Special Article

Dengue Vaccine

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Dengue is an expanding health problem. About two-fifths of the world population are at risk for acquiring dengue with 50-100 million cases of acute febrile illness yearly including about 500,000 cases of DHF/DSS. No antiviral drugs active against the flavivirus exist. Attempts to control mosquito vector has been largely unsuccessful. Vaccination remains the most hopeful preventive measure. Dengue vaccine has been in development for more than 30 years, yet none has been licensed. The fact that enhancing antibody from previous infection and high level of T cell activation during secondary infection contribute to immunopathology of DHF, the vaccine must be able to induce protective response to four dengue serotypes simultaneously.

Inactivated vaccine is safe but needs a repeated booster thus, development is delayed. Tetravalent live attenuated vaccine and chimeric vaccine using yellow fever or dengue viruses as a backbone are being carried out in human trials. DNA vaccine and subunit vaccine are being carried out in animal trials.

Keywords: *Dengue, DHF, DSS, Vaccine*

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Dengue is a mosquito borne flavivirus infection caused by four dengue virus serotypes (type 1 to 4). It is found in tropical and subtropical regions around the world and has recently emerged as a global health problem. In April 2002 the World Health Organization (WHO) estimated that at least 2.5 billion people - two fifths of the world's population were at risk of contracting dengue and that the number of infections worldwide may reach 50 million cases per year[1].

Dengue viruses

Genus flavivirus of family flaviviridae includes about 70 members, 34 of which are mosquito borne, 17 are tick borne and 22 have no known vector[2]. Forty species of flavivirus have been associated with human illness. Flavivirus carries a positive sense single stranded RNA genome of about 11 kilobases with single open reading frame encoding 3 structural proteins: capsid (C) protein, membrane precursor (prM) protein which is proteolytically cleaved by a cellular protease to form the M protein in mature virions, envelope (E) protein and seven non-structural (NS) protein[3]. The E glycoprotein is the primary target for virus specific neutralizing antibodies[4]. A monoclonal antibody directed against the prM protein has also been shown to protect mice from dengue virus (DENV) challenge[5].

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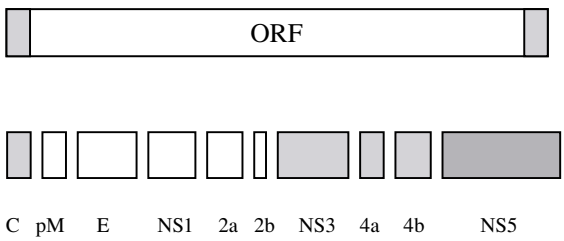


Fig. 1 Flavivirus genome and its protein: Single open reading frame (top) and ten viral proteins (bottom)

Based on hemagglutination-inhibition and cross neutralization tests, flaviviruses are grouped into antigenic complexes, dengue serocomplex comprises four closely related serotypes DENV-1, DENV-2, DENV-3 and DENV-4[2]. Although, there is extensive cross reactivity among theses viruses in serological tests there is no or only short lived[6] cross protection immunity in humans, so a person living in an endemic area can have as many as four infections, one with each serotype during their lifetime. However, some data suggested that sequential exposure to two or more flaviviruses induced rapid onset of very broad anamnestic responses with high titre heterotypic antibodies following the second infection which may be long lasting and protective against subsequent infection with related and unrelated flaviviruses[7,8,9].

Flaviviruses are zoonosis that depend on animal species other than humans for their maintenance in nature with the exception of dengue viruses. Humans are usually incidental and dead end hosts that do not contribute to the natural transmission cycle. Dengue viruses however, have adapted completely to humans and are maintained in large urban areas in the tropics in human - mosquito - human transmission cycles that no longer depend on animal reservoirs[10].

Pathogenesis

Dengue virus is transmitted to humans by Aedes mosquito bites. The incubation period is 4-6 days. Infection may be asymptomatic or may lead to undifferentiated fever (UF), dengue fever (DF) or dengue hemorrhagic fever / dengue shock syndrome (DHF/DSS).[11] Infants and young children usually develop undifferentiated febrile illness that can be accompanied by a maculopapular rash. Older children and adults may develop mild febrile illness or classical dengue fever characterized by acute febrile illness lasting 3-7 days (may be longer or with biphasic curve) accompanied by severe headache , retroorbital pain, muscle joint and bone pain, less often maculopapular rash, leukopenia, thrombocytopenia and mild elevation of hepatic transaminases.[11] They eventually recover without sequelae. Dengue hemorrhagic fever, occurring in no more than 3% of infected individuals[12] is distinguished from DF on clinical ground by plasma leakage, multifactorial hemostatic abnormalities including marked thrombocytopenia and a bleeding diatheses which may lead to shock and / or hemorrhage with a case fatality rate ranging from 1-5%.

