals vaccine was found to be less effective when they were exposed to adenovirus based vaccines and adenoviruses in the past. Previously it was found that HIV vaccine strategy utilizing adenovirus vector had applied higher doses of vaccines but phase II trials results were inconsistent.^{51,52}

Venezuelan equine encephalitis (VEE) virus has also been used to develop a Dengue Vaccine. DENV1 prM and E were expressed in a VEE vector which was able to form virus replicon. Potent neutralizing antibody titer was seen with three doses in immunized macaques however this vaccine failed to provide full protection against live

DENV1. Heterologous priming with two-dose naked DNA, followed by boosting with the VEE replicon dengue particles at the third dose was demonstrated to induce a complete protection in immunized macaques.⁵³ These results demonstrate that a DNA vaccine along with enhancing agent can provide lasting immunity in the vaccinated individual. In a recent study live-attenuated Schwarz vaccine strain of measles viruses has been engineered to encode the domain III and/or M ecto-domain of DENV.⁵⁴ Mice were immunized by a combinational vaccine (DENV-measles) and an enhanced immune response was seen with neutralizing antibodies.⁵⁵ This approach has the advantage of developing a single-dose combination vaccine against measles virus and DENV infection.

PcDNA3 based DNA vaccines using PrM and E gene prM and E expressed together produced extracellular sub-viral particles (EPS) in mammalian cells and were highly immunogenic.^{56,57} In one of such vaccine strategy a pcDNA3-based DNA vaccine containing PrM/E genes of JEV, DENV1 and DENV2 was successful in producing strong neutralizing antibodies response in mice models.^{44,58} This study was able to conclude that a DNA vaccine can induce high levels of DENV neutralization in mice models.⁵⁹ When co-expressed, DENV prM and E can produce a very strong immune response in animal models, highlighting the importance of DNA vaccine in immunization strategy.⁶⁰ One of the studies was aimed at designing tetravalent vaccine encoding domain III of E, this vaccine was able to generate antibody response against all four serotypes in mice models.⁶¹ Currently Konishi et al.⁵⁸ are trying a novel approach in DENV vaccine development by combining a tetravalent DNA vaccine with a protein based vaccine such as DENV2 EPS or an inactivated JE vaccine to increase the vaccine effectiveness.

Konishi et al.⁵⁸ used the ability to induce anamnestic responses to peripheral challenge to evaluate the tetravalent vaccine in a mouse model, as an indicator of the presence of vaccination-induced memory B cells. The production of immune responses to a peripheral amplification of virus is expected in humans who become infected with dengue virus. Studies have shown that an individual exposed to DENV infection gets a lifelong immunity against that specific serotype at both humoral and T cells dependent immunity limiting the chances of future exposure to that DENV serotype.

DNA vaccines based on the envelop protein of DENV2 along with t-PA

Vaccine studies conducted on two constructs which were encoding all three domains (pE1D2) of E protein and domain III (pE2D2) along with plasminogen activator signal peptide (t-PA). Both plasmids were expressed in Baby hamster kidney (BHK) cells. Both epitopes were detected by fluorescent labelled antibodies. This study proved that outer domain of E protein can be secreted in mammalian cells cultures without the involvement of prM protein which acts as a chaperon for proper folding of E protein. When Balb/c mice were inoculated both pE1D2 and pE2D2 DNA vaccines and later challenged with DENV2, all pE1D2-vaccinated mice survived challenge, while 45% of animals immunized with the pE2D2 died after infection. Furthermore, only 10% of pE1D2-immunized mice presented some clinical signs of infection after challenge, whereas most of animals inoculated with the pE2D2 showed effects of the disease with high morbidity degrees. Antibody analysis revealed that mice which were administrated with pE1D2 had several folds high antibody titers than compared with the group injected with pE2D2, these findings suggest that pE1D2 had a better potential as a vaccine candidate.⁶²

