Dengue Vaccines Approach the Finish Line

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The spread of dengue virus (DV) via its *Aedes* mosquito vector throughout most of the tropics has led to a worldwide resurgence of epidemic dengue, including dengue hemorrhagic fever. For the first time in 60 years, the pipeline of dengue vaccines looks promising. Strains of each of the 4 DV serotypes, attenuated by passage in tissue culture or by recombinant DNA technology, have been formulated into tetravalent vaccines and have entered successful phase 1 and 2 clinical trials in the United States and Southeast Asia. Antibody-dependent enhancement of wild-type DV infections by the vaccine represents a unique safety issue, which is under investigation. The Pediatric Dengue Vaccine Initiative (funded by the Bill and Melinda Gates Foundation), the World Health Organization, industry, the US military, and governments of tropical countries are collaborating to accelerate dengue vaccine development and phase 3 vaccine efficacy trials in countries where dengue is endemic. A protective tetravalent vaccine must be licensed soon if dengue is to be brought under control.

BACKGROUND

Dengue fever (DF) is an acute *Flavivirus* infection transmitted by several species of *Aedes* mosquitoes. Dengue virus (DV) has 4 antigenically related serotypes: DEN-1, DEN-2, DEN-3, and DEN-4. Infection with any one of the 4 serotypes can produce a broad spectrum of clinical illness, including asymptomatic infection, mild febrile illness, classic DF, and the lethal dengue hemorrhagic fever and shock syndrome (DHF/DSS). During the past 50 years, dengue has evolved into one of the world’s major infectious diseases [1]. More than 2.5 billion people are at risk for dengue in >100 countries, and *Aedes aegypti* and dengue epidemic activities are now widely distributed in the tropics and subtropics (figure 1).

It is estimated that 50–100 million DV infections and 500,000 DHF/DSS cases occur annually and are concentrated in Southeast Asia, the Western Pacific, and Latin America [1]. Case-fatality rates vary from <1% to >30%, depending on diagnostic acumen and the availability of intravenous fluids and blood for the treatment of hypovolemic shock caused by massive hemorrhage and capillary plasma leakage [3]. The simultaneous or sequential cocirculation of >2 DV serotypes in a locale is associated with frequent outbreaks of severe DF and DHF/DSS, with 90% of cases occurring in children <15 years of age (mean age, 5–10 years, depending on the locale).

The disease is occurring throughout the year in urban and rural areas and is no longer confined to the 4- to 6-month rainy season [4]. DF is now a leading cause of morbidity in American and European travelers and military personnel, rivaling or exceeding malaria in many countries [5].

A BRIEF HISTORY OF DENGUE VACCINE DEVELOPMENT

The first successful dengue vaccine was reported in 1945 by Sabin and Schlesinger, who attenuated the “Hawaiian” strain (serotype DEN-1) of DV in mouse brain by serial passage and then used this mouse brain vaccine to protect 16 volunteers against the bites of infected *A. aegypti* mosquitoes [6]. The US Army ultimately halted production of mouse brain vaccine and, in 1971, began the modern era of DV propagation in tissue culture, which promised a safer vaccine substrate [7]. Recombinant DNA technology has catalyzed more recent advances in vaccine development [8].
RATIONAL FOR DENGUE VACCINE DEVELOPMENT

The World Health Organization (WHO) and the US military classify dengue as a major international health concern. Dengue has become impossible to eradicate and difficult to control because of massive urbanization, overpopulation, and substandard living conditions; increased regional and international travel; failure to sustain A. aegypti control programs; and the global emergence of more virulent genotypes of DV [9]. Policy makers in Southeast Asian countries universally have agreed that a dengue vaccine is urgently needed [4]. This sense of urgency is shared by the US military and the WHO [8].

Rationale for Tetravalent Dengue Vaccines (TDVs)
The current strategy to develop a combined vaccine against all 4 DV serotypes is supported by 3 key epidemiologic and immunologic facts. First, primary infection with one serotype may induce long-term protective immunity to reinfection with the homologous serotype that persists for years; however, immunity against heterotypic serotypes lasts only for several months [6]. Thus, no single serotype can provide long-term protection against the other 3 serotypes.

