

THE ROLE OF BIOTECHNOLOGY IN DENGUE VIRAL INFECTION : Effort for The Development of A Dengue Vaccine

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Abstract

Introduction: Dengue fever and Dengue Haemorrhagic Fever (DF/DHF) are caused by dengue viruses (DENV). There are four antigenically related but distinct DENV serotypes (DENV-1 through DENV-4). Humans are the amplifying vertebrate host and *Aedes* mosquitoes are the primary mosquito vector as well as the reservoir of infection. DENV infection causes a spectrum of diseases, ranging from asymptomatic infections to infections complicated by haemorrhagic shock and death (Yoksan, 2008).

Epidemiology: WHO (2008) estimates that about 2.5 billion people or 40% of the world's population live in areas where there is a risk of dengue transmission. About 500,000 cases of dengue's severest form (DHF/DSS) occur annually, resulting in about 24,000 deaths, mostly among children.

Tropical and subtropical areas of South East Asia and Latin America are the hardest hit by dengue infection. Although dengue rarely occurs in the continental United States, it is endemic in Puerto Rico and Hawaii, a U.S. territory. Mosquitoes capable of transmitting the virus have been found in the U.S. (in Florida, Georgia, Louisiana, Alabama, Mississippi, and Texas) over the last 10 years (Cano and Bannister, 2001; CDC, 2009; Science News, 2010). Facing this problem, vaccination is the answer.

Vaccine Development: Dengue is an expanding public health problem, and an effective vaccine remains elusive. Significant influence of sequential infections with different dengue virus serotypes on the severity of disease can be viewed in terms of beneficial and detrimental effects of the heterologous immunity. A more complete understanding of these effects is likely to be critical for predicting optimal vaccine – induced immune responses, with the aim of protecting the global population from emerging infectious disease threats (Rothman, 2004).

In 1980, Mahidol University committed to develop a live-attenuated tetravalent DENV vaccine. They were subjected to general safety test and monkey neurovirulence tests in accordance with the U.S. FDA requirements. (Yoksan 2008)

Results: All vaccine recipients developed either a mild or no adverse reaction to the vaccine, the immunogenicity data were discussed. The current strategy of creating tetravalent DENV vaccine formulation can lead to an unbalanced immune response. This is attributed to viral interference that apparently comes into play when three monovalent vaccine viruses DENV-1, DENV-2, DENV-4 are mixed with DENV-3 to create a tetravalent formulation (Yoksan, 2008).

Summary: More research is needed on a priority basis to work out the viral interference factor in order to make the production of a tetravalent vaccine out of the attenuated DENV-3 candidate vaccine strain a success.

Key words: Dengue Haemorrhagic Fever, Shock and death, tetravalent dengue vaccine, viral interference factor.

History

The origins of the word Dengue are not clear, but one theory is that it is derived from the Swahili phrase 'Ka-dinga pepo', which describes the disease as being caused by an evil spirit. The Swahili word "dinga" may possibly have its origin in the Spanish word "dengue" meaning fastidious or careful, which would describe the gait of a person suffering the bone pain of dengue fever. The first confirmed case report dates from 1789

and is by Benjamin Rush, who coined the term "breakbone fever" because of the symptoms of myalgia and arthralgia. The viral etiology and the transmission by mosquitoes were discovered in the 20th century by Sir John Burton Cleland. Population movements during World War II spread the disease globally. A pandemic of dengue began in Southeast Asia after World War II and has spread around the globe since then (Wikipedia, 2010).

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Etiology

Dengue Fever and Dengue Haemorrhagic Fever (DF/DHF) are caused by dengue viruses (DENV). There are four antigenically related but distinct DENV serotypes (DENV-1 through DENV-4). These DENV are single stranded RNA viruses that belong to the family Flaviviridae and the genus *Flavivirus* – a family which includes other medically important vector borne viruses (e.g. West Nile virus, Yellow Fever virus, Japanese Encephalitis virus, etc.). Dengue viruses are arboviruses (arthropod-borne virus) that are transmitted primarily to humans through the bite of an infected *Aedes* species mosquito (CDC, 2009).