Most approaches involving DNA based flavivirus vaccines were designed with both structural proteins i-e PrM and E genes, due to the

known fact that precise folding of E, prM is an important contributor in dengue virus life cycle.⁵² However, these proteins contain highly hydrophobic regions that may interfere with their expression and/or secretion.⁶³ In fact, some studies with DNA vaccines encoding prM/E sequences showed the induction of low titers of neutralizing antibodies and partial protection, even after several DNA doses. For addressing concerns about limited or low neutralizing antibodies for prM/E based DNA vaccines alternative methods were applied.⁴⁶ These strategies included lysosomal based targeted delivery, immune stimulatory and/or cytokine sequences, prime/booster immunization regimen, which improved the immunogenicity.⁶⁴ Studies also revealed that anti-prM antibodies were contributing in enhancement of infection by generating partially neutralizing monoclonal antibodies; these antibodies are also very cross reactive which is one of the factors in the progression of DF to DHF and DSS.⁶⁵ These findings highlight the fact that anti DENV DNA vaccine should specifically target the E protein which is more immunogenic and has no significant role in antibody based enhancement of infection.

Chimeric DNA vaccines

Among several other techniques for formulation of DNA vaccine, one of the techniques is designing of chimeric constructs expressing antigenic epitopes of all four DENV serotypes. Rhesus macaques were inoculated with three constructs (sA, sB, and sC). Constructs sA and sC had both PrM and E genes whereas sB was encoding the ecto-domain of E. It was seen that these DNA chimeric vaccines were able to produce neutralizing antibodies against all four serotypes however When challenged with live dengue-1 or dengue-2 virus, partial protection against dengue-1 was observed. Thus a variable immune response was observed, nevertheless further optimization might fix this problem and a uniform immune response would be possible.⁶⁶

DNA shuffling and screening technologies have previously been used in evolving interferon- α , IL-12, co-stimulatory molecules and viruses.^{67,68} When this strategy was applied for formulating tetravalent DENV vaccine, it showed promising results by the production of strong neutralizing antibody response against all four serotypes. One of the study conducted on mice with intra-cerebral inoculation of DENV2 showed protection owing to chimeric DNA vaccine. Similar results of chimeric DNA vaccines were observed in rhesus macaques.⁶⁹ Chimeric DNA vaccine with booster dose regimen has been tested against several deadly pathogens including DENV. A collaborated effort involving researchers from National medical research Centre (NMRC), Walter Reed Army Institute of Research (WRAIR) and NHP showed protection in mice model vaccinated with chimeric DENV DNA vaccine.⁷⁰

Discussion

The pathogenesis of any infectious disease is the most essential query; however the progress of dengue virus infection is not well understood. It is shown that the macrophages, dendritic cells and other cells of reticuloendothelial source essentially support dengue virus infections.⁷¹ It is also noticed that dendritic cells i.e. Langerhans cells, dermal and interstitial dendritic cells are extra permissive for the entry of dengue virus as compared to the macrophages and monocytes by employing DC-SIGN (Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin) also known as CD209 as a receptor for dengue viruses.⁷² Furthermore vascular endothelial cells and hepatocytes are the sites of DENV infection in DHF patients.⁷³ The infection of B cells and fibroblasts at in vitro level are known but at in-vivo it is still a question mark.⁷⁴ Based on these observations, it might be possible that multiple cell types which include macrophages,

dendritic cells and hepatocytes are the cells that are responsible for enhancing dengue virus infection.⁷⁵

The ideal condition for the prevention of dengue virus infection would be that the invading virus will be ingested, digested and eliminated by macrophages. In the case of DENV, however the macrophages also serve as the host cells for proficient replication of DENV which in turn complicates the immune functions. A successful confrontation to dengue virus infection needs a balance to be maintained between the induction of proficient anti-viral effector mechanisms and the prevention of harmful tissue damage.⁵ Cytokines that are produced by the macrophages also play important role in innate and adaptive immune systems. These cytokines involve

include tumor necrosis factor (TNF), IL-1, IL-6, IL-10, IL-12, IL-15, IL-18, interferon-alpha (IFN- α), IFN- β , transforming growth factor-beta (TGF- β) and chemokines like IL-8, macrophage inflammatory protein (MIP)-1 α , MCP-1 etc. Recent advances in understanding the regulation of macrophages' function in infection by STAT-activating cytokines, their receptors or signaling mechanism signify the value of the Stat-pathway in the control of infection and immunopathology. Through genomics and accessibility of newer technologies it has been now possible to define the molecular pathways of macrophages activation and depression. In the near future the target genes can be identified which recognize the molecular mediators inhibiting the subversion of macrophages by viruses (and intracellular pathogens) and control tissue damage.⁵