Pathogenesis of dengue hemorrhagic fever (DHF) has not been well characterized, multiple factors seem to contribute including strains of viruses[13,14], previous infection with a heterotypic DENV[15] and host factors.

Seroepidemiological studies by Halstead et al in Thailand during the 1960s first suggested an association of increased risk for DHF with a secondary DENV infection[15]; a new DENV infection in an individual who had previously experienced one or more dengue infections. He proposed a model of antibody dependent enhancement whereby upon secondary infection, persisting non neutralizing antibodies from previous infection may opsonize the virus and enhance its uptake and replication in the macrophage[16]. This has been confirmed by a large outbreak of DHF associated with DENV-2 in Cuba[17] as well as cohort studies in Southeast Asia[18,19]. Passive transfer

of antibody against DENV did increase viremia titer in non human primates and a positive correction between peak viremia titer and disease severity in humans has been demonstrated[20]. Once infected by a different serotype, virus-antibody complex lead to complement activation and enhanced infection of monocytic cells. Immune complex formation in vivo has been detected in association with complement activation in patient with severe disease[21]. The targeting of dengue virus infected monocytic cells by T-cells results in release of cytokines, lysis cells and the release of intracellular enzymes and activators leading to subsequent plasma leak and shock. Elevations of circulating levels of activation markers including soluble TNF receptors, soluble IL-2 receptors and soluble CD_8 have been shown to correlate with disease severity[22].

Increased production of cytokines including TNF_∞, IFN_γ, IL-10 and chemokines has also been detected in acute DENV infection[23-25].

Mongkolsapaya J et al reported that a substantial fraction of T cells activated during the secondary infection has poor affinity for the antigenic peptides of this second virus serotype. Instead, T cells have higher affinity to primary dengue virus serotype (original antigenic sin). These inappropriate T cells contribute to immunopathology while incapable to clear virus[26].

Different strains of the same dengue serotype circulating in different parts of the world

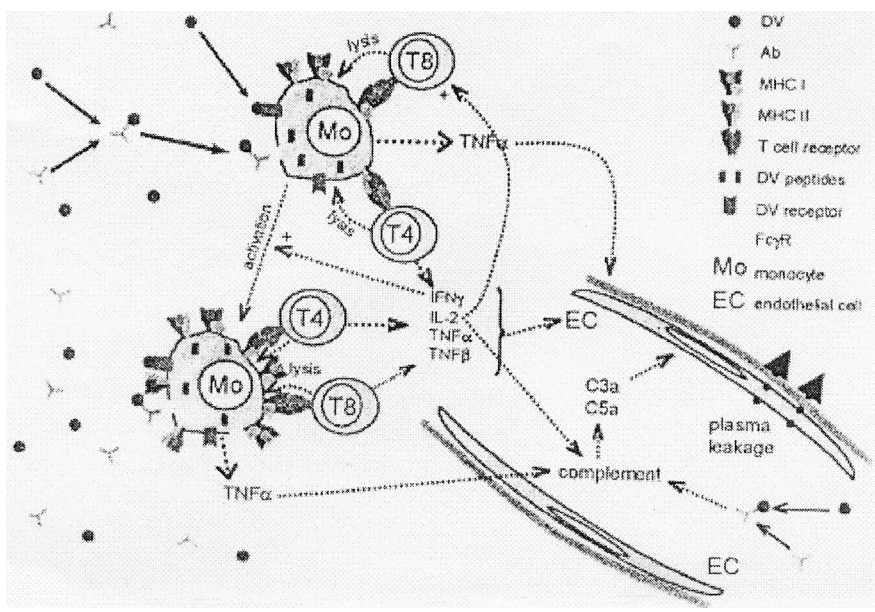


Fig. 2 Model of immunopathogenesis of plasma leakage in dengue hemorrhagic fever

Capillary leak in dengue virus infection Proposed model by which dengue virus (DV) produces a capillary leak syndrome. Monocytes (Mo) are thought to be the primary cellular target for DV. Serotype crossreactive antibodies (Ab), present at the time of second DV infection, bind to virions without neutralization and then enhance the entry of virus into monocytic cells expressing immunoglobulin receptors (FCR), as shown in the left side of the picture. Serotype crossreactive memory T cells, also present at the time of secondary DV infection, recognize viral antigens in the context of class I and II major histocompatibility complex (MHC) molecules. These T cells produce cytokines, such as interferon gamma (IFN) and tumor necrosis factors (TNF) alpha and beta, and lyse DV-infected monocytes. TNF-alpha is also produced in monocytes in response to DV infection and/or interactions with T cells. These cytokines have direct effects on endothelial cells (EC) to induce plasma leakage. Interferon gamma activates monocytes to increase the expression of MHC molecules and immunoglobulin receptors and the product of TNF-alpha. The complement cascade, activated by virus-antibody complexes and by several cytokines, releases the complement anapylatoxins C3a and C5a which further increase capillary permeability. Interleukin-2 may contribute by facilitating T cell proliferation.