DENV infections cause a spectrum of diseases, ranging from asymptomatic infections to infections complicated by haemorrhagic, shock, and death. Infections with DENV of one serotype results in apparent life-long monotypic immunity against that serotype, but not against any other serotype; thus, separate infections with all four DENV serotypes are theoretically possible in a single host. It should be noted that in Thailand, all of the four DENV serotypes co-circulate, thereby resulting in multiple exposures and potential for re-infection with different serotypes (Yoksan, 2008; CDC, 2009).

Epidemiology

Dengue virus (DENV) is one of the most important global pathogens and may represent a global pandemic. Approximately 2.5 billion people worldwide at risk for dengue infections, or 40% of the world's population. Between 50-100 millions dengue infections are estimated to occur each year, with upwards of 1.5 millions infected individuals presenting with clinical symptoms and about 500,000 cases of dengue's severest form (DHF/DSS) occur annually, resulting in about 24,000 deaths, mostly among children (Tomlinson, 2009, Science News, 2010).

After Malaria, DENV is the most common mosquito borne pathogen that infects humans. Again, population growth, urbanization, increased air travel, and climate change may also be factors in the global reemergence of dengue disease. In the U.S., a Texas girl died from DHF in 1999 and 29 other cases were reported in the state. Between 1977 and 1994 there were 2,248 suspected cases

and 481 confirmed in the U.S. according to the CDC. Carrier mosquitos have been found in Florida, Georgia, Louisiana, Alabama, Mississippi, and Texas (Cano and Bannister, 2001). After decades of absence in the United States, experts say the disease is causing illness again along the Texas – Mexico border, so do in Hawaii, and that widespread dengue infections in the Continental U.S. is a real possibility (Williams, 2009).

The first recognized Dengue epidemics occurred almost simultaneously in Asia, Africa, and North America in the 1780s, shortly after the identification and naming of the disease in 1779. A pandemic began in Southeast Asia in the 1950s, and by 1975 DHF had become a leading cause of death among children in the region. Epidemic dengue has become more common since the 1980s. By the late 1990s, dengue was the most important mosquito-borne disease affecting humans after malaria, with around 40 million cases of dengue fever and several hundred thousand cases of dengue hemorrhagic fever each year (Wikipedia, 2010).

In 2008, for the South East Asia region as a whole, there is about 18% increase in the number of reported cases and about 15% increase in the number of reported dengue deaths as compared to the same period in the previous year. There was substantial increase in the reported cases of dengue in Thailand, Indonesia and Myanmar. The peak months in 2008 of dengue transmission was in Indonesia was February, June in Thailand and July in Myanmar.

The case fatality rate in Thailand is below 0.2%, in Indonesia and Myanmar it is around 1%. However there are some focal outbreaks away from the urban areas that have case fatalities even up to 3 to 5% in India, Indonesia and Myanmar.

Table 1. DHF Cases and Case Fatality Rate in South East Asia Region, 2006-2008
(SEARO, WHO, 2008)

| Country | Cases | | | DHF | | | CFR | | |
|----------------|---------|---------|---------|-------|-------|------|------|------|-------|
| | 2006 | 2007 | 2008 | 2006 | 2007 | 2008 | 2006 | 2007 | 2008* |
| Bangladesh | 2,198 | 466 | 1181 | 11 | 0 | 0 | 0.50 | 0 | 0 |
| Bhutan | 106 | 112 | 37 | 0 | 5 | 3 | 0 | 4.46 | 8.11 |
| DPR Korea | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| India | 12,317 | 5,534 | 11,476 | 184 | 69 | 79 | 1.49 | 1.25 | 0.69 |
| Indonesia | 106,425 | 188,115 | 101,656 | 1,132 | 1,599 | 737 | 1.06 | 1.01 | 0.73 |
| Maldives | 2,836 | 1,570 | 1,476 | 10 | 2 | 3 | 0.35 | 0.13 | 0.20 |
| Myanmar | 11,049 | 15,033 | 14,480 | 130 | 171 | 100 | 1.21 | 1.10 | 0.70 |
| Nepal | 22 | 3 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| Sri Lanka | 11,964 | 7,135 | 5,644 | 44 | 24 | 19 | 0.37 | 0.33 | 0.33 |
| Thailand | 46,829 | 65,581 | 76,059 | 59 | 95 | 91 | 0.13 | 0.14 | 0.12 |
| Timor Leste | 144 | 156 | 108 | 0 | 1 | 1 | 0 | 0.64 | 0.56 |