Table 2 Dengue vaccine candidates in preclinical development

Vaccine developer	Details	Development stage
Inovio Pharmaceuticals, U.S	Tetravalent chimeric EDIII with inbuilt cleavage sites expressed from plasmid vector.(Ramanathan et al., 2009)	Tetravalent Candidate Vaccine Evaluated in non-Human Primates.
Kobe University, Japan	prM/E expressed from plasmid vector.(Imoto and Konishi, 2007)	Tetravalent Candidate Vaccine Evaluated in Mice.
Centers for Disease Control & Prevention (CDC), U.S	prM/E expressed from plasmid vector.(Mota et al., 2005, Purdy and Chang, 2005)	Tetravalent Candidate Vaccine Evaluated in non-Human Primates.
National Medical Research Council (NMRC), Singapore	Tetravalent "shuffled" prM/E expressed from plasmid vector. (Raviprakash et al., 2006)	Tetravalent Candidate Vaccine Evaluated in non-Human Primates.

Human dendritic cells are responsible for the active replication of dengue virus. After infection with dengue virus, dendritic cells secrete cytokines that possibly lead to cell maturation. These DC's also stimulate the activation of T lymphocytes and start an adaptive immune response. While dengue virus infection might step up the apoptotic progression in cytokine-withdrawn DC but both cell maturation and cytokine production can holdup or block the enduring process of apoptosis. The dengue virus-stimulated dendritic cells express maturation markers such as HLA-DR, B7-1, CD11b, B7-2 and CD83. Additionally they also stimulate secretion of TNF- α and IFN- α . Though dendritic cells undergo spontaneous apoptosis without the presence of feeding cytokines, this procedure appears to be delayed after dengue virus infection.⁷⁶

Interaction of host antibodies with the dengue virus is another important interaction of dengue virus with the host. The majority of dengue-specific antibodies in human sera is inadequately neutralizing and unites to multiple serotypes of dengue virus. Furthermore, these neutralizing antibodies appear to bind novel epitopes which include intricate, quaternary epitopes that are only conserved on the intact virion. Previous studies have established the fact that human and mouse antibodies identify different epitopes on the dengue virion. The leading assumption that projected to clarify the increased risk of brutal disease in secondary cases is antibody dependent enhancement (ADE), which postulates that inadequately neutralizing antibodies from the first exposure bind to the second serotype and augment infection of Fc γ R bearing myeloid cells which includes macrophages and monocytes. By understanding how human antibodies counteract or augment dengue virus infection, it will facilitate in better assessment of the existing vaccines and also in the development of next generation novel vaccines.⁷⁷

Comparison with other DENV vaccine approaches DNA vaccine is best suited for the development of chimeric tetravalent vaccine.⁷⁸⁻⁸⁰ DNA vaccine has shown no when combined with other candidates⁵⁷ (Table 2). In one of the combined immunization approaches DNA vaccines against tick-borne encephalitis viruses showed negligible interference thus DNA vaccines can be used in combination for

achieving maximum results.⁵⁴ DNA vaccines are stable, easy to produce and transported with less cost, and easy to facilitate endemic dengue populations. Putnak et al.⁵⁶ showed that many flavivirus DNA vaccines including vaccines against dengue also have been developed.⁵⁶

Further studies showed the more advantage of DNA vaccine durability, that when gene is induced intramuscularly, it persists to continue gene expression for weeks to months.⁷⁷ In combined immunization, this advantage should overcome the dose quantity.⁸¹ Although DNA vaccine are easy to manufacture and maintain however weak immunogenicity should be addressed. This can be achieved by addition of adjuvants or increasing the expression of immunogenic proteins.⁴⁵ With the development of efficient promotors and constructs and delivery mechanisms DNA vaccine will be an important tool in modern world of medicine.