Adapted with permission from Rothman A.L. Pathogenesis of dengue virus infection. In: UpToDate, Rose, BD (E2), UpToDate, Wellesley, MA, 2005. Copyright 2005 UpToDate, Inc.

are not equally capable of inducing severe disease. For instance, the South American strains appear to be less virulent compared to the Asian strains[27]. Thus, virus strain-specific factors may play a role. Despite the presence of the American DENV-2 genotypes and some co-circulation of DENV-1 and DENV-3 in America in 1960-1970s, the first major epidemic of DHF / DSS in the Americas occurred only after the introduction of DENV-2 Asian genotype in 1981[17]. Rico Hesse et al have defined genetic elements with the genome of DENV-2 American genotype virus which distinguishes them from members of the Asian genotype[27]. American genotype DENV-2 exhibits decreased infectivity for Aedes mosquitoes in comparison to Asian DENV-2[28].

Changing epidemiology

Epidemics that were clinically compatible with DF occurred as early as 1635 and 1699 in the West Indies and Central America restpectively[10]. Early literature revealed an epidemic of knee fever in Cairo, Egypt and its suburbs described by Al Jabarti in 1979[29], an epidemic in Batavia (Djakarta) in Asia in the same year by David Bylon[30] and an epidemic in Philadelphia USA in 1780 by Benjamin Rush[31]. Since then several outbreaks have been reported from all five continents involving nearly all tropical and subtropical countries.

In Asia Pacific epidemic DF was common in the first 50 years of the 20th century. Epidemic waves move through the region every 10 to 40 years depending on introduction of new viruses and become endemic in many cities[10]. During and after world war II hundreds of thousands of susceptible allied and Japanese soldiers moved into endemic areas of Asia causing epidemics among the troops. Asian cities became hyperendemic[10]. It was thought that hyperendemicity and co-circulation of multiple serotypes resulted in the emergence of epidemic DHF in 1950s. The first outbreak of DHF in Asia was recognized in Manila, Phillippines in the year 1954[32] followed by an outbreak in Bangkok, Thailand and Hanoi, Vietnam in 1958, Singapore, Malaysia and Cambodia between 1960 - 1964[33] and by 1975 it had become a leading cause of hospitalization and death among children in many countries[34]. In the 1980s, DHF began a second expansion into Asia when Sri Lanka, India and Maldive islands had their first DHF epidemics[34]. In the 1990s, DHF continued to show a higher incidence in South - East Asia particulary in Vietnam and Thailand which together accounted for more than two-thirds of the DHF cases reported in Asia[34]. However, an increase in the number of reported cases has been noted in the Phillippines, Laos, Cambodia,Myanmar, Malaysia, India, Singapore and Sri Lanka during the period 1991 - 1995 compared to the preceding 5 year period[34].

In America *Ae. aegypti* control programs targeting urban yellow fever prevention were very effective against DF as well. Epidemic DF declined or disappeared in many regions that had successfully eliminated the mosquito vector[10]. A characteristic DF during the first 70 years of the 20th century was the epidemics of classical DF caused by a single virus serotype commonly involving thousands of cases with self limited transmission and disappearance of virus after several months[10]. Severe hemorrhagic disease and fatalities were rare[10]. In 1970 only DENV-2 was present in the Americas although DENV-3 may have had a focal distribution in Columbia and Puerto Rico[35]. In 1977 DENV-1 was introduced and caused major epidemics throughout the region[35]. DENV-4 was introduced in 1981 and caused similar widespread epidemics[35]. Not until 1981 that Cuba reported the first major outbreak of DHF associated with the Southeast Asian strain of DEN-2 in the Americas, during which a total of 344,203 cases of dengue were notified including 10,312 severe cases