*Up to September 2008

In Indonesia, DHF is endemic throughout the country. All four serotypes (DENV 1 - 4) are endemic in most of the large cities of the country. In Indonesia dengue is predominantly endemic in urban areas where more than 35% of the country's population lives. In 2007 altogether 188115 cases were reported. In 2007 maximum cases were reported from Jakarta and West Java (each reported more than 25,000 cases). East Java and Central Java reported between 10,000 to 20,000 cases and Bali, East Kalimantan and Lampung, South Sumatra, Yogyakarta, West Sumatra, North Sumatra, north Sulawesi, South Kalimantan, South Sulawesi reported between 1000 to 5000 cases each and the rest of the provinces reported less than 1000 cases.

During 2008^{*} maximum number of the cases (17,604) reported during January 2008. The trend of the diseases is in reducing during the consecutive months. The month wise trend of the year 2007 and 2008 were compared and data shows that there is significant reduction in the morbidity of DHF in during all the months. The case fatality rate in 2006 and 2007 were almost similar and it is slightly higher than 1% and 0.73% in 2008.

Clinical Dengue

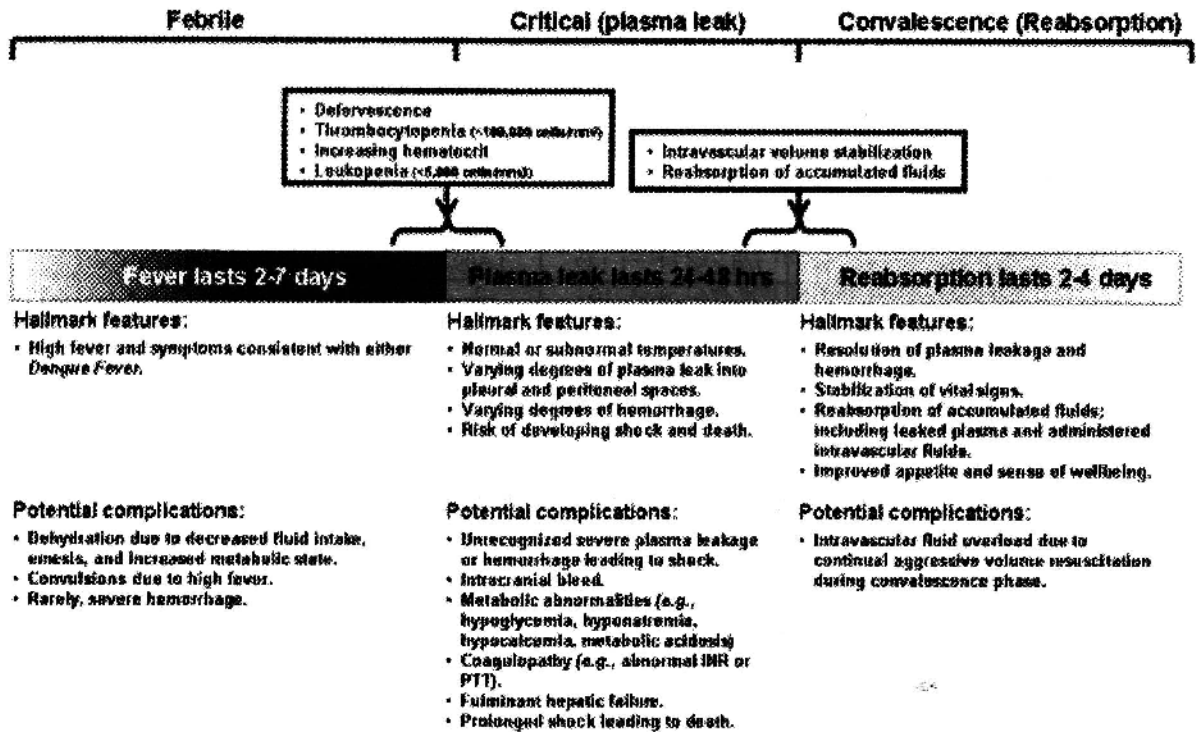
As the early presentations of DF and DHF/ DSS are similar and the course of infection is short, timely identification of persons that will develop severe manifestations can be challenging.

