

Dengue and Yellow Fever — Challenges for the Development and Use of Vaccines

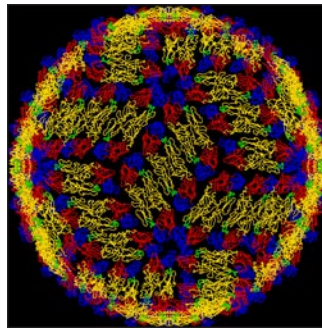
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Dengue is an important human viral disease transmitted by insects. Although nearly half the world's population is at risk for infection and as many as 100 million cases occur annually,¹ we have no antiviral drugs to treat it and no vaccines to prevent it. A closely related but much more lethal mosquito-borne virus, yellow fever, used to be one of the great scourges among humans. Although yellow fever is now largely controlled by vaccination, many regions are susceptible to a reemergence if the disease is introduced by travelers, and substantial recent problems with vaccine safety will no doubt change vaccination policy.

Both dengue and yellow fever are single-stranded RNA viruses in the family Flaviviridae, which includes West Nile virus and approximately 50 others. Substantial progress has been made in understanding the mechanism of the entry of these viruses into cells, the atomic structure of the viral envelope (see figure), the interactions between the molecular determinants and the host antibody, and the mechanism underlying the neutralization of the virus by antibodies.² The unraveling of virus–cell and virus–antibody interactions at the molecular level may lead to the development of antiviral drugs, improved vaccines, and tests for protective and pathological antibodies.

Dengue and yellow fever are endemic to and epidemic in tropical regions (see map). Both are zoonoses maintained in nature by transmission to humans from

monkeys or mosquitoes that breed in tree holes. Infected humans have high blood levels of virus and can therefore infect vector mosquitos. After an incubation period of about 10 days, during which the virus replicates in the salivary-gland tissues of blood-

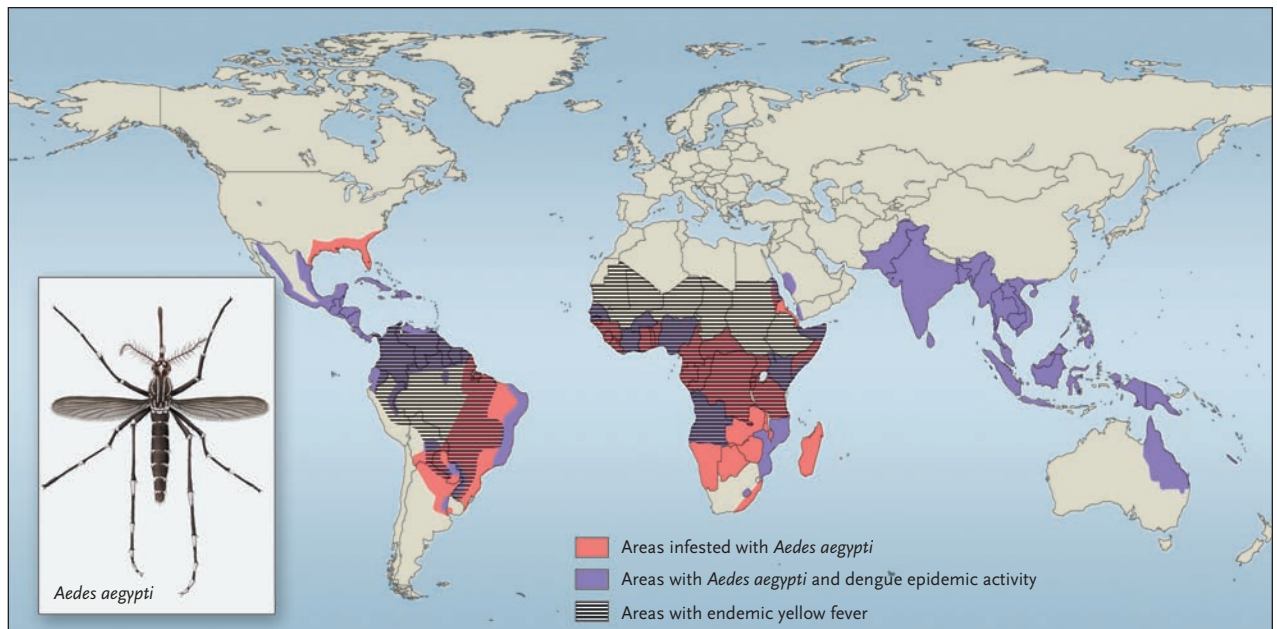


Structure of the Dengue Virus on Cryoelectron Microscopy.

feeding aedes mosquitoes, they can transmit the virus to another person. Throughout the tropics, the principal vector for endemic and epidemic spread, *Aedes aegypti*, has adapted to living among humans in domestic environments. Increasing human population density, urbanization, poor sanitation (creating breeding sites for larval mosquitoes), reinfestation (in the 1970s) of South America by *A. aegypti* after a successful eradication campaign, and the movement of infected persons by airplanes have contributed to a substantial increase in dengue incidence during the past 50 years.² *A. aegypti* mosquitoes are prevalent in the southern United States, which is therefore receptive to the introduction and spread of both dengue and yellow fever (see map).

