Deadly Dengue: New Vaccines Promise to Tackle This Escalating Global Menace

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As global killers go, dengue does not get much press. But with an annual toll of nearly 100 million infections and 20,000 deaths, it ranks second only to malaria among deadly mosquito-borne diseases. A major public health threat in most tropical countries, the disease has been steadily spreading and growing more virulent. Unlike its close cousin that causes yellow fever, the wily dengue virus has confounded vaccine researchers for decades. But recent scientific advances and a coordinated global effort could soon change that. "We now have several rich pipelines of vaccines and several entities promoting them," says Joachim Hombach, who heads the World Health Organization's dengue prevention program. "I am confident we will see a vaccine in a few years."

An unprecedented collaboration among vaccine manufacturers, research institutes, regulatory agencies, and other stakeholders is generating the momentum needed to make the dengue vaccine a reality.

Most patients infected with dengue show no symptoms, or get dengue fever—a self-limiting, flu-like illness. However, about 1% of the cases turn into dengue hemorrhagic fever, a serious condition in which blood leaks from capillaries and collects in body cavities. Nearly 1 patient in 20 with this condition dies. Most victims are children. "You have a child that has a nonspecific febrile illness, and 12 hours later the child is in profound shock and can die if not treated effectively," says Duane Gubler, director of the Asia-Pacific Institute of Tropical Medicine and Infectious Diseases in Honolulu. "This causes panic among the parents."

The dengue virus's partner in crime, the *Aedes Aegypti* mosquito, is a highly domesticated insect that thrives in crowded urban settings and transmits the disease very efficiently. Population growth and migration to already congested cities provide the deadly duo with an ever-increasing supply of victims. Airline travel spreads the disease further by moving infected people and mosquitoes between population centers. As a result, the number of countries that have experienced dengue epidemics has quadrupled since 1970. "We've seen unprecedented urbanization during the past 20–30 years, and that's what is driving epidemic dengue," says Gubler.

Attempts to eradicate *A. Aegypti* have largely failed, since dengue-affected countries typically lack the resources to do this. As an alternative, researchers are racing to develop a vaccine. But with dengue, this is easier said than done. The virus comes in four major strains, or serotypes, any of which can cause the disease. Immunity against one serotype does not protect against infection by another; on the contrary, it may increase the risk of getting hemorrhagic fever. To avoid this possibility of immune-enhanced disease, the vaccine has to protect simultaneously and equally against all four serotypes, according to Alan Barrett, director of research at the Sealy Center for Vaccine Development at the University of Texas in Galveston. "With a vaccine cocktail like this, it's going to be a balancing act," he says.

Testing a potential vaccine is another hurdle. Unlike other mosquito-borne diseases such as malaria, yellow fever, or Japanese encephalitis, dengue causes disease only in humans—the virus replicates in other primates, but produces no symptoms. Currently, the best method available to test a dengue vaccine's efficacy in a preclinical setting is to see if it prevents viremia in nonhuman primates challenged with the virus. However, this does not predict how the vaccine will act in people. "The great challenge from the scientific point of view is that we have no animal model for the disease," says Barrett. "The only good animal model is humans."

Despite these challenges, dengue vaccine developers have made major strides during the past few years, and two are now within striking distance of licensing a product.

Researchers from the Walter Reed Army Institute of Research and GlaxoSmithKline have collaborated to develop a "live attenuated virus" vaccine that combines weakened versions of all four dengue types. Prior to the collaboration, Walter Reed researchers had developed three prototype vaccines for one type, using either a live or a killed virus or a viral envelop protein. When Walter Reed and GlaxoSmithKline researchers compared the three candidates in primates, they found that the live virus vaccine worked the best. But when vaccines for the four serotypes were mixed together and administered in clinical trials, the immune response was weak unless the dose was repeated. "What that taught us was, when you mix four dengue viruses together, they interfere," says Bruce Innis, a vice president and director at the United Kingdom-based GlaxoSmithKline. "As with other combination live viral vaccines, it is going to take more than one dose to immunize." After trial and error, the team has found that the vaccine could be given in two doses spaced about 6 months apart.

Close on their heels is a live attenuated virus vaccine from Sanofi Pasteur. This formulation is based on

a "chimera" that encodes dengue envelope and premembrane proteins into the genome of a virus used in the company's very successful yellow fever vaccine. Four such chimeras combine to make a tetravalent dengue vaccine that is "safe and immunogenic for all four wild-type dengue strains," according to Jean Lang, a senior director at the Lyon, France-based multinational company. In contrast, vaccines based on weakened dengue viruses have "failed to consistently achieve both goals in the last decades," he says.

Innis points out that GlaxoSmithKline's vaccine might prove more effective because it contains not just two dengue proteins, but the entire virus. "The question is, which vaccine gives you the minimum information required to get an adequate immune response," he says. "That will have to be settled through experimentation." Both vaccines are currently in Phase II trials on adults and children. Two more chimeric vaccines are in early development: one from the National Institutes of Health and one from the Centers for Disease Control, licensed to Fort Collins, Colorