NEW TREATMENT STRATEGIES FOR DENGUE AND OTHER FLAVIVIRAL DISEASES
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NEW TREATMENT STRATEGIES FOR DENGUE AND OTHER FLAVIVIRAL DISEASES
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diseases, held at the Novartis Institute for Tropical Diseases in Singapore, 26–27
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There are three strategies that we can consider if we are thinking about controlling flavivirus infections. The one that has worked well in the past has been mosquito control. Then vaccination was used, with vaccines such as those against the yellow fever 17D strain. This is still an effective means of prevention when it is actually implemented. The third strategy, which is the main focus of this meeting, is therapy, although we shouldn’t neglect these other aspects of flavivirus control.

The following lists some of the issues that I think we should consider during our discussions. What is the scientific feasibility of a particular kind of approach for dealing with dengue fever, and is it likely to be effective? How much is it going to cost? Ideally, we don’t want therapies to be so expensive that they will be restricted to developed countries. Related to this, what is the target population going to be for a particular kind of therapy or prevention?

With respect to therapies, what about antibodies? We are not going to be talking too much about these, but they have been proven to work. What about small molecules? There are state-of-the-art technologies that can be employed to elucidate target structures, develop biochemical assays, screen for small molecules and find those with good pharmacological properties. If we can develop these kinds of therapies, how are they going to be used? Are they more applicable in a prophylactic setting, or in a post-exposure context? One of the key issues for therapeutics is the diagnosis of flavivirus infections. In order to implement post-exposure therapeutics, we will need rapid diagnosis. Diagnostic methods will probably have to be low-tech and low cost.

Another important issue in therapeutics is the nature of the best small molecule targets: we will be touching on a number of the flavivirus proteins involved in host cell entry. Are viral targets best, or should we also go after host targets? The latter might be better in providing a broad protection against different flaviviruses. Should we be considering targets that are highly specific at all, where resistance might be an issue?

In terms of vaccines, what are the various strategies that can be considered? In the case of dengue, what is the feasibility of the ‘holy grail’—a tetravalent vaccine that provides long-lasting protective immunity? We would like to highlight some
of the vaccine progress on ‘simpler’ flavivirus diseases such as Japanese encephalitis. Again, what is the cost of vaccination? This will be important for implementation in developing countries. One of the things we run into in the USA and other countries is the complexity of liability issues associated with vaccines. We are now also seeing this also in small molecule therapeutics.

Another area we will discuss is that of hi-tech approaches such as microarrays and proteomics. What can these kinds of techniques tell us about flavivirus biology? They can be applied to state-of-the-art epidemiology and evolution studies, and they can be used to study the effects of virus infection at the organismal and cellular levels. One thing that we would like to see come from such studies is a better understanding of the pathogenic mechanisms of the more severe sequelae of dengue infection. Might this provide us with new therapeutic insights in a post-exposure setting? Much of the pathology associated with these diseases is after the peak of viraemia.

I have been working on hepatitis C in recent years, and in terms of preclinical models it is clear that the flavivirus field has a tremendous advantage. We have biochemical assays for a number of the virus-specific enzymes, and viruses have never been difficult to coax to replicate in cell culture, so you can actually do cell-based evaluation of potential antivirals.

Finally, another issue which I would like to invoke some discussion on is where we stand in terms of the animal models. We have models that can be used to support flavivirus infection, but these don’t necessarily recapitulate the pathology in human disease. How important is it to try to develop models that do that? These are some of the issues that I hope we can address in our time together here. Let’s begin!
Dengue/dengue haemorrhagic fever: history and current status