Second, one or several serotypes can circulate simultaneously in any locale, and serotypes can change unpredictably from one season to the next. Thus, one cannot reliably predict whether a monovalent vaccine would protect during the first dengue season and in subsequent seasons in any locale. A tetravalent vaccine will better protect travelers and troops who are rapidly deployed to tropical areas where several DV serotypes cocirculate over time.

Third, dengue differs from other hemorrhagic infections in that DV infections are more severe in individuals who have acquired DV antibodies either actively from a previous DV infection or passively from mothers before birth [10, 11]. Thus, DHF/DSS cases are associated with prior infection and are apparently mediated by nonneutralizing antibodies that are residual from an earlier DV infection. Antibody-dependent enhancement has provided an explanatory hypothesis whereby preexisting, cross-reactive DV antibodies facilitate entry of DV into target cells, thereby increasing the virus burden [12, 13]. A massive cellular release of virus and soluble DV protein triggers complement activation, which, together with inflammatory cytokines, synergizes locally to trigger the vascular leakage observed in severe DV infections [14]. The secondary-infection hypothesis and antibody-dependent enhancement suggest that dengue vaccines must induce protective neutralizing antibodies to all 4 DV serotypes simultaneously rather than sequentially, to avoid enhancement of dengue after subsequent infection.

Rationale for Live Attenuated Dengue Vaccines (LAVs)
Immune control of dengue will probably require an LAV rather than a nonreplicating killed whole virus, recombinant subunit, or recombinant DNA vaccine [15]. First, control of epidemics requires rapid immunization with a single inoculation or 2 closely spaced inoculations. Second, the vaccine should induce long-lasting neutralizing antibody levels that mimic those associated with natural DV infection. Antibody titers should not decrease to nonprotective levels that may leave individuals susceptible to the immunopathological events associated with DHF/DSS if they are subsequently infected by a heterotypic DV serotype. Third, low-cost, efficient vaccines are needed to protect children in the tropics.

By contrast to attenuated vaccines, inactivated and subunit vaccines require multiple inoculations, elicit short-term im-
munity, and fail to induce robust major histocompatibility complex class 1–restricted T cell immunity that contributes to full protection against dengue. These vaccines are likely to be more expensive than LAV, and their introduction would increase the cost of immunization programs in developing countries. Most developing countries are unlikely to field a vaccine that costs more than $1.00 per dose [4]. For these reasons, nonreplicating dengue vaccines will remain a second choice for clinical development—or at least until the risk associated with LAVs outweighs their benefit [15]. Industry support will be essential for the prolonged and expensive field trials that lead to licensure. The leading live attenuated TDV candidates currently undergoing clinical trials are discussed below.

**Primary dog kidney (PDK) tissue culture–passaged vaccines.** For the past 20 years, live attenuated monovalent vaccine candidates, propagated and attenuated in primary and diploid cell cultures, have been evaluated in humans by US Army investigators [7]. Most of these candidates were either underattenuated, making the volunteers ill, or overattenuated, lacking suitable immunogenicity. The proper balance between immunogenicity and reactogenicity was achieved by Halstead and Marchette [16], who used PDK cell culture to grow the vaccine candidates. The Mahidol University group in Bangkok, Thailand, and the US Army group at the Walter Reed Army Institute of Research (Silver Spring, MD) have each developed acceptably safe and immunogenic PDK-passaged monovalent vaccines representing each of the 4 DV serotypes [17, 18]. Both research groups have combined their successful monovalent strains into several TDV formulations for phase 1 and 2 trials involving North American adult volunteers [19, 20] or Thai adults and children [21, 22].

In summary, the Mahidol University vaccines were more reactogenic after the first of 2 or 3 vaccinations, and, for >80% of volunteers, seroconversion to all 4 DV serotypes occurred only after the second or third booster inoculation was administered 3–12 months after the priming vaccination. The Mahidol vaccines appear to be unacceptably reactogenic in children and adults [21, 25]; for this and other reasons, Sanofi Pasteur ceased codevelopment of the Mahidol formulations. Of note, no severe cases of dengue occurred in 104 Thai vaccine recipients who were monitored 5–6 years after immunization.

One promising formulation of the TDV developed by the Walter Reed Army Institute of Research has been administered twice (at 0 and 6 months) to 7 Thai children and 34 Thai infants. The formulation has been acceptably immunogenic, well tolerated, and free of serious vaccine-associated adverse effects. Phase 2 studies of this and another new formulation are pending. GlaxoSmithKline is a codeveloper of these formulations with the Walter Reed Army Institute of Research [8].

