Dengue fever (DF) is rapidly evolving into one of the world’s major infectious diseases [1]. DF is an acute flavivirus infection transmitted by several species of Aedes mosquitoes. Dengue virus has 4 antigenically related serotypes: DEN-1, DEN-2, DEN-3, and DEN-4. Infection with any 1 of the 4 serotypes can produce a broad spectrum of effects, including asymptomatic infection, mild febrile illness, classic DF, and the lethal dengue hemorrhagic fever/shock syndrome (DHF/DSS). Dengue virus has become impossible to eradicate and difficult to control, because of massive urbanization, overpopulation, ever-increasing regional and international travel, and failure to sustain Aedes aegypti control programs. Moreover, there is no specific treatment for DHF/DSS. Mortality rates vary from <1% to >30%, depending on diagnostic acumen and availability of intravenous fluid and blood for treatment of the hypovolemic shock caused by massive hemorrhage and capillary plasma leak [2].

An estimated 50–100 million dengue infections and 500,000 DHF/DSS cases occur annually in the tropics, and DF is well known in North American and European travelers and military personnel [3]. In tropical areas where dengue virus is highly endemic, DHF/DSS is typically confined to children younger than age 15 years, with a mean age of 5–10 years. Dengue infection has spread progressively to most tropical countries during the past 40 years, particularly to countries in Southeast Asia, the western Pacific, and Latin America [1]. To illustrate, the number of cases of DF and DHF/DSS in the Philippines has increased 700% between the 1970s and the early 1990s. In Indonesia, dengue infection was recognized in only 2 cities in 1968; by 2001, it was reported in all of the nation’s provinces and in 93% of its 310 districts. DHF is the second-most-frequent cause of pediatric admissions at Jakarta’s largest public hospital, after acute respiratory infections. The spread of dengue virus from cities to rural areas has impeded the diagnosis and management of DHF/DSS; this, in turn, has resulted in higher case fatality rates, which may be as high as 30% in rural areas, compared with 1% in cities. The disease now occurs throughout most months of the year and is no longer confined to the 4–6-month rainy season. Prominent media attention and the fact that DHF/DSS affects poor and rich children alike have contributed to its notoriety. Dengue epidemics cause the population to panic and to overwhelm hospitals and outpatient clinics. There is near-universal agreement among policy makers in Southeast Asian countries that a dengue vaccine is urgently needed [4]. This sense of urgency is shared by the US military and the World Health Organization [5].

A key fact driving dengue vaccine development is that a primary infection with 1 serotype may induce long-term protective immunity to reinfection with the homologous serotype but only short-term immunity, lasting several months, to heterologous serotypes [6]. Dengue differs from other hemorrhagic infections in that dengue infection is more severe in individuals who have acquired dengue antibodies either passively, from their mothers before birth, or actively, from a previous dengue infection [7–9]. Antibody-dependent enhancement (ADE) has provided an explanatory hypothesis, whereby preexisting, cross-reactive dengue virus antibodies facilitate dengue virus entry into Fc-receptor-bearing cells (e.g., macrophages), thereby increasing virus burden and disease severity [10–12]. The secondary-infection hypothesis and ADE suggest that dengue vaccines must induce protective neutralizing antibodies to all 4 serotypes simultaneously rather than sequentially, to avoid enhancement of dengue illness after subsequent infection. Also, a tetravalent vaccine will better protect travelers and troops rapidly deployed to tropical areas where several dengue virus serotypes cocirculate.

For the past 20 years, live attenuated monovalent vaccine candidates, propagated and attenuated in primary and dip-
laid cell cultures, have been evaluated in humans by US Army investigators [13–16]. Most of these vaccine candidates were either underattenuated, making the volunteers ill, or overattenuated, lacking suitable immunogenicity. The proper balance between immunogenicity and reactogenicity was achieved by Halstead, through use of primary dog kidney (PDK) cell culture to grow the vaccine candidates [17–19]. The Mahidol University group in Thailand and the US Army group at the Walter Reed Army Institute of Research (WRAIR) have each developed acceptably safe and immunogenic PDK-passaged monovalent vaccines representing each of the 4 dengue virus serotypes [20–22]. Both research groups have combined their successful monovalent strains into several tetravalent vaccine formulations for phase 1/2 trials in North American adult volunteers [23–25] or in Thai adults and children [26, 27]. To summarize the results of these trials: the vaccines were more reactogenic after the first of 2 or 3 vaccinations, and seroconversion to all 4 dengue serotypes in >80% of volunteers occurred only after the second or third booster inoculation, administered many months after the priming vaccination. The Mahidol vaccines appear to be unacceptably reactogenic in children [26]. One promising formulation of the WRAIR tetravalent vaccine is currently being tested in Thai children and infants. Industry support will be essential for any vaccine candidates selected for the prolonged and expensive field trials that lead to licensure.