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Abstract. Dengue fever (DF) is an old disease; the first record of a clinically compatible disease being recorded in a Chinese medical encyclopaedia in 992. As the global shipping industry expanded in the 18th and 19th centuries, port cities grew and became more urbanized, creating ideal conditions for the principal mosquito vector, Aedes aegypti. Both the mosquitoes and the viruses were thus spread to new geographic areas causing major epidemics. Because dispersal was by sailing ship, however, there were long intervals (10–40 years) between epidemics. In the aftermath of World War II, rapid urbanization in Southeast Asia led to increased transmission and hyperendemicity. The first major epidemics of the severe and fatal form of disease, dengue haemorrhagic fever (DHF), occurred in Southeast Asia as a direct result of this changing ecology. In the last 25 years of the 20th century, a dramatic global geographic expansion of epidemic DF/DHF occurred, facilitated by unplanned urbanization in tropical developing countries, modern transportation, lack of effective mosquito control and globalization. As we go into the 21st century, epidemic DF/DHF is one of the most important infectious diseases affecting tropical urban areas. Each year there are an estimated 50–100 million dengue infections, 500 000 cases of DHF that must be hospitalized and 20 000–25 000 deaths, mainly in children. Epidemic DF/DHF has an economic impact on the community of the same order of magnitude as malaria and other important infectious diseases. There are currently no vaccines nor antiviral drugs available for dengue viruses; the only effective way to prevent epidemic DF/DHF is to control the mosquito vector, Aedes aegypti.


History

The first reports of major epidemics of an illness thought to possibly be dengue occurred on three continents (Asia, Africa and North America) in 1779 and 1780 (Rush 1789, Hirsch 1883, Pepper 1941, Howe 1977). However, reports of illnesses compatible with dengue fever occurred even earlier. The earliest record found to date was in a Chinese ‘encyclopaedia of disease symptoms and remedies,’ first published during the Chin Dynasty (AD 265 to 420) and formally edited in AD 610.
(Tang Dynasty) and again in 992 during the Northern Sung Dynasty (Nobuchi 1979). Outbreaks of illness in the West Indies in 1635 and in Panama in 1699 could also have been dengue (Howe 1977, McSherry 1982). Thus, a dengue-like illness had a wide geographic distribution before the 18th century, when major epidemics of dengue-like illness occurred widely. It is uncertain that the epidemics in Batavia (Jakarta), Indonesia, and Cairo, Egypt, in 1779 were actually dengue (Carey 1971).

At some point in the past, probably with the clearing of the forests and development of human settlements, dengue viruses moved out of the jungle and into a rural environment, where they were, and still are, transmitted to humans by peridomestic mosquitoes such as *Aedes albopictus*. Migration of people and commerce ultimately moved the viruses into the villages, towns, and cities of tropical Asia, where the viruses were most likely transmitted sporadically by *Aedes albopictus* and other closely related peridomestic *Stegomyia* mosquito species.

The slave trade between West Africa and the Americas, and the resulting commerce, were responsible for the introduction and the widespread geographic distribution of an African mosquito, *Aedes aegypti*, in the New World during the 17th, 18th and 19th centuries. This species became highly adapted to humans and urban environments and was spread throughout the tropics of the world by sailing ships. The species first infested port cities and then moved inland as urbanization expanded. Because *Ae. aegypti* had evolved to become intimately associated with humans, preferring to feed on them and to share their dwellings, this species became a very efficient epidemic vector of dengue and yellow fever viruses (Gubler 1997). Therefore, when these viruses were introduced into port cities infested with *Ae. aegypti*, epidemics occurred. It was in this setting that major epidemics of dengue fever occurred during the 18th, 19th and early 20th centuries, as the global shipping industry developed and port cities were urbanized in response to increased commerce and ocean traffic. The last major dengue pandemic began during World War II and continues through the present (Gubler 1997, Halstead 1992).

The earliest known use of the word *dengue* to describe an illness was in Spain in 1801 (Soler 1949). However, the most likely origin of the word is from Swahili (Christie et al 1872, Christie 1881). In both the 1823 and 1870 epidemics of dengue-like illness in Zanzibar and the East African coast, the disease was called *Ki-Dinga pepo*. From this came the name *dinga* or *denga*, which was used to describe the illness in both epidemics. Christie (Christie et al 1872, Christie 1881) speculates that the name denga was taken via the slave trade to the New World, where it was called ‘Dandy fever’ or ‘The Dandy’ in the St. Thomas epidemic of 1827. The illness was first called *dunga* in Cuba during the 1828 epidemic, but later changed to dengue, the name by which it has been known ever since (Munoz 1828). Most likely, the Spanish recognized the disease in Cuba as the same one that was called dengue in Spain in 1801. If the word *dengue* did originate in East Africa from *dinga* or *denga*,
this suggests the disease was occurring before the 1823 epidemics described by Christie. This is not unlikely since epidemics were reported in Africa, the Middle East and Spain in the late 1700s.