DF follows an uncomfortable but relatively benign self-limited course. DHF may at first appear as a relatively benign infection, but can directly develop into life-threatening illness as fever abates.

There are three predictable pathophysiological phases that DF can usually be distinguished from DHF (CDC, 2009):

- Febrile phase: viremia-driven high fevers
- Critical/plasma leak phase: Sudden onset of varying degrees of plasma leak into the pleural and abdominal cavities
- Convalescence or reabsorption phase: Sudden arrest of plasma leak with concomitant reabsorption of extravasated plasma and fluids

Figure 1. Phases of Infections Resulting in Dengue Haemorrhagic Fever (CDC, 2009)

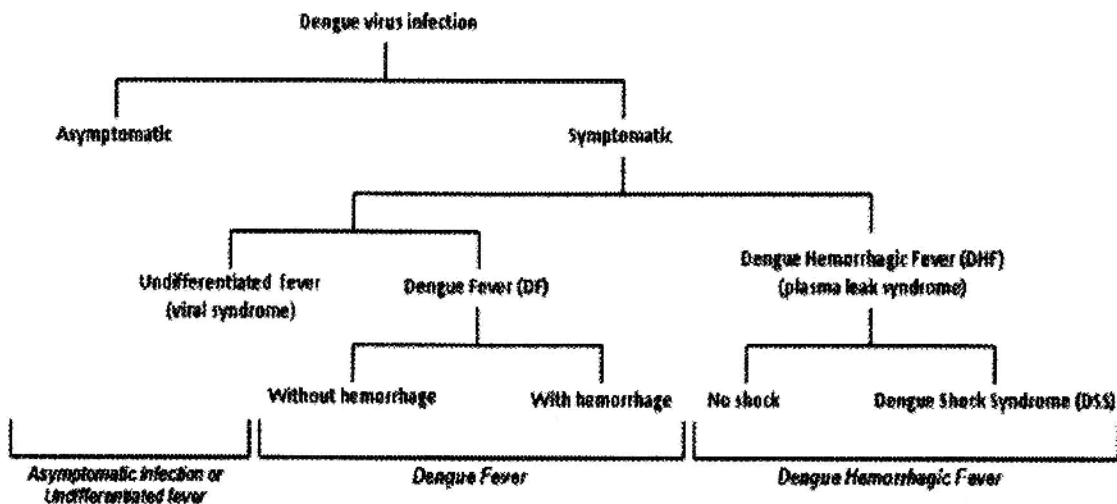


Dengue infected patients are either asymptomatic or they have one of the three clinical presentations:

- Undifferentiated fever

- Dengue Fever with OR without haemorrhagic; OR
- Dengue Haemorrhagic Fever OR Dengue Shock Syndrome

Figure 2. Clinical Manifestation (WHO, Geneva, 1997)



*Adapted from *Dengue Hemorrhagic Fever: Diagnosis, Treatment, Prevention and Control*, 2nd edition. WHO, Geneva, 1997

Immune Responses to DENV

The E glycoprotein is the principal component of the external surface of the DENV virion and is a dominant target of the response consisting of antibodies against DENV. Antibodies against E have been shown to inhibit viral binding to cells and to neutralize viral infectivity *in vitro*. Passive transfer of antibodies against E protected mice from DENV challenge. Antibodies against E show variable degrees of cross-reactivity among the DENV serotypes, although neutralization of infectivity by antibodies is usually more serotype specific than virion binding.

Antibody dependent enhancement of infection (ADE) is a phenomenon where binding of antibody to virus at non-neutralizing epitopes, or at concentration below the neutralization endpoint, can enhance the uptake of virion into monocyte cell lines through interaction with cell surface Ig receptors (Rothman, 2004).