Acknowledgments

None.

Conflicts of interest

None.

References

1. Rigau-Pérez JG, Clark GG, Gubler DJ, et al. Dengue and dengue hemorrhagic fever. *Lancet*. 1998;352(9):971-977.
2. Vaughn DW, Green S, Kalayanarooj S, et al. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *J Infect Dis*. 2000;181(1):2-9.
3. Agarwal R, Kapoor S, Nagar R, et al. A clinical study of the patients with dengue hemorrhagic fever during the epidemic of 1996 at Lucknow, India. *Southeast Asian J Trop Med Public Health*. 1999;30(4):735-740.
4. HALSTEAD S. Pathophysiology and pathogenesis of dengue hemorrhagic fever. *Monograph on dengue/dengue haemorrhagic fever*. 1993;80:103.

5. Chaturvedi UC, Nagar R, Shrivastava R. Macrophage and dengue virus: friend or foe? *Indian J Med Res.* 2006;124(1):23-40.
6. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature.* 1998;392(6673):245-252.
7. Bender A, Bui LK, Feldman MA, et al. Inactivated influenza virus, when presented on dendritic cells, elicits human CD8+ cytolytic T cell responses. *J Exp Med.* 1995;182(6):1663-1671.
8. Yeh WC, Shahinian A, Speiser D, et al. Early lethality, functional NF- κ B activation, and increased sensitivity to TNF-induced cell death in TRAF2-deficient mice. *Immunity.* 1997;7(5):715-725.
9. Wong BR, Josien R, Lee SY, et al. TRANCE (tumor necrosis factor [TNF]-related activation-induced cytokine), a new TNF family member predominantly expressed in T cells, is a dendritic cell-specific survival factor. *J Exp Med.* 1997;186(12):2075-2080.
10. Avirutman P, Malasit P, Seliger B, et al. Dengue virus infection of human endothelial cells leads to chemokine production, complement activation, and apoptosis. *J Immunol.* 1998;161(11):6338-6346.
11. Green S, Rothman A. Immunopathological mechanisms in dengue and dengue hemorrhagic fever. *Curr Opin Infect Dis.* 2006;19(5):429-436.
12. King CA, Marshall JS, Alshurafa H, et al. Release of vasoactive cytokines by antibody-enhanced dengue virus infection of a human mast cell/basophil line. *J Virol.* 2000;74(15):7146-7150.
13. Green S, Vaughn DW, Kalayanarooj S, et al. Early immune activation in acute dengue illness is related to development of plasma leakage and disease severity. *J Infect Dis.* 1999;179(4):755-762.
14. Conceição TM, El-Bacha T, Villas-Boas CS, et al. Gene expression analysis during dengue virus infection in HepG2 cells reveals virus control of innate immune response. *J Infect.* 2010;60(1):65-75.
15. Talavera D, Castillo AM, Dominguez MC, et al. IL8 release, tight junction and cytoskeleton dynamic reorganization conducive to permeability increase are induced by dengue virus infection of microvascular endothelial monolayers. *J Gen Virol.* 2004;85(Pt 7):1801-1813.
16. Halstead SB. Dengue. *Lancet.* 2007;370:1644-1652.
17. Kurosu T, Chaichana P, Yamate M, et al. Secreted complement regulatory protein clusterin interacts with dengue virus nonstructural protein 1. *Biochem Biophys Res Commun.* 2007;362:1051-1056.
18. Nielsen DG. The relationship of interacting immunological components in dengue pathogenesis. *Virol J.* 2009;6:211-218.
19. Kaufmann SH, Medzhitov R, Gordon S. The innate immune response to infection, ASM Press, USA. 2004.
20. Lozach PY, Burleigh L, Staropoli I, et al. DC-SIGN-mediated enhancement of dengue virus infection is independent of DC-SIGN internalization signals. *Journal of Biological Chemistry.* 2005.
21. Arturo Cabrera-Hernandez, Duncan RS. Mammalian dengue virus receptors. *Dengue Bulletin.* 2005;29:119.
22. Halstead SB, O'Rourke EJ, Allison AC. Dengue viruses and mononuclear phagocytes. II. Identity of blood and tissue leukocytes supporting in vitro infection. *J Exp Med.* 1977;146(1):218-229.
23. Halstead SB, Porterfield JS, O'Rourke EJ. Enhancement of dengue virus infection in monocytes by flavivirus antisera. *Am J Trop Med Hyg.* 1980;29(4):638-642.