With documentation that yellow fever was transmitted by mosquitoes, many early workers suspected that dengue fever was also mosquito-borne. In the previrology era, work was slow and relied on use of human volunteers. Work done by Graham (1903), Bancroft (1906) and Cleland et al (1918) documented dengue transmission by mosquitoes.

Although it had been shown that dengue fever was caused by a filterable agent, (Ashburn et al 2004, Siler et al 1926) the first dengue viruses were not isolated until the 1940s, during World War II (Kimura & Hotta 1944, Hotta 1952, Sabin & Schlesinger 1945, Sabin 1952). Dengue fever was a major cause of morbidity among Allied and Japanese soldiers in the Pacific and Asian theatres. Sabin and his group were able to show that some virus strains from three geographic locations (Hawaii, New Guinea and India) were antigenically similar (Sabin & Schlesinger, Sabin 1952). This virus was called dengue 1 (DENV-1), and the Hawaii virus was designated as the prototype strain (Haw-DENV-1). Another antigenically distinct virus strain isolated from New Guinea was called dengue 2 (DENV-2), and the New Guinea C strain (NGC-DENV-2) was designated the prototype. The Japanese virus isolated by Kimura and Hotta (Kimura & Hotta 1944, Hotta 1952) was subsequently shown to be DENV-1 as well. Two more serotypes, dengue 3 (DENV-3) and dengue 4 (DENV-4), were later isolated from patients with a haemorrhagic disease during an epidemic in Manila, in 1956 (Hammon et al 1960). Since these original isolates were made, thousands of dengue viruses have been isolated from all parts of the tropics; all have fit into the four-serotype classification.

The occurrence of severe and fatal haemorrhagic disease associated with dengue infections is not unique to the twentieth century. Patients with disease clinically compatible with dengue haemorrhagic fever (DHF) have been reported sporadically since 1780, when such cases were observed in the Philadelphia epidemic (Rush 1789). Significant numbers of cases of haemorrhagic disease were associated with several subsequent epidemics, including Charters Towers, Australia, in 1897, Beirut in 1910, Taiwan in 1916, Greece in 1928 and Taiwan in 1931 (Copanaris 1928, Akashi 1932, Halstead & Papaevangelou 1980, Rosen 1986, Hare 1898, Koizumi et al 1916). However, epidemic occurrences such as these were relatively rare, and the long intervals between them made each a unique event that was not considered important in terms of a long-term, continuous public health problem. Understanding the emergence of dengue and DHF as a global public health problem in the last half of the 20th century requires a review of the ecological and demographic changes that occurred in the Asian and American tropics during this period. The detailed history of dengue has been recently reviewed (Gubler 1997).
Natural history

There are four dengue virus serotypes: DENV-1, DENV-2, DENV-3 and DENV-4. They belong to the genus *Flavivirus*, family *Flaviviridae* (of which yellow fever is the type species), which contains approximately 56 viruses (ICTV 2005).

Humans are infected with dengue viruses by the bite of an infective *Ae. aegypti* mosquito (Gubler 1988). *Ae. aegypti* is a small, black-and-white, highly domesticated urban mosquito that prefers to lay its eggs in artificial containers commonly found in and around homes in the tropics, for example, flower vases, old automobile tires, buckets that collect rainwater, and trash in general. Containers used for water storage, especially 55-gallon drums and cement cisterns, are especially important in producing large numbers of adult mosquitoes in close proximity to dwellings where people live and work. The adult mosquitoes prefer to rest indoors, are unobtrusive, and prefer to feed on humans during daylight hours. The female mosquitoes are very nervous feeders, disrupting the feeding process at the slightest movement, only to return to the same or a different person to continue feeding moments later. Because of this behaviour, *Ae. aegypti* females will often feed on several persons during a single blood meal and, if infective, may transmit dengue virus to multiple persons in a short period of time even if they only probe without taking blood (Gubler & Rosen 1976). It is not uncommon to see several members of the same household become ill with dengue fever within a 24 to 36 h time frame, suggesting transmission by a single infective mosquito (D. J. Gubler, unpublished data). It is this behaviour that makes *Ae. aegypti* such an efficient epidemic vector. Inhabitants of dwellings in the tropics are rarely aware of the presence of this mosquito, making its control difficult.