Another more detail explanations is about a second feature of antibodies (Ab), one which is the same across all antibodies: the crystallisable fragment (Fc). The Fc is designed to bind to proteins called the Fc receptors on the surfaces of macrophages, immune cells that roam the bloodstream seeking to engulf and "dissolve" viruses and bacteria. Coated with Fc receptors, macrophages constantly stick to the Fc end of antibodies, which brings whatever the antibody has locked onto into close contact with the cells capable of destroying it. In most people infected with their first dengue serotype, antibodies bind tightly to the viral surface (high avidity) and escort the virus via the Fc/Fc receptor link to macrophages where the virus is destroyed. The immune system then stores away a few of the successful antibodies in case that same virus is ever encountered again. When the system encounters a second dengue serotype, however, the antibodies from the first infection do not attach as securely to the new version in many cases, enabling the virus to break away from its antibody partner and begin copying itself. In this scenario, the antibody's Fc/Fc receptor interaction has served only to deliver the virus into cells that it could not otherwise penetrate. The latter phenomenon, called antibody-dependent enhancement (ADE), has delayed the development of dengue vaccines for decades (Scientistlive, 2008).

It is believed that primary infection with a single serotype of dengue virus elicits short lived cross-protective immunity against other heterologous serotypes, however the duration of the cross-protection has not been explicitly estimated using epidemiological data. Nishiura (2008) in his study elicited the length of cross-protection lasted only 1-2 weeks, far shorter than previously believed.

The ADE phenomenon was first hypothesized in 1978 by Scott Halstead, one of the world's top experts in dengue virus infection. He has been looking for 40 years for a model to be able to test the ADE phenomenon. At last the Shresta group, by using the mouse model, has already made a key and surprising observation that a type of liver cells, called liver sinusoidal endothelial cells (LSECs), but not the previously expected cells types (such as the macrophages and dendritic cells) support ADE of dengue infection (Zellweger et.al. 2010). The kind of model Dr. Shresta has done will be important as researchers work to develop a vaccine.

Diagnosis (Wikipedia, 2010)

The diagnosis of dengue is usually made clinically. The classic picture is high fever with no localizing source of infection, a rash with thrombocytopenia and relative leukopenia - low platelet and white blood cell count. Dengue infection can affect many organs and thus may present unusually as liver dysfunction, renal impairment, meningo-encephalitis or gastroenteritis.

1. Fever, headaches, eye pain, severe dizziness and loss of appetite.
2. Hemorrhagic tendency (positive tourniquet test, spontaneous bruising, bleeding from mucosa, gingiva, injection sites, etc.; vomiting blood, or bloody diarrhea)
3. Thrombocytopenia (<100,000 platelets per mm³ or estimated as less than 3 platelets per high power field)
4. Evidence of plasma leakage (hematocrit more than 20% higher than expected, or drop in hematocrit of 20% or more from baseline following IV fluid, pleural effusion, ascites, hypoproteinemia)

5. Encephalitic occurrences (hepatomegali, WHO, 1977).

Dengue Shock Syndrome is defined as Dengue Hemorrhagic Fever plus:

- * Weak rapid pulse
- * Narrow pulse pressure (less than 20 mm Hg)
- * Cold, clammy skin and restlessness.

Dependable, immediate diagnosis of dengue can be performed in rural areas by the use of Rapid Diagnostic Test kits, which also differentiate between primary and secondary dengue infections. Serology and polymerase chain reaction (PCR) studies are available to confirm the diagnosis of dengue if clinically indicated. Dengue can be a life threatening fever.

Treatment

There are no specific treatments for dengue viral infection. While healthcare improvements over the last several years in many of the affected regions have resulted in a decrease in the mortality rate associated with the disease, many regions still suffer from inadequate healthcare facilities and inability to treat disease symptoms.

The mainstay of treatment is timely supportive therapy to tackle shock due to hemoconcentration and bleeding. Close monitoring of vital signs in the critical period (up to 2 days after defervescence) is critical. Increased oral fluid intake is recommended to prevent dehydration. Supplementation with intravenous fluids may be necessary to prevent dehydration and significant concentration of the blood if the patient is unable to maintain oral intake. A platelet transfusion is may be indicated if the platelet level drops significantly (below 20,000) or if there is significant bleeding. The presence of melena may indicate internal gastrointestinal bleeding requiring platelet and/or red blood cell transfusion.