24. Moreno-Altamirano MM, Sánchez-García FJ, Muñoz ML. Non Fc receptor-mediated infection of human macrophages by dengue virus serotype 2. *J Gen Virol.* 2002;83(Pt 5):1123-1130.
25. Fujiwara N, Kobayashi K. Macrophages in inflammation. *Curr Drug Targets Inflamm Allergy.* 2005;4(3):281-286.
26. Pacsa AS, Agarwal R, Elbishi EA, et al. Role of interleukin-12 in patients with dengue hemorrhagic fever. *FEMS Immunol Med Microbiol.* 2000;28(2):151-155.
27. Ray G, Kumar V, Kapoor AK, et al. Status of antioxidants and other biochemical abnormalities in children with dengue fever. *J Trop Pediatr.* 1999;45(1):4-7.
28. Alvarez G, Piñeros JG, Tobón A, et al. Efficacy of three chloroquine-primaquine regimens for treatment of *Plasmodium vivax* malaria in Colombia. *Am J Trop Med Hyg.* 2006;75(4):605-609.
29. Mady BJ, Erbe DV, Kurane I, et al. Antibody-dependent enhancement of dengue virus infection mediated by bispecific antibodies against cell surface molecules other than Fc gamma receptors. *The journal of immunology.* 1991;147(9):3139-3144.
30. Ennis FA, Meager A. Immune interferon produced to high levels by antigenic stimulation of human lymphocytes with influenza virus. *J Exp Med.* 1981;154(5):1279-1289.
31. Kontny U, Kurane I, Ennis FA. Gamma interferon augments Fc gamma receptor-mediated dengue virus infection of human monocytic cells. *Journal of virology.* 1988;62(11):3928-3933.
32. Lok SM, Kostyuchenko V, Nybakken GE, et al. Binding of a neutralizing antibody to dengue virus alters the arrangement of surface glycoproteins. *Nat Struct Mol Biol.* 2008;15(3):312-317.
33. Taura Y, Fujiyama S, Kawano S, et al. Clinical evaluation of titration of hepatitis C virus core antibody and its subclasses. *J Gastroenterol Hepatol.* 1995;10(3):270-276.
34. Lin CF, Lei HY, Shiao AL, et al. Antibodies from dengue patient sera cross-react with endothelial cells and induce damage. *J Med Virol.* 2003;69(1):82-90.
35. Chuang YC, Lei HY, Lin YS, et al. Dengue virus-induced autoantibodies bind to plasminogen and enhance its activation. *J Immunol.* 2011;187(12):6483-6490.
36. Markoff LJ, Innis BL, Houghten R, et al. Development of cross-reactive antibodies to plasminogen during the immune response to dengue virus infection. *J Infect Dis.* 1991;164(2):294-301.
37. Johnston S, Sykes K, Barry M. DNA-based vaccines: extending the technology. In: Levine et al. (Eds.), new generation vaccines. 1997;pp.279-286.
38. Kutzler MA, Weiner DB. DNA vaccines: ready for prime time? *Nat Rev Genet.* 2008;9(10):776-788.
39. Gubler DJ. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. *Trends Microbiol.* 2002;10(2):100-103.
40. Gibbons RV, Kalayanarooj S, Jarman RG, et al. Analysis of repeat hospital admissions for dengue to estimate the frequency of third or fourth dengue infections resulting in admissions and dengue hemorrhagic fever, and serotype sequences. *Am J Trop Med Hyg.* 2007;77(5):910-913.
41. Gurunathan S, Klinman DM, Seder RA. DNA vaccines: immunology, application, and optimization*. *Annu Rev Immunol.* 2000;18:927-974.
42. Durbin AP, Whitehead SS. Next-generation dengue vaccines: novel strategies currently under development. *Viruses.* 2011;3(10):1800-1814.
43. Kochel TJ, Raviprakash K, Hayes CG, et al. A dengue virus serotype-1 DNA vaccine induces virus neutralizing antibodies and provides protection from viral challenge in Aotus monkeys. *Vaccine.* 2000;18(27):3166-3173.
44. Konishi E, Yamaoka M, Kurane I, et al. A DNA vaccine expressing dengue type 2 virus premembrane and envelope genes induces neutralizing antibody and memory B cells in mice. *Vaccine.* 2000;18(11-12):1133-1139.
45. Danko JR, Beckett CG, Porter KR. Development of dengue DNA vaccines. *Vaccine.* 2011;29(42):7261-7266.