After a person is bitten by an infective mosquito, the virus undergoes an incubation period of 3–14 days (average, 4–7 days), after which the person may experience acute onset of fever accompanied by a variety of non-specific signs and symptoms. During this acute febrile period, which may be as short as 2 days and as long as 10, dengue viruses may circulate in the peripheral blood. If other *Ae. aegypti* mosquitoes bite the ill person during this febrile viraemic stage, those mosquitoes may become infected and subsequently transmit the virus to other uninfected persons, after an extrinsic incubation period of 8 to 12 days (Gubler 1988).

Changing disease patterns

The disease pattern associated with dengue, which was characterized by relatively infrequent epidemics until the 1940s, changed with the ecological disruption in Southeast Asia during and after World War II. The economic development and

Epidemiological changes in the Americas have been the most dramatic. In the 1960s and most of the 1970s, epidemic dengue was rare in the American region because the principal mosquito vector, *Ae. aegypti*, had been eradicated from most of Central and South America (Gubler 1987, 1989, 1997, 1993, Pinheiro 1989). The eradication program was discontinued in the early 1970s, and this species then began to reinvade those countries from which it had been eradicated. By the 1990s, *Ae. aegypti* had regained the geographic distribution it had before eradication was initiated (Fig. 1).


While Africa has not yet had a major epidemic of DHF, sporadic cases of severe disease have occurred as epidemic DF has increased markedly in the past 25 years. Before the 1980s, little was known of the distribution of dengue viruses in Africa. Since then, however, major epidemics caused by all four serotypes have occurred in both East and West Africa (Gubler 1997, 2002). In 2006, dengue viruses and

FIG. 2. Expanding geographic distribution in the Americas from 1981 to 2006.
Aedes aegypti mosquitoes have a worldwide distribution in the tropics with over 2.5 billion people living in dengue-endemic areas (Fig. 3) (Gubler 1997, 2002).

Currently, DF causes more illness and death than any other arboviral disease of humans. The number of cases of DEN/DHF reported to WHO has increased dramatically in the past two decades (Fig. 4).

![Map showing global distribution of Aedes aegypti mosquitoes and recent epidemic dengue.](image)

**FIG. 3.** Global distribution of *Aedes aegypti* mosquitoes and recent epidemic dengue.

![Bar graph showing global average dengue fever/dengue haemorrhagic fever cases reported to WHO annually, by decade.](image)

**FIG. 4.** Global average dengue fever/dengue haemorrhagic fever cases reported to WHO annually, by decade.
Each year, an estimated 50–100 million dengue infections and several hundred thousand cases of DHF occur, depending on epidemic activity (Gubler & Clark 1995, Gubler 2002, Monath 1994, World Health Organization 2000). DHF is a leading cause of hospitalization and death among children in many Southeast Asian countries (World Health Organization 1997).

**Factors responsible for increased incidence**

The emergence of epidemic dengue and DHF as a global public health problem in the past 25 years is closely associated with demographic and societal changes that have occurred over the past 50 years (Gubler & Trent 1994, Gubler & Clark 1995, Gubler 2002). A major factor has been the unprecedented population growth which has been the primary driving force for unplanned and uncontrolled urbanization, especially in tropical developing countries. The substandard housing and the deterioration in water, sewer and waste management systems associated with unplanned urbanization have created ideal conditions for increased transmission of mosquito-borne diseases in tropical urban centres.