Aspirin and non-steroidal anti-inflammatory drugs should be avoided as these drugs may worsen the bleeding tendency associated with some of these infections. Patients may receive paracetamol preparations to deal with these symptoms if dengue is suspected (Wikipedia, 2010).

Dengue Vaccine

Dengue is a flaviviral disease that is currently a public health problem of global proportions. Since vector control elicited unsatisfactory result, vaccination seems to be the only hope. Vaccines for other flaviviral diseases have been successfully made. But a dengue vaccine has been elusive despite decades of effort. Several factors such as the existence of four antigenically distinct viruses that cause disease, the immune enhancement phenomenon underlying disease pathogenesis and the lack of a good animal model of the disease have collectively contributed to making the task of developing a dengue vaccine a formidable one. Global awareness of dengue in recent years has kindled renewed interest in developing dengue vaccine (Swaminathan, 2010). Immunomics is the new specific 'omics' science for the study of epitope for production of new vaccines. For dengue, the disease without an effective vaccine, immunomics can be useful, e.g. Mazumder et.al. (2007) reported on computational analysis and identification of amino acid sites in dengue E proteins relevant to development of diagnostics and vaccines (Wiwanitkit, 2008).

History (Okonek and Peters, 2009)

Long before the causes of disease were known and long before the processes of recovery were understood, an interesting thing was observed: if people recovered from a disease, rather than succumbing to it, they appeared to be immune from a second bout with the same illness. Perhaps it was these types of observations that led the Chinese to try to prevent smallpox—a deadly disease characterized by pus-filled blisters—by exposing uninfected individuals to matter from smallpox lesions. This process, known as “variolation,” took a variety of forms. One form consisted of removing pus and fluid from a smallpox lesion and using a needle to place it under the skin of the person to be protected. Another method involved peeling scabs from lesions, drying and grinding them to a powder, and letting an uninfected person inhale this powder. The third method involved picking up a small amount of the scab powder with a needle and then using the needle to place the powder directly into the individual's veins. Lady Mary Wortley

Montagu, wife of the British Ambassador to Turkey, observed this third method in the early 1700s and brought it back to England.

One person who experienced variolation as a child in the late 1700s was Edward Jenner, a young boy who survived the process and grew up to become a country doctor in England. Jenner was interested when a milkmaid told him that she could not catch smallpox because she had had cowpox. Jenner noted that there were many people like the milkmaid - people who milked cows and who did not get smallpox even when exposed repeatedly. With this in mind, Jenner undertook a daring experiment in 1796: he infected a young boy with cowpox in hopes of preventing subsequent smallpox infection. After allowing the boy to recover fully from cowpox, Jenner - in an experiment that would be considered unethical by today's scientific community - intentionally infected the boy with smallpox by injecting pus from a smallpox lesion directly under his skin. As Jenner had predicted, the boy did not contract smallpox.

Jenner's process came to be called "vaccination," after "vacca," the Latin word for cow, and the substance used to vaccinate was called a "vaccine." Now, some 200 years later, we have progressed from a time when vaccination was a rare event, and Jenner's theories about vaccination were not widely accepted, to the late 1900s when vaccines are so commonplace that most children receive multiple vaccinations before they reach their first birthdays.

The smallpox virus is now found only in freezers in high-containment laboratories at the Centers for Disease Control and Prevention (CDC) in Atlanta and the Institute for Viral Preparations in Moscow.