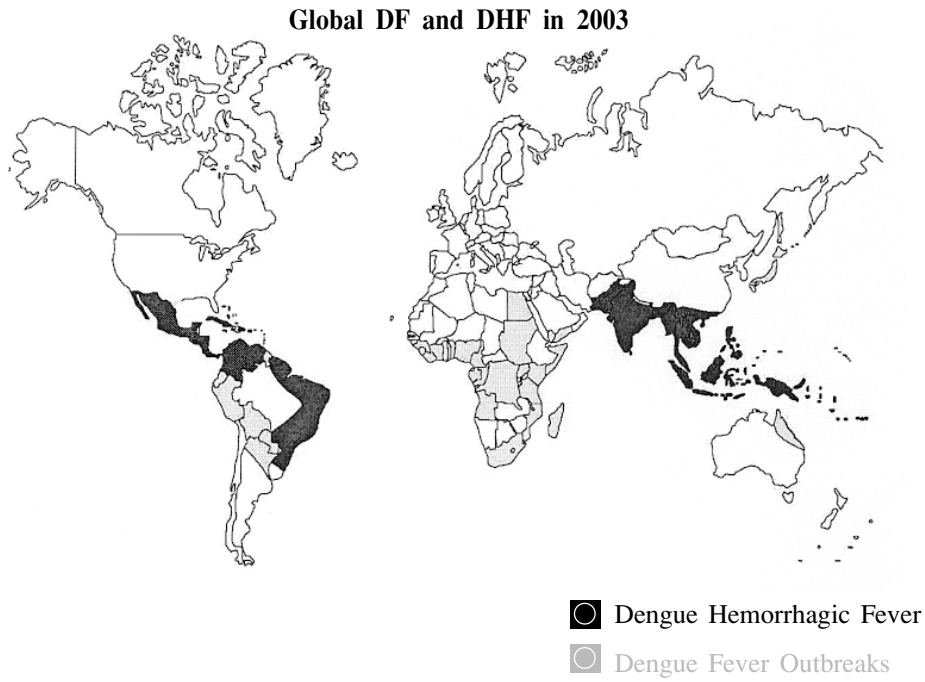


Fig. 3 Dengue Epidemiology

Thomas SJ, Strickman D, Vaughn Dw. Adv Virus Res. 2003; 61: 235-89. Dengue epidemiology: virus epidemiology, ecology, and emergence

and 158 deaths. Between 1981-1996 a total of 42,246 cases of DHF and 582 deaths were reported in 25 countries in the Americas[34]. The main cause of resurgence of DHF was the failure of the hemispheric campaign to eradicate *Ae. aegypti.* Following a successful period that resulted in elimination of the mosquito from 18 countries in 1962, the program began to decline and as a result progressive dissemination of the vector occurred, to the same level as before. Other factors included rapid growth and urbanization of populations and increased travelling of persons. Presently there are all four dengue serotypes circulating in the Americas[34].

In Africa, Dengue viruses had not been isolated on the continent prior to 1960s, epidemics of DF were reported in the early 1900s primarily in South Africa and Senegal[36]. The first DEN viruses isolated in Africa were from humans in Nigeria[37]. Subsequently all four serotypes have been isolated from both East and West Africa[10,36]. Epidemic DHF has not been reported from Africa.

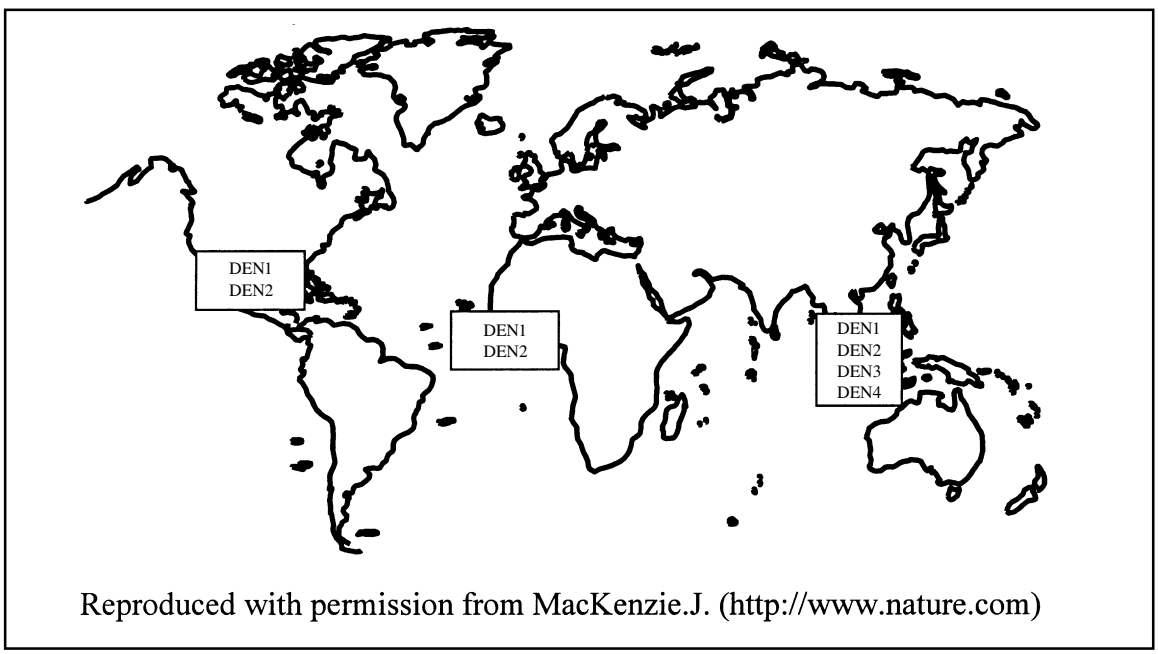
In 2004, DF/DHF was one of the most important epidemic diseases affecting tropical and subtropical countries worldwide with great impact on public health, social and economics of the communities.

There are no antiviral drugs active against the flaviviruses. Attempts to control mosquito vector populations have proven difficult. Vaccination remains the most effective measure.