A second major factor has been the lack of effective mosquito control in dengue-endemic areas (Gubler & Trent 1994, Gubler & Clark 1995, Gubler 1989, 2002). Emphasis during the past 25 years has been on space spraying with insecticides to kill adult mosquitoes; this has not been effective (Gubler 1989, Newton & Rieter 1992) and, in fact, has been detrimental to prevention and control efforts by giving citizens of the community and government officials a false sense of security (Gubler 1989). Additionally, the geographic distribution and population densities of *Ae. aegypti* have increased, especially in urban areas of the tropics, because of increased numbers of mosquito larval habitats in the domestic environment. The latter include non-biodegradable plastics and used automobile tires, both of which have increased dramatically during this same period of time.

Another major factor in the global emergence of dengue and DHF is globalization and increased movement of humans, animals and commodities via aeroplane, which provides the ideal mechanism for the transport of dengue and other urban pathogens between population centres of the world (Gubler & Trent 1994, Gubler & Clark 1995, Gubler 1989, 2002). For instance in 2004, an estimated 1 billion persons travelled somewhere via aeroplane. Many travellers become infected while visiting tropical dengue endemic areas, but become ill after returning home, resulting in a constant movement of dengue viruses in infected humans to all areas of the world, and ensuring repeated introductions of new dengue virus strains and serotypes into areas where the mosquito vectors occur. The result is increased epidemic activity, the development of hyperendemicity, and the emergence of epidemic DHF (Fig. 5).
FIG. 5. Geographic distribution of dengue virus serotypes in 1970 (A) and in 2006 (B).
The USA and Europe are not immune to the introduction of dengue viruses. Each year for the past 25 years, imported dengue cases to the USA have been documented by the Centers for Disease Control and Prevention (CDC) (Gubler 1996, Rigau-Pérez et al 1994, CDC 2004, unpublished data). These cases represent introductions of all four virus serotypes from all tropical regions of the world. Most dengue introductions into the USA come from the American and Asian tropics, and reflect the increased number of Americans travelling to those areas. Overall, from 1976 to 2003, 3697 suspected cases of imported dengue were reported to the CDC (Gubler 1996, Rigau-Pérez et al 1994, CDC 2004, unpublished data). Although adequate blood samples were received from only a portion of these patients, 875 (24%) were confirmed as dengue. A similar increase in reported dengue has been seen in Europe in recent years (Wichmann et al 2003).

These cases represent only the tip of the iceberg because most physicians in the United States and Europe have a low index of suspicion for dengue, which is often not included in the differential diagnosis, even if the patient recently travelled to a tropical country (Gubler 1996, Wichmann et al 2003). As a result, many imported dengue cases are never reported. It is important to increase awareness of dengue and DHF among physicians in temperate areas, however, because the disease can be life-threatening. For example, two cases of the severe form of DHF, dengue shock syndrome, were described in Swedish tourists returning from holiday in Asia (Wittesjo et al 1993). In the USA, severe disease also occurs among imported cases of dengue (CDC 1995). It is important, therefore, that physicians in the USA and Europe consider dengue in the differential diagnosis of viral syndrome in all patients with a travel history to any tropical area.

The potential for epidemic dengue transmission in the USA and Europe still exists, since both have infestations of at least one of the principal mosquito vectors. On eight occasions in the past 25 years (in 1980 after an absence of 35 years, and in 1986, 1995, 1997, 1998, 1999, 2001 and 2005), autochthonous transmission occurred in the USA, secondary to importation of the virus in humans. Of interest was the 2001 Hawaii outbreak, which was the first dengue transmission in that state in 56 years (Effler et al 2005) caused by DEN-1 virus introduced from Tahiti where a major epidemic of DHF was occurring. Transmission in Hawaii was sporadic and illness mild; 122 cases were confirmed (Effler et al 2005). Although the outbreaks in the USA have been small, they underscore the potential for dengue transmission in areas where two competent mosquito vectors occur (Gubler & Trent 1994). *Ae. aegypti*, the most important and efficient epidemic vector of dengue viruses, has been in the USA for over 200 years and has been responsible for transmitting major epidemics in the past (Ehrankramz et al 1971). Currently, this species is found only in the Gulf Coast states from Texas to Florida, although small foci have recently been reported in Arizona. *Ae. albopictus*, another, but less efficient epidemic vector of dengue viruses, was introduced
to the continental USA in the early 1980s and has since become widespread in the eastern half of the country. Although CDC has ceased surveillance, at last count it occurred in 1044 counties in 36 of the continental states (C. G. Moore, Colorado State University, 2004, personal communication); this species has also been found in Hawaii for over 90 years. *Ae. albopictus* has recently been introduced and has become established in several European countries. Both *Ae. aegypti* and *Ae. albopictus* can transmit dengue viruses to humans and their presence increases the risk of autochthonous dengue transmission, secondary to imported cases (Gubler 1988, 1996).