Historical Development (Yoksan, 2008)

Dengue haemorrhagic fever (DHF) was first recognized as a new disease in Manila in 1954. The disease affected mainly children and was characterized by the acute onset of high fever, petechial haemorrhage and shock. The second large outbreak occurred in Manila again in 1956 which resulted in more than 1200 cases, with 10 to 15 per cent case-fatality rate. In 1958, an outbreak of DHF occurred in Bangkok and its nearby areas. Almost 2500 cases with 10 percent case-

fatality rate were recorded. Since then, DHF has become a serious public health problem, causing large-scale morbidity and mortality among children in the South-East Asia and the Western Pacific regions of WHO. Well-established epidemics have also been reported from Myanmar, China, Cambodia, Indonesia, Laos, Malaysia, Philippines, Thailand and Viet Nam. In the WHO South-East Asia Region, DHF is a major public health problem in Indonesia, Myanmar and Thailand.

A meeting of the Research Study Group on DHF was held in New Delhi on 24-25 February 1977. Several measures with potential for the prevention and control of this disease were considered. After detailed discussions, the group made its recommendations, of which the two important ones were: (i) vaccine research; and (ii) control of *Aedes aegypti*.

Rationale (Yoksan, 2008)

The scientific hypothesis behind the development of a tetravalent DENV vaccine against DHF can be summarized as follows:

- (1) Adults developed a higher rate of seroconversion of antibody response against DENV viruses and appeared to be less susceptible to DHF. The naturally-acquired immunity appeared to protect the individuals against the infection. The immunization of target populations could result in the development of a protective antibody response in individuals and could help in protection against the disease.
- (2) It had also been shown that a mono or bitypic antibody response could be a risk factor for DHF if sequential infection by other serotypes of DENV viruses occurred. It was imperative that the DENV vaccine should be able to confer the protective immunity against all four serotypes of DENV infection and provide life-long immunity. This called for the development of a live-attenuated tetravalent DENV vaccine.
- (3) The target population for immunization against DHF should be toddlers 1-3 years old.

Technical Consideration on Dengue Vaccine Development at Mahidol University (Yoksan, 2008)

The objectives of this program were to select strains of DENV-1, -2, -3, -4 which showed promise of being attenuated for human use and produced in cell substrates. All the four DENV virus serotypes being developed in Thailand were passaged serially in cell culture without specific selection. Clinical trials of monovalent dengue vaccines were done in Lamphoon and Loei provinces, an area where there was low prevalence of *Aedes aegypti* mosquitos.

Conclusion of Present Status of PDK-based live-attenuated Dengue Vaccine (Yoksan, 2008)

Peer review of the vaccine development project gave recommendations to the Ministry of Public Health of Thailand and to WHO-SEARO based on their assessment whether the candidate vaccines were suitable for vaccine trials in human beings or not. The peer group summarized the progress as follows:

- (1) Monovalent candidate vaccines
 - (a) DENV-1: A usable candidate vaccine
 - (b) DENV-2: A near-perfect candidate vaccine
 - (c) DENV-3: The most recently developed candidate vaccine, somewhat more reactogenic than the other candidate vaccines. A search for better vaccine should proceed.
 - (d) DENV-4: A very good product
- (2) Bivalent and trivalent combinations using DENV-1 PDK 13, DENV-2 PDK 53 and DENV-4 PDK 48 had undergone phase 1 trials in adults with satisfactory results
- (3) Tetravalent vaccine was acceptably safe. Interference was noticed after mixing of the DENV-3 PGMK-30/F3 in the combination.

Second Generation Recombinant Vaccines (Yoksan, 2008)

The second-generation recombinant vaccines using complementary DNA (cDNA) technology was developed by the Centers for Disease Control and Prevention (CDC), Fort Collins, Colorado, USA. Vaccine development studies were realized at CDC while biological marker testing was partially done in Thailand.

Lessons Learned (Yoksan, 2008)

There was a general consensus that vaccination can be done of the most cost-effective ways to prevent DF and DHF. The aim of this project was to develop a safe and immunogenic vaccine against the four DENV serotypes. Formal phase 1 and phase 2 clinical trials had proven the vaccines to be both safe and immunogenic in humans. Human trials of the tetravalent vaccine were successfully concluded.