Recombinant DNA technology has facilitated the development of live attenuated vaccines for dengue virus and other flavivirus uses. The article by Durbin et al. [28] in this issue of the Journal of Infectious Diseases adds a valuable new chapter to the 70-year-old saga of dengue vaccine development. The authors have provided convincing evidence that their prototype vaccine candidate, rDEN4ΔA30, has a promising future. The vaccine is derived from a cDNA clone of DEN-4 and contains a 30-nt deletion in the 3’ untranslated region of the virus [29, 30]. It is safe, clinically well tolerated, robustly immunogenic, and genetically stable in healthy, adult US volunteers after a single inoculation. The low dose needed to induce immunity should make it economical to manufacture. The vaccine seems to be restricted in its ability to infect mosquitoes [31], and, therefore, there is little risk of loss of the attenuation phenotype that is possible after sustained transmission of live virus vaccines. The Δ30 mutation provides a genetic backbone for the creation of chimeric viruses containing the structural genes for the C protein, premembrane (prM) protein, and envelope (E) glycoprotein of DEN-1, DEN-2, and DEN-3. The E gene product binds to host cells and represents the major protective antigen [32]. Durbin et al.’s results justify construction and clinical trial of Δ30 chimeras expressing E antigens of each of the 3 remaining dengue serotypes and their final incorporation into a candidate tetravalent vaccine. Two important unanswered questions involve the duration of the neutralizing antibody response and whether virus-virus interference in a tetravalent formulation inhibits the antibody response to 1 or more dengue serotypes in the vaccine.

An equally promising advance is the ChimeriVax vaccine technology, developed by Acambis. The genes encoding the prM and E proteins of the licensed yellow fever vaccine virus 17D (YF-VAX) have been replaced with those of heterologous flaviviruses, including the 4 dengue serotypes [33, 34] and other flavivirus [35–38]. Phase 1 clinical trials of these chimeric vaccine candidates are under way.

Several other chimeric dengue vaccines are in the late stages of preclinical development [5], and the preclinical development of other vaccine candidates is in progress. These alternative candidates include purified inactivated dengue virus [39]; infectious DNA or RNA; expression vector-based and naked DNA; and recombinant subunit dengue vaccines [40].

Many research and public health questions remain unresolved. For example:

1. Can tetravalent vaccines consistently achieve acceptable reactogenicity and >80% antibody response to all 4 serotypes and in all populations at risk for dengue infection? RNA sequence data indicate that the dengue viruses are evolving and diverging [41, 42]. The molecular basis of virulence and pathogenesis of DHF/DSS must be better understood, to ensure that vaccine development stays ahead of dengue virus evolution. The mechanisms of vaccine-induced protection need to be clarified in future field trials. The consensus immunogenic target of a neutralizing antibody response to all 4 serotypes in at least 80% of volunteers may not be appropriate in all populations and clinical settings.

2. Are tetravalent vaccines safe and immunogenic in flavivirus-seropositive persons? There is a theoretical concern that prior natural infection (or vaccination) with a serologically related flavivirus, such as Japanese encephalitis virus (in Asia) or yellow fever virus, St. Louis encephalitis virus, or West Nile virus (in the Americas), would sensitize individuals and lead to more severe vaccine reactions than in flavivirus-naïve persons. The benign clinical course and robust immune response in volunteers immunized with monovalent DEN-2 vaccine after vaccination against yellow fever [13, 14] provides some reassurance that severe reactions would not occur and that dengue titers may be enhanced.

3. Would tetravalent live-virus vaccines be safe and immunogenic in HIV-infected persons? HIV infection is increasing in populations at risk for dengue infection, particularly in Southeast Asia. Attenuated live-virus vaccines are generally contraindicated in HIV-infected persons. With the exception of 1 patient, who recovered uneventfully from DHF [43], there have been no published reports of dengue infection in HIV-seropositive persons. Dengue vaccine candidates may need to be tested carefully in

EDITORIAL COMMENTARY • JID 2005:191 (1 March) • 651
HIV-positive and other immunosuppressed individuals, but the ethics of such studies are problematic.

4. Do tetravalent vaccines elicit virus-enhancing antibody similar to that induced by wild-type dengue virus infection [10]? If so, what is the clinical significance of such antibody [44]?

5. How do the vaccine responses in infants and children differ from those in adults? Infants often respond to wild-type dengue virus infection with few symptoms, and preadolescent children are less incapacitated by dengue infection than are adults. Similarly, PDK-attenuated vaccines, which tend to be reactogenic in adults, may be less reactogenic in infants and young children. Clinical attenuation as a function of decreasing age has, in fact, been noted in the first modern, tetravalent, live attenuated vaccine trial in dengue- and Japanese encephalitis virus–seronegative children [26]. However, most children (60%) still had mild to moderate dengue-like illness after the first of 3 vaccinations, and seroconversion to the 4 serotypes in more than 80% of volunteers was achieved only after the third inoculation, at 12 months. A WRAIR PDK-attenuated vaccine formulation is currently undergoing a phase 1 trial in children and infants in Bangkok; I await the outcome with anticipation.

An opportunity now exists to put newly developed dengue vaccines into the field quickly. In July 2003, the Bill and Melinda Gates Foundation funded the Pediatric Dengue Vaccine Initiative (PDVI) for 5 years and US $55 million. The International Vaccine Institute in Seoul, South Korea, serves as the PDVI secretariat. A 4-point program will accelerate the development and field testing of dengue vaccines [45]. I am optimistic that field trials of 1 or more dengue vaccines will commence within 3 years in Latin America and in Southeast Asia. The licensing of a protective vaccine must come soon, if dengue is to be brought under control.

References
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