**Prevention**

Prevention and control of dengue fever/DHF currently depends on controlling the mosquito vector, *Ae. aegypti*, in and around the home where most transmission occurs. Space sprays with insecticides to kill adult mosquitoes are usually ineffective, unless they are sprayed indoors where the mosquitoes are resting. The most effective way to control the mosquitoes that transmit dengue is larval control, including eliminating, cleaning or chemically treating water-holding containers that serve as the larval habitats for *Ae. aegypti* in the domestic environment (Gubler 1989, World Health Organization 2000, Newton & Rieter 1992, Reiter & Gubler 1997). At present, there is no vaccine for dengue viruses, although several candidates are at various stages of development (Kinney & Huang 2001, Chang et al 2004). To be effective, a dengue vaccine must protect against all four virus serotypes, i.e. be a tetravalent formulation. For effective use in dengue endemic countries, a dengue vaccine should be safe for use in children 9–12 months of age, must be economical and should provide long-lasting protective immunity (ideally >10 years). Currently, there are at least six tetravalent candidate dengue vaccines that are in or near clinical trials in humans. The Pediatric dengue Vaccine Initiative funded by the Bill and Melinda Gates Foundation, was founded to facilitate bringing one or more of these promising candidate vaccines to fruition (Accelerating the Development and Introduction of a Dengue Vaccine for Poor Children. Hosted by: Children’s Hospital No. 1 and Pasteur Institute of Ho Chi Minh City, December 5–8, 2001, Ho Chi Minh City, Vietnam).

There is no completely effective method of preventing dengue infection in travellers to tropical areas. The risk of infection can be significantly reduced, however, by understanding the basic behaviour and habits of the mosquito vectors and by taking a few simple precautions, such as using aerosol bomb insecticides to kill adult mosquitoes indoors, using a repellent containing diethylmetatoluamide (DEET) on exposed skin, and wearing protective clothing treated with a similar repellent. The risk of exposure may be lower in modern, air-conditioned hotels with well-kept grounds, and in rural areas.
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DISCUSSION

Fairlie: I am not sure what you are attributing the explosion of dengue fever in Singapore to. You mentioned that one of the factors encouraging spread was urbanization, but Singapore has always been urbanized. It was very successful in the 1960s and 1970s at controlling the mosquito population. Why do you think this explosion in Singapore has occurred in the last few years?

Gubler: It’s difficult to know all of the factors involved. The Singapore problem began in the 1980s when Dr Chan Kai-Lok, who devised the program, retired. He had been successful for the better part of 20 years, and then left to work in the University. After this, dengue wasn’t taken seriously for a few years until the resurgence began. Singapore has maintained its mosquito population at a relatively low level. Serological surveys indicate that the herd immunity is low. If there are foci of higher mosquito population densities you can have transmission even with low mosquito populations. *Aedes aegypti* is a highly efficient epidemic vector because each mosquito will bite multiple people. If there was one in this room today and it was infected, it would bite four or five of us. It doesn’t need to take blood; just probing will inject virus, and all of us would become infected. There have been some reorganization issues that may have contributed to the problem. Another possibility is that we know that dengue viruses change genetically and there are some strains with higher infectivity and epidemic potential. Singapore has thousands of migrant workers coming in from around the region, resulting in a constant introduction of new viruses. It is likely that this combination of factors is responsible, although it is not possible to be specific.