Dengue vaccines

DENV-1 was first isolated independently during world war II in the Pacific by Japanese and American investigators[6,38]. DENV-2 was also isolated by the latter[6]. DENV-3 and DENV-4 were subsequently isolated in the 1950s during DHF

Global distribution of dengue virus serotypes, 1970



Global distribution of dengue virus serotypes, 2004

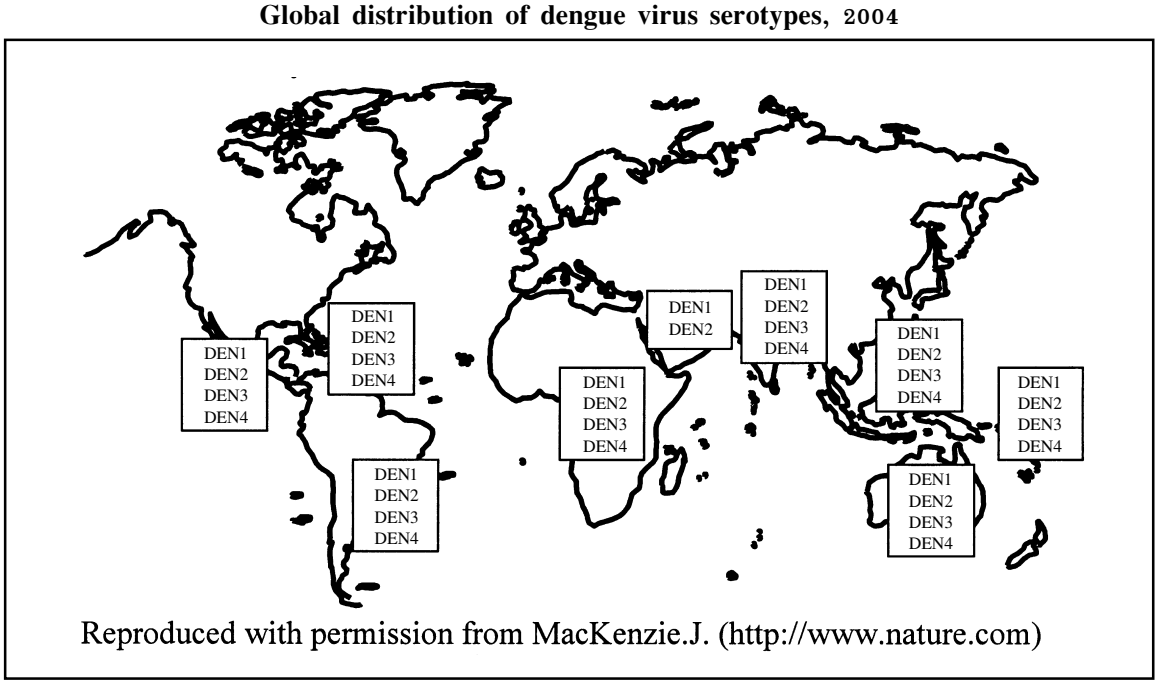


Fig. 4 The change in distribution of dengue serotypes over the past 30 years

epidemics in the Phillippines and Thailand[39]. Since then thousands of viruses have been isolated from the tropics but no new serotype has been documented[10]. Early research on dengue vaccine was supported by the United States Army which was concerned about epidemic dengue during troop deployments in endemic areas.

1. Inactivated vaccine

Wild type dengue viruses grow well in suckling mouse brain and mosquito cell lines but their replication in certified mammalian cell line was low which will not yield large enough quantities of virus as an inactivated antigen. Together with the need for booster immunization, this vaccine did not receive much attention until Putnak et al from WRAIR increased DENV-2 (S 16803 strain) production upto 10^9 plaque-forming units (PFUs) / cell in the certified Vero cell culture[40]. Mice and monkeys vaccinated with this vaccine developed high neutralization antibody titer and were protected from homologous virus challenge[40]. This may be followed by the development of similar inactivated vaccines of other DENV serotypes. Inactivated vaccine is being encouraged by some authors who caution about the possibility of untoward recombination effects of live vaccines[41].

2. Live attenuated vaccine

The first attempts to immunize with attenuated dengue vaccine was undertaken by Sabin using mouse brain passaged virus DENV-1 in 36 human volunteers (4 experiments) producing a rash but few other symptoms[42]. Field trial of this strain in Puerto Rico in 1962 showed partial protection of subjects administered DENV-1 vaccine during an outbreak of predominantly DENV-3[43]. Although considered a useful vaccine, further studies were discontinued when propagation of DENV in cell culture, a safer production substrate, was achieved. Between 1975 and 1985 four vaccine candidates, produced and tested in non human primates were studied in human volunteers. The results showed that DENV-2 was less immunogenic, DENV-4 was over attenuated, DENV-1 and DENV-3 were under attenuated and caused illness, suggesting an alternative approach[44,45,46]. A solution was reached when Halstead, at the University of Hawaii discovered that DENV could be propagated and serially attenuated in primary dog kidney (PDK) cells[47]. Mahidol University, Thailand and the WRAIR, U.S.A. both received one or more seed virus that had been adapted to grow in PDK cells. Over the past 20 years both institutions have developed monovalent, bivalent and then tetravalent DENV vaccines that entered into successful phase I and II trials to the stage of co-development partnership with vaccine manufacturers.