Two recurrent problems have slowed the development of PDK-attenuated TDVs. The problems include (1) achieving the proper level of attenuation of each serotype strain to provide minimal reactogenicity and maximal immunogenicity [19] and (2) viral interference between different immunogenicity comprising the final vaccine mixture [19, 20, 24]. The solution to these problems has required empirical testing of multiple passage levels and vaccine dilutions. It is unclear whether these impediments have been overcome for the current PDK formulations.

**Live attenuated chimeric vaccines.** In addition to the classic attenuation by serial passage in tissue culture, recombinant DNA technology has facilitated the development of LAVs for dengue and other flaviviruses. Because of space limitations, this review is focused on vaccines that have emerged from preclinical studies into clinical trial.

One such prototype vaccine candidate, rDEN4Δ30, 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 [25]. The monovalent vaccine is safe, clinically well tolerated, robustly immunogenic, and genetically stable in healthy, adult American volunteers after a single inoculation. The low dose needed to induce immunity should make the vaccine economical to manufacture [26]. The vaccine seems to be restricted in its ability to infect mosquitoes [27]; therefore, there is little risk of loss of the attenuation phenotype 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 C, premembrane (PrM), and envelope (E) glycoprotein of DEN-1, DEN-2, and DEN-3. The envelope gene product binds to host cells and represents the major protective antigen. Phase 1 clinical trials of each of the 4 chimera serotypes will be completed soon. Their admixture and testing as a candidate TDV are scheduled for 2007. Two important unanswered questions include the duration of the neutralizing antibody response and whether virus–virus interference in a tetravalent formulation inhibits the antibody response to ≥1 serotype in the vaccine. Several industrial sponsors in Europe and Brazil have been awarded nonexclusive licenses for the rDEN4Δ30 formulations (S. Whitehead, personal communication).

Another promising advance is the ChimeriVax vaccine technology developed by Acambis [28] The genes encoding pre-membrane and envelope proteins of the licensed 17D yellow fever vaccine virus (YF-VAX; Aventis Pasteur) have been replaced with envelope proteins of other flaviviruses, including the 4 DV serotypes. The ChimeriVax-DEN 1–4 tetravalent virus mixture demonstrated a restricted ability to replicate in Aedes vector mosquitoes, similar to the rDEN4Δ30 chimera. In the first dengue phase 1 clinical trial [29], a single dose of the monovalent ChimeriVax-DEN 2 was well tolerated, with a safety profile consistent with that of YF-VAX. Of 42 yellow fever–naïve volunteers, 92% had seroconversion to DEN 2, with low cross-reactivity to heterotypic DEN serotypes noted. Preimmunity to yellow fever virus did not interfere with ChimeriVax-DEN 2 immunization.
[29]. In the subsequent TDV trial, ChimeriVax-DEN 1–4 was well tolerated in 66 volunteers after inoculations were given at 0 and 6 months. Approximately 67% of volunteers had seroconversion to at least 3 serotypes after the first inoculation; immunogenicity after the second inoculation is pending (N. Kanesa-Thasan, personal communication). Sanofi Pasteur and Acambis are codeveloping ChimeriVax-Dengue 1–4; if ongoing phase 2 trial results are favorable, Sanofi Pasteur is prepared to conduct phase 3 trials to justify licensure (J. Lang, personal communication).

OTHER DENGUE VACCINE CANDIDATES

Preclinical development of other candidates is progressing. Examples include attenuated chimeric dengue vaccines, inactivated whole virus, infectious DNA or RNA, expression vector–based and naked DNA, and recombinant subunit dengue vaccines [8, 30].

SOME UNRESOLVED VACCINE AND PUBLIC HEALTH QUESTIONS

A sampling of other unique and complex issues that surround field trials of attenuated TDVs (aside from the theoretical risk of vaccine-associated DHF/DSS) is summarized below.