46. Raviprakash K, Apt D, Brinkman A, et al. A chimeric tetravalent dengue DNA vaccine elicits neutralizing antibody to all four virus serotypes in rhesus macaques. *Virology*. 2006;353(1):166-173.

47. Mukhopadhyay S, Kuhn RJ, Rossmann MG. A structural perspective of the flavivirus life cycle. *Nat Rev Microbiol*. 2005;3(1):13-22.

48. Lorenz IC, Allison SL, Heinz FX, et al. Folding and dimerization of tick-borne encephalitis virus envelope proteins prM and E in the endoplasmic reticulum. *J Virol*. 2002;76(11):5480-5491.

49. Simasathien S, Watanaveeradej V. Dengue vaccine. *J Med Assoc Thai*. 2005;88(Suppl 3):S363-S377.

50. Jon Cohen. Did Merck's failed HIV vaccine cause harm? *Science*. 2007;318(5853):1048-1049.

51. Raviprakash K, Wang D, Ewing D, et al. A tetravalent dengue vaccine based on a complex adenovirus vector provides significant protection in rhesus monkeys against all four serotypes of dengue virus. *J Virol*. 2008;82(14):6927-6934.

52. Chen L, Ewing D, Subramanian H, et al. A heterologous DNA prime-Venezuelan equine encephalitis virus replicon particle boost dengue vaccine regimen affords complete protection from virus challenge in cynomolgus macaques. *J Virol*. 2007;81(21):11634-11639.

53. Brandler S, Lucas-Hourani M, Moris A, et al. Pediatric measles vaccine expressing a dengue antigen induces durable serotype-specific neutralizing antibodies to dengue virus. *PLoS Negl Trop Dis*. 2007;1(3):e96.

54. Zuniga A, Wang Z, Liniger M, et al. Attenuated measles virus as a vaccine vector. *Vaccine*. 2007;25(16):2974-2983.

55. Chang GJ, Davis BS, Hunt AR, et al. Flavivirus DNA vaccines. *Ann N Y Acad. Sci*. 2001;951:272-85.

56. Putnak R, Porter K, Schmaljohn C. DNA vaccines for flaviviruses. *Adv Virus Res*. 2003;61:445-468.

57. Konishi E, Terazawa A, Fujii A. Evidence for antigen production in muscles by dengue and Japanese encephalitis DNA vaccines and a relation to their immunogenicity in mice. *Vaccine*. 2003;21(25-26):3713-3720.

58. Konishi E, Kosugi S, Imoto J. Dengue tetravalent DNA vaccine inducing neutralizing antibody and anamnestic responses to four serotypes in mice. *Vaccine*. 2006;24(12):2200-2207.

59. Azevedo AS, Yamamura AM, Freire MS, et al. DNA vaccines against dengue virus type 2 based on truncate envelope protein or its domain III. *PloS one*. 2011;6(7):e20528.

60. Putnak R, Fuller J, Vander Zanden L, et al. Vaccination of rhesus macaques against dengue-2 virus with a plasmid DNA vaccine encoding the viral pre-membrane and envelope genes. *Am J Trop Med Hyg*. 2003;68(4):469-476.

61. Brett DL, Heinz JT, Charles MR. Flaviviridae: the viruses and their replication. *Fields virology*. 2001;1:991-1041.

62. Raviprakash K, Porter KR, Kochel TJ, et al. Dengue virus type 1 DNA vaccine induces protective immune responses in rhesus macaques. *J Gen Virol*. 2000;81(Pt 7):1659-1667.

63. Dejnirattisai W, Jumnainsong A, Onsirisakul N, et al. Cross-reacting antibodies enhance dengue virus infection in humans. *Science*. 2010;328(5979):745-748.

64. Lazetic S, Leong SR, Chang JC, et al. Chimeric co-stimulatory molecules that selectively act through CD28 or CTLA-4 on human T cells. *J Biol Chem*. 2002;277(41):38660-38668.

65. Steven RL, Jean CCC, Randal O, et al. Optimized expression and specific activity of IL-12 by directed molecular evolution. *Proceedings of the National Academy of Sciences*. 2003;100(3):1163-1168.

66. Apt D, Raviprakash K, Brinkman A, et al. Tetravalent neutralizing antibody response against four dengue serotypes by a single chimeric dengue envelope antigen. *Vaccine*. 2006;24(3):335-344.

67. Simmons M, Burgess T, Lynch J, et al. Protection against dengue virus by non-replicating and live attenuated vaccines used together in a prime boost vaccination strategy. *Virology*. 2010;396(2):280-288.

68. Liu MA. DNA vaccines: a review. *J Intern Med*. 2003;253(4):402-410.

69. Schultz J, Dollenmaier G, Mölling K. Update on antiviral DNA vaccine research (1998-2000). *Intervirology*. 2000;43(4-6):197-217.

70. Schmaljohn C, Vanderzanden L, Bray M, et al. Naked DNA vaccines expressing the prM and E genes of Russian spring summer encephalitis virus and Central European encephalitis virus protect mice from homologous and heterologous challenge. *J Virol*. 1997;71(12):9563-9569.

71. Wolff JA, Lutzke JJ, Acsadi G, et al. Long-term persistence of plasmid DNA and foreign gene expression in mouse muscle. *Hum Mol Genet*. 1992;1(6):363-369.

72. Bozza FA, Cruz OG, Zagne SM, et al. Multiplex cytokine profile from dengue patients: MIP-1beta and IFN-gamma as predictive factors for severity. *BMC Infect Dis*. 2008;8:86.

73. Lin YL, Liu CC, Chuang JI, et al. Involvement of oxidative stress, NF-IL-6, and RANTES expression in dengue-2-virus-infected human liver cells. *Virology*. 2000;276(1):114-126.

74. Mathew A, Rothman AL. Understanding the contribution of cellular immunity to dengue disease pathogenesis. *Immunol Rev*. 2008;225:300-313.

75. Yauch LE, Zellweger RM, Kotturi MF, et al. A protective role for dengue virus-specific CD8+ T cells. *J Immunol*. 2009;182(8):4865-4873.

76. Veiga-Fernandes H, Walter U, Bourgeois C, et al. Response of naive and memory CD8+ T cells to antigen stimulation *in vivo*. *Nat Immunol*. 2000;1(1):47-53.

77. Wahala WM, Silva AM. The human antibody response to dengue virus infection. *Viruses*. 2011;3(12):2374-2395.

78. Raviprakash K, Kochel TJ, Ewing D, et al. Immunogenicity of dengue virus type 1 DNA vaccines expressing truncated and full length envelope protein. *Vaccine*. 2000;18(22):2426-2434.

79. Mota J, Acosta M, Argotte R, et al. Induction of protective antibodies against dengue virus by tetravalent DNA immunization of mice with domain III of the envelope protein. *Vaccine*. 2005;23(26):3469-3476.

80. Purdy DE, Chang GJ. Secretion of noninfectious dengue virus-like particles and identification of amino acids in the stem region involved in intracellular retention of envelope protein. *Virology*. 2005;333(2):239-250.

81. Ramanathan MP, Kuo YC, Selling BH, et al. Development of a novel DNA SynCon™ tetravalent dengue vaccine that elicits immune responses against four serotypes. *Vaccine*. 2009;27(46):6444-6453.