Mahidol candidates DENV-1 (16007) PDK13, DENV-2 (16681) PDK53, DENV-3 (16562) PGMK30 / FRhL-3 and DENV-4(1036) PDK48 were obtained by passages in primary dog kidneys cell, primary African green monkey kidney cells or Simian faetal Rhesus lung cells[48]. Phase I Clinical trial in 10 adult volunteers of Aventis Pasteur tetravalent vaccine composed of 3.47-3.9 $\log_{10}$ PFU/dose of each component, given as a single dose, caused no serious adverse events although it was slightly more reactogenic compared with individual candidates. The most common side effects were headache, malaise, eye pain, maculopapular rash and a brief fever of 38°C. Viremia were detected in all subjects between days 7 and 12 all with DENV-3 and one with also DENV-4 on day 11[48]. Antibody response was predominantly against DENV-3. Seven volunteers had antibody response against more than one serotype. Dengue specific T cell response was induced in all volunteers. The result indicated that there was interference among the four viruses and that DENV-3 was not fully attenuated[48]. Mahidol / Aventis phase Ib study was a dose ranging study in 49 healthy Thai adults aged 19-30 years who received 2 doses of tetravalent vaccine in 7 different formulations given 6 months apart compared with placebo given in 10 subjects[49]. The first dose was more reactogenic. Five subjects were hospitalized for dengue like fever related to vaccination. Most volunteers experienced clinically moderate fever, headache, myalgia or rash after 7-14 days lasting within 3 days. Some decrease in

platelet count, neutrophil count and elevated liver enzymes were observed. DENV-3 viremia was detected in 47 / 49 subjects[49]. Fourteen of 49 subjects had preexisting antibodies against DENV and / or JEV before the first dose. After one dose 58% of dengue vaccine recipients seroconverted against ≥ 3 serotypes and 35 % against all four serotypes. After the second dose seroconversion was 76% and 71% against ≥ 3 and 4 serotypes respectively[49]. The latest trial of Mahidol / Aventis live attenuated dengue vaccine was on safety and immunogenicity of a three dose regimen of two formulations given to 82 healthy flavivirus naive children aged 5-12 years[50]. The duration between dose 1 and 2 was 3-5 months and between dose 2 and 3, 8-12 months with purified vero cell rabies vaccine in 21 children as a control. Children were randomized to receive either formula 3212 which contained 3, 2, 1 and 2 $\log_{10}$ $CCID_{50}$ of DEN-1, DENV-2, DENV-3 and DENV-4 respectively or formula 3313 which contains 3, 3, 1 and 3 $\log_{10}$ $CCID_{50}$ of DEN-1, DENV-2, DENV-3 and DENV-4 respectively. No serious adverse event related to dengue vaccine occurred. Most children experienced mild to moderate fever, rash, headache and myalgia within 12 days of dose 1, lasting 3 days or less[50]. One subject in F 3212 had a one week dengue like fever. Transient mild elevation of liver enzymes and hematologic indices abnormality were noted mainly after dose 1. After the third dose 89 % of subjects in group F3212 seroconverted while all the children in group F3313 seroconverted[50].

Walter Reed Army Institute of Research (WRAIR) took Phase I studies on candidate attenuated dengue vaccine to select safe and immunogenic monovalent vaccines and selected DENV-1 45AZ5 PDK20, DENV - 2 S16803 PDK-50, DENV-3 CH53489 PDK20 and DENV-4 341750 PDK20 for expansion of clinical testing[51]. Phase I trial of 16 formulations of a tetravalent live attenuated dengue vaccine was studied in 64 flavivirus non-immune adult volunteers using combinations of high (10^{5-6} PFU / dose) or low ($10^{3.5-4.5}$ PFU / dose) dosage. Formulations 1-15 were each inoculated into three or four volunteers (total 54) on day 0 and 28. Formulation 16 was tested in 10 volunteers, five inoculated on day 0 and 30 on day 0 and 120 and four on day 0, 20 and 120. The 16 formulations were safe, variably reactogenic after dose 1 and nearly nonreactogenic after dose 2 and 3. Volunteers seroconverted to DENV-1 69%, DENV-2 78%, DENV-3 69%, DENV-4 38%. Seven formulations met the serologic criteria required (raising of neutralizing antibodies in at least 75% of volunteers to at least 3 of four dengue serotypes following one or two inoculations) and three of these were sufficiently attenuated clinically to justify further testing[52]. The study also suggested that longer dosing interval may improve seroconversion rate. Gwinn J et al found that volunteers vaccinated with monovalent or trivalent DENV vaccines developed cell mediated immunity predominantly Th 1[53]. After three more studies in adults, two in flavivirus naive, one in flavivirus immune volunteers it was shown that partial immunity to yellow fever, Japanese encephalitis and dengue viruses increased neutralizing antibody response to a single dose of tetravalent dengue vaccine and that formula 13, 14 and 17 looked promising. A phase I study was performed in 7 children 6-7 years of age using formula 17 live attenuated tetravalent dengue vaccine given in 2 doses 6 months apart. The vaccine was well tolerated with no serious adverse events. One child was eliminated from the protocol due to preexisting antibody to DENV-2 before dose 1, all the remaining subjects showed 100% tetravalent seroconversion. A phase I/II trial of tetravalent live attenuated dengue vaccine in 50 flavivirus antibody naive infants is in progress. The result is pending.