Sanofi Pasteur Dengue Vaccine Enters Pediatric Efficacy Clinical Study (Sanofi Pasteur, 2009)

In February 2009, Sanofi Pasteur started investigational tetravalent dengue vaccine in children, in collaboration with Mahidol University of Thailand, the Ministry of Public Health, and the Pediatric Dengue Vaccine Initiative (PDVI). The pediatric clinical study in Thailand was aimed to assess the efficacy of the vaccine in protecting children against dengue. As Dr. Pratap Singhasivanon put it, such large scale pediatric studies are instrumental for the development of a safe and efficacious dengue vaccine to protect against a disease that primarily affects children. Dr. Joachim Hombach, the Coordinator Implementation Research for IVR in WHO, provided support by stating that WHO is committed to the availability of a dengue vaccine that will ultimately benefit children in endemic countries. This research program includes ongoing clinical studies in Mexico, Peru, and The Philippines with adults and children.

Cost-Effectiveness of a Pediatric Dengue Vaccine (Shepard, 2004)

The gross cost per 1000 population of all ages of the vaccination program would be US\$154. Due to projected savings in dengue treatment, the net cost per capita would be only U\$17 (89% below the gross cost). The cost per disability adjusted life year (DALY) saved by a pediatric vaccine would be US\$50, making the potential vaccine highly cost-effective. Eventually, vaccination may be able to replace environmental control as a strategy for dengue prevention and be cost saving.

Research

Wolbachia

In a study at the School of Integrative Biology at the University of Queensland, superfine needles were used to inject 10,000 mosquito embryos with Wolbachia bacterium. Once an insect was infected, the bacterium would spread via its eggs to the next generation (McMeniman, et.al., 2009). It takes risk for immediate spreading of dengue virus in the population.

Antiviral Approaches

Polymerase inhibitors are reportedly under active investigation by several pharmaceutical companies (Tomlinson, 2009). The NS3 protease is a primary target for development of dengue antiviral drugs since the NS2-NS3B is required for virus replication. Selective inhibition of *in vitro* DENV-2 multiplication was achieved with diverse entry inhibitors such as polysulfates, polyoxotungstates, lectins, peptides, and tetracycline derivatives (Acosta, 2008).

Sterile Insect Technique (Wikipedia, 2010)

The sterile insect technique, a form of biological control, has long proved difficult with mosquitos because of the fragility of the males. However, a transgenic strain of *Aedes aegypti* was announced in 2010 which might alleviate this problem: the strain produces females that are flightless due to a mis-development of their wings, and so can neither mate nor bite. The genetic defect only causes effects in females, so that males can act as silent carriers.

New Test (Williams, 2009)

To construct the new test of cross-neutralization, researchers took CV-1 fibroblast cells, which share some traits with macrophages, and genetically engineered them to include a gene that directs for the building of an FC receptor on their surfaces. The new test captured for the first time the contribution of antibodies to more severe disease via fc/fc receptor delivery of virus to target cells. Hopefully, this new test will be adopted widely and soon because it is more likely to catch enhancement.

Use as Biological Weapon (Wikipedia, 2010)

Dengue fever was one of more than a dozen agents that the United States researched as potential biological weapons before the nation suspended its biological weapons program.

Summary

1. The way forward for prevention and control of dengue is by implementing the following six elements: dengue surveillance; case management; outbreak response; integrated vector management; social mobilization and communication for dengue; and dengue research.
2. Plasma leakage and haemorrhagic complication, if managed successfully, often recovered fully without sequelae.
3. Although dengue virus is considered one of the most significant mosquito-borne pathogens that threaten global health, there are no approved antiviral drugs to combat dengue infection.
4. Much additional research is needed to identify and characterize protective and pathological immune responses in order to make an effective DENV vaccine a reality.
5. Surprising observation by the Shresta group showed that a type of liver cells, called liver sinusoidal endothelial cells (LSCs), support the ADE phenomenon of dengue infection.
6. A vaccine has been developed to prevent dengue fever, but it still under trial. It is not yet available in the market. Scientific progress

is likely to help in prevention of dengue fever by vaccination in the years to come. More specifically, together with those for Hepatitis E and Malaria, the New York Academy of Sciences hoped that Dengue vaccines will be available in 2012.

7. In the end, the world was unprepared for the outbreak which has added to the many problem super-bugs we are not dealing from dengue, avian and swine flu among some of the most recent.

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