In its classic form, dengue is an acute illness characterized by fever, headache, muscle and joint pain, and rash. There are four serotypes, and neutralizing antibodies are serotype-specific, so in regions where multiple serotypes cocirculate, people may have sequential infections. Immunity against a specific serotype is lifelong, but previous infection with one serotype is a risk factor for a more severe form of dengue — dengue hemorrhagic fever — upon subsequent infection with another serotype. Dengue hemorrhagic fever is characterized by the capillary-leak syndrome, thrombocytopenia, hemorrhage, hypotension, and shock. Its incidence has increased dramatically during the past several decades, as multiple dengue serotypes introduced into new environments by air travelers with viremia have become endemic. Approximately 500,000 cases occur annually, with a case fatality rate ranging from 1 to 3% to as high as 10 to 20%, depending on the sophistication of the available fluid management and intensive care.

Since we lack an animal model of dengue hemorrhagic fever, our knowledge of pathogenic mechanisms relies on evidence from in vitro studies and patients. Differences in virulence among dengue-virus strains as well as host factors — principally antibody-mediated enhancement of dengue replication³ — contribute to disease expression. Central to the immune-enhancement mechanism in dengue hemorrhagic fever is the usurping of



Distribution of Dengue, Yellow Fever, and Their Principal Vector, the *Aedes aegypti* Mosquito.

Areas infested with *A. aegypti* are receptive to the introduction (by air travelers with viremia) and epidemic transmission of the dengue and yellow fever viruses. Yellow fever has never occurred in Asia — possibly because immunity to dengue provides a barrier to interhuman transmission by mosquitoes and because Asian strains of *A. aegypti* are less efficient vectors than strains from Africa and Latin America — but spread to Asia is an important future threat.

Fc receptors on mature dendritic cells and macrophages as a means of entry for complexes of dengue virus and subneutralizing antibody. These complexes form in the presence of cross-reactive antibody induced by a previous infection with a heterologous dengue serotype or, in infants with maternally derived IgG, when antibody levels drop below neutralizing levels. In such instances, the viral load is increased by means of the infection of an increased proportion of Fc-receptor-bearing cells and an increased level of virus in each cell. The T-cell activation and clearance of infected cells by killer cells and cross-reactive cytotoxic T cells then elicits a proinflammatory “cytokine storm” that causes endothelial damage and capillary leakage.

These immunopathological mechanisms create a conundrum for vaccine developers: since se-

quential infection and heterotypic antibodies cause dengue hemorrhagic fever, a successful vaccine must simultaneously generate long-lasting protective immunity against all four dengue serotypes.⁴ This vexing problem is the reason that no dengue vaccine has yet been approved for use, despite considerable efforts (see table) and substantial funding from the Bill and Melinda Gates Foundation for the Pediatric Dengue Vaccine Initiative. Two live, attenuated vaccines are in phase 2 clinical development. One, developed by the Walter Reed Army Institute of Research and GlaxoSmithKline, consists of viruses empirically attenuated by means of serial passage in cell culture. The second was rationally designed by Acambis through the genetic engineering of the envelope genes of dengue (which contain the epitopes for neutralizing antibodies) into a clone of

the licensed yellow fever 17D vaccine. The resulting live, attenuated dengue–yellow fever chimeric viruses elicit antibodies only to dengue. This vaccine was licensed to Sanofi Pasteur and will soon enter phase 3 trials.

Both vaccines contain mixtures of four dengue serotypes designed to induce long-lasting neutralizing antibodies. The co-administration of four live viruses is associated with competition among serotypes with respect to replication and the ability to stimulate neutralizing antibodies, probably owing to the activation of toll-like receptors and the induction of innate immunity. These effects have been associated with the generation of an incomplete repertoire of neutralizing antibodies after a single inoculation; therefore, complete immunization may require multiple doses. Moreover, our limited understanding of viral neutral-

Vaccines against Dengue and Yellow Fever.*

Developer	Type of Vaccine	Stage of Development
Yellow fever		
7 Manufacturers	17D (live, attenuated)	Licensed
Dengue		
Acambis and Sanofi Pasteur	Live, attenuated chimeric dengue–yellow fever	Phase 2; soon to enter phase 3
WRAIR and GlaxoSmithKline	Live, attenuated	Phase 2
NIH, Biologicals E (India), Panacea (India)	Live, attenuated chimeric dengue–dengue	Phase 1
Mahidol University (Bangkok)	Live, attenuated	Preclinical†
CDC, Inviragen, Shantha (India)	Live, attenuated chimeric dengue–dengue	Preclinical
Hawaii Biotech	Recombinant, subunit	Preclinical
U.S. Navy	DNA	Preclinical

* WRAIR denotes Walter Reed Army Institute of Research, NIH National Institutes of Health, and CDC Centers for Disease Control and Prevention.

† A similar vaccine developed by this group was previously investigated in early-stage clinical trials by Mahidol University and Sanofi Pasteur.

ization and immune correlates of protection, and the difficulty of distinguishing cross-reactions from the development of type-specific antibodies, create challenges for vaccine development. Ultimately, large, controlled field trials will be needed to demonstrate vaccine effectiveness, with follow-up lasting for several seasons of viral transmission to ensure that sensitization to dengue hemorrhagic fever has not occurred.

Yellow fever, for its part, is a fearsome systemic illness characterized by high levels of virus in the blood, jaundice, midzonal coagulative necrosis (apoptosis) of the liver, renal failure, myocardial injury, hemorrhage, and shock — with case fatality rates as high as 50%. The true incidence of yellow fever is unknown but is likely to be a few thousand cases per year, with intermittent, large epidemics involving more than 100,000 cases. In contrast to dengue, yellow fever has only one serotype — a fact that simplified the development of a vaccine. A

live, attenuated (17D) vaccine was developed in 1936 by means of serial passage in chicken-embryo tissue; it has since been used in more than 400 million people and induces long-lasting neutralizing antibodies in about 99% of those who are vaccinated. Many countries in which yellow fever is endemic routinely immunize infants at 9 months of age. In the United States, about 250,000 persons per year are vaccinated to prevent infection during travel or military assignments in tropical regions.

Until 2001, the 17D vaccine was believed to be extremely safe, but that view has been altered by the recognition of a new syndrome: viscerotropic disease associated with the yellow fever vaccine,⁵ an extensive infection of vital organs by a 17D virus that is indistinguishable from wild-type yellow fever disease and has a 60% case fatality rate. Genetic factors of the host (possibly in genes involved in interferon responses) and acquired factors (advanced age and thymectomy) ap-

pear to underlie susceptibility to this condition. The overall incidence is about 1 case for every 200,000 to 400,000 vaccinations, but among persons over 60 years of age, the incidence is as high as 1 for every 50,000 vaccinations — which makes 17D one of the least safe vaccines in use. In addition, neurotropic adverse events (mainly encephalitis caused by invasion of the brain by the 17D virus) have a similar incidence but a much lower case fatality rate (about 6%).

Such risks result in difficult choices, given that the reported incidence of yellow fever among unvaccinated travelers is lower than that of serious vaccine-related adverse events. The problem is that unvaccinated persons traveling to an area of active transmission of yellow fever are in great danger, but such areas are often epidemiologically silent, since the indigenous population is immune or surveillance is poor — rendering it impossible to selectively vaccinate only persons who are at a real risk for exposure. Until a safer



S. Burt Wolbach and John L. Todd Crossing Kandong Bolon, a Creek near Somita, Gambia, Where They Were Researching Yellow Fever and Other Tropical Diseases, in February 1911.

vaccine is developed, travelers and their physicians must exercise judgment on the basis of geographic and epidemiologic data. Travel plans should be carefully assessed to determine whether the traveler will in fact enter a region in which yellow fever is endemic (see map). Improved surveillance for adverse events must be instituted wherever vaccinations are routinely given. Finally, research is needed on the individual risk factors for vaccine-associated viscerotropic disease and on the care of patients in whom this potentially lethal complication develops.

Dr. Monath reports being the former chief scientific officer of Acambis, for which he has served as an expert witness and has received consulting fees; holding multiple patents for recombinant, chimeric vaccines that use the yellow fever 17D strain as a live vector; and serving on the Board of Counselors of the Pediatric Dengue Vaccine Initiative and as a nonexecutive director of Xcellerex, which is developing a yellow fever vaccine.

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An interview with Dr. Monath and a slide show are available at www.nejm.org.

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