Chimeric live attenuated vaccine

Chimeric vaccine have been constructed by replacing the prM-E gene segment of another flavivirus (back bone) with the corresponding gene segment of the desired virus selected for the vaccine. The backbone segment could come from an attenuated wild type virus or an engineered vaccine candidate.

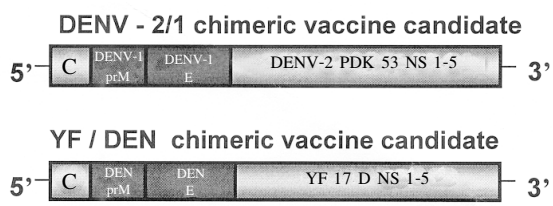


Fig. 5 Chimeric Dengue Vaccine Candidates

U.S. CDC and Mahidol University develop DEN/DEN chimera using DENV-2 live attenuated vaccine candidate 16681, PDK 53 as a back bone for structural prME genes of the other dengue viruses. The vaccine has shown safety and good immunogenicity in cynomolgous monkeys[54].

Acambis developed a tetravalent chimeric DENV vaccine using yellow fever virus (17 D) as a back bone (chimeriVAX-DEN) prepared by electroporation of Vero cells with RNA transcript from viral cDNA. DENV-1 strain was PUO359, DENV-2 strain was PUO218 and DENV-3 strain was PaH881 all isolated in 1980 in Thailand. DENV-4 strain was 1228 isolated in Indonesia in 1978[55]. Preclinical studies demonstrated that the vaccine candidates are replication competent and genetically stable and do not become more neurovirulent upon 20 passages in vero cells. The safety of a tetravalent vaccine compared to that of YF-VAX in a formal monkey neurovirulent test showed a significantly less brain lesions. Immunogenicity and protective efficacy of four different formulations were evaluated in cynomolgus monkeys following a single dose subcutaneous vaccination followed by a virulent virus challenge 6 months later. All monkeys developed low levels of viremia postimmunization, and all the monkeys that had received equal concentrations of either a high - dose (5,5,5,5) or a low-dose (3,3,3,3) formulation seroconverted against all four DEN virus serotypes. Twenty-two (92%) of 24 monkeys were protected as determined by lack of viremia post-challenge[56]. A randomized double blind phase I clinical trial using chimeriVax DENV-2 in both flavivirus naive and YF immune individuals showed high NTAb and 100% seroconversion at 12 months from either dose[57]. The vaccine was well tolerated without serious adverse events. Phase I study of chimeric tetravalent vaccine has been in trial in adults. The result is pending.

DNA vaccine

A vaccine in which one or more genes encoding protective antigen of the pathogen are delivered to the hosts (animals or human) in the form of plasmid DNA containing appropriate flavivirus gene[58].

When given by intramuscular route or into intracutaneous dendritic cells via gene gun DNA is transcribed into nuclei of cells resulting in subsequent protein expression and induction of immunity[58].

The advantage of DNA vaccines include easy manufacturing, stability of DNA no need for cold chain and no concern for adventitious agent that result from propagation in cell culture. The draw back are higher costs, relatively large quantities of DNA required and that plasmid can integrate into chromosome at random loci[58]. The strong promotor used in plasmid theoretically could result in up regulation of some cellular gene e.g. oncogenic protein[58]. Plasmid DNA vaccine encoding prM and E gene, NS_1, NS_3 have been in animal trials

and were reported to be protective[59,60]. There was no direct competition between monovalent components.

Subunit vaccine

Individual immunogens of flaviviruses can be expressed in bacteria, yeast, mosquitoes or mammalian cells and then purified to prepare subunit vaccine. These immunogens can be given by direct inoculation or through vectors such as baculovirus, adenovirus[61]. A recombinant fusion protein containing amino acids 298 - 400 of B domain of DENV-2 E protein fused to the maltose binding protein (MBP) of E.coli was shown to induce anti DENV neutralizing antibody and confirmed protection in mice. Several trials have been in animals[62,63].

Mutant strain vaccine candidates

1. DENmutF

A substitution mutation of DENV-2 in which portions of wild type DENV-2 3'SL nucleotide sequence were replaced by analogous portions of West Niles virus resulted in DEN2mutF which is markedly defective for replication in mosquito cell line (C6/36 cells) but replicated to titer comparable to those of wild type (wt) DENV-2 parent in continuous monkey cell line (LL2 MK2 cells). DEN1 mutF constructed accordingly was evaluated in rhesus monkey model and found to be attenuated and highly immunogenic with NTAb response detectable 17 months after single dose and those monkeys were protected from DENV-1 viremia after challenge[64].

2. rDEN delta[30]

Stem-loop identified as TL2 in secondary structures of 3' UTR was removed by deletion of 30 nucleotides from DENV-4 genome. The resultant virus rDEN4 delta30 was shown to be attenuated in rhesus monkeys compared to parent virus containing TL2 sequence and was highly immunogenic and well tolerated in adult volunteers[65]. Phase 2 clinical trial was of single inoculation of rDEN4delta30 to 3 separate dose (10^3, 10^2, 10^1) cohorts of 20 volunteers per group with 4 placebo control. Serum was collected on day 28 and 42. The vaccine was well tolerated. The most common adverse events were transient asymptomatic rash $\geq$ 50% and mild neutropenia ~20%. Fifty percent developed low grade viremia (0.5 - 0.7 $\log_{10}$). Ninety five percent of vaccinees in each dose cohort developed $\geq$ 4 fold increase in serum NTAb against DENV-4. All 4 serotypes of Dengue viruses have gone through this type of engineering. They can be combined to form tetravalent live attenuated dengue vaccines or be used as a back bone to form tetravalent chimeric vaccines[66].

In conclusion dengue vaccines that are currently being carried out in human trials include live attenuated vaccines and chimeric vaccines.

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เดงกี่วัคซีน

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โรคที่เกิดจากไวรัสเดงกี่ เป็นปัญหาสุขภาพที่กำลังขยายตัวกว้างขึ้นเรื่อย ๆ ปัจจุบันประมาณ 40 เปอร์เซ็นต์ ของประชากรในโลกอาศัยอยู่บนพื้นที่ที่มีความเสี่ยงต่อการติดเชื้อเดงกี่ โดยมีรายงานไข้เดงกี่ ปีละ 50 - 100 ล้านคน รวมทั้งไข้เลือดออกและภาวะช็อกจากไข้เลือดออก ปีละประมาณ 5 แสนคน ยังไม่มียาใดที่จะฆ่าไวรัสในกลุ่มนี้ได้ ความพยายามกำจัดควบคุมยุงที่ผ่านมาไม่ประสบผลสำเร็จ วัคซีนซึ่งเป็นความหวังสูงสุดในการป้องกันโรค ได้มีการค้นคิดทำวัคซีนมานานกว่า 30 ปีแล้ว แต่ก็ยังไม่มีวัคซีนตัวใดสามารถจดทะเบียนใช้ได้ ปัญหาคือ แอนติบอดีที่เกิดจากการติดเชื้อครั้งก่อน ไม่สามารถทำลายไวรัสต่างซีโรทัยป์ในการติดเชื้อครั้งใหม่ แต่กลับส่งเสริมให้เกิดอาการของไข้เลือดออกเช่นกัน การกระตุ้นที-ลิมโฟไซต์อย่างมากมายในการติดเชื้อครั้งใหม่ ก็ไม่สามารถกำจัดไวรัส แต่กลับส่งเสริมให้เกิดอาการรุนแรงของไข้เลือดออก วัคซีนที่จะใช้จึงควรต้องสามารถกระตุ้นให้เกิดการป้องกันการติดเชื้อเดงกี่ไวรัสทั้ง 4 ซีโรทัยป์พร้อม ๆ กัน

วัคซีนป้องกันไวรัสเดงกี่ชนิดตัวตายนั้นปลอดภัย กระตุ้นภูมิคุ้มกันได้ดี แต่ต้องฉีดกระตุ้นหลายครั้ง จึงไม่ได้รับการสนใจทำต่อไป วัคซีนที่ทำให้อ่อนฤทธิ์ลงทั้ง 4 ซีโรทัยป์ และวัคซีนไคเมอริค 4 ซีโรทัยป์ ที่ใช้ไวรัสไข้เหลือง หรือไวรัสเดงกี่ เป็นตัวยืนพื้นนั้นปลอดภัย และกระตุ้นให้เกิดภูมิคุ้มกันดี กำลังได้รับการทดลองในคนอย่างกว้างขวาง วัคซีนดีเอ็นเอและวัคซีนรีคอมบีแนนท์สับยูนิตกำลังทำการทดลองในสัตว์