1. Can a TDV consistently achieve acceptable reactogenicity and 80% protective efficacy to all 4 serotypes in all countries where individuals are at risk for dengue infection for at least 3–5 years [4]? Is this consensus vaccine response appropriate in all populations and clinical settings?
2. Phylogenetic and epidemiologic analyses suggest that more virulent genotypes are displacing those that have a lower epidemiologic impact [9]. TDV components must stay current or ahead of DV evolution.
3. Because there are no acceptable animal models, field trials must be designed to elucidate DV evolution and pathogenesis, in addition to vaccine safety and efficacy.
4. The relative protection afforded by neutralizing antibody versus cellular immunity should be clarified in future field trials. In the absence of antibody, can unequivocal evidence of immune priming provide a reliable correlate of protection?
5. We need to develop in vitro tests capable of distinguishing protective from nonprotective vaccine responses. The WHO should endorse a standardized DV antibody microneutralization assay. Several quantitative PCR assays that are close to being validated will permit detection of each of the 4 DV serotypes in the blood of trial participants.
6. In what epidemiologic settings can herd immunity, provided by partial vaccination of a population, decrease DV transmission and protect against dengue? To what extent will herd immunity confound the analysis of vaccine protection?
7. Will TDVs be safe and immunogenic in persons with preimmunity to other flaviviruses, such as West Nile virus, Japanese encephalitis virus, and yellow fever virus? We are reasonably reassured that severe reactions would not occur in persons immune to yellow fever [7, 29]. Safety needs to be confirmed in other individuals who are immune to flaviviruses.
8. Would TDVs be safe and immunogenic in HIV-infected and other immunosuppressed persons? LAVs are generally contraindicated in such individuals, but attenuated TDV candidates will need to be tested eventually. The ethics of such studies are thorny, but they can be addressed by a consensus of vaccine stakeholders and institutional review boards.
9. How do vaccine responses in infants and children differ from those in adults? Infants often respond to wild DV infection with few symptoms, and preadolescent children are less incapacitated by DV infection than are adults. In fact, clinical attenuation as a function of decreasing age was noted in the first PDK-attenuated tetravalent vaccine trial involving DV-seronegative children [21]. The immune and clinical responses to the vaccine need to be stratified by age in future field trials, to include flavivirus-negative and -positive participants.
10. There is growing consensus that the short-term objective of phase 3 field trials of TDV should be to protect against virally confirmed dengue of any severity (from mild dengue to DHF) and DV of any serotype. The long-range objective would be to confirm efficacy against all 4 DV serotypes. To do this, geographically diverse field sites must be developed to ensure that the vaccine can be tested against the 4 circulating DV serotypes and against the full range of clinical dengue.

PEDIATRIC DENGUE VACCINE INITIATIVE

For the first time in 60 years, an opportunity exists to introduce newly developed dengue vaccines into the field quickly. In July 2003, the Bill and Melinda Gates Foundation funded the Pediatric Dengue Vaccine Initiative with 55 million dollars for 5 years. The Pediatric Dengue Vaccine Initiative has been collaborating with vaccine manufacturers, governments, and the WHO to fund a 4-point program to accelerate the development and field-testing of dengue vaccines. This program will (1) better define the global burden of dengue; (2) elucidate the social and economic costs of dengue, particularly in countries that may introduce dengue vaccines; (3) identify phase 3 vaccine test sites in different countries where dengue is endemic, as well as build local capacity for dengue research; and (4) fund basic research on how dengue vaccines can be administered safely. Guidelines published by the WHO in 2002 to facilitate dengue vaccine trials in countries where dengue is endemic are being revised and updated in anticipation of field trials of ≥1 TDVs to begin within the next 3 years in Latin America and Southeast Asia [31]. A protective vaccine must be licensed soon if dengue is to be brought under control.
DR. THEODORE E. WOODWARD

Although Ted had no direct influence on my research on dengue, we interacted in different venues on many occasions starting in 1967, when I first met him at an Armed Forces Epidemiological Board meeting held at the Walter Reed Army Institute of Research. I was a National Institutes of Health postdoctoral fellow in preventive medicine at Case Western Reserve University. I remember how he encouraged me by his obvious interest in our data on the interaction of flaviviruses and human WBCs. We continued to meet between 1968 and 1970, during my assignment in the US Army Medical Research and Development Command, where, as an Armed Forces Epidemiological Board member, Ted helped me to guide the Army’s extramural research program in communicable diseases. Ted had already been retired for 7 years from his position as chairman of the Department of Medicine at the University of Maryland when I joined the Center for Vaccine Development in 1988. However, he was still active, and we spoke on several occasions about our vaccine trials. In addition, we and other faculty taught a course on disease pathogenesis to third-year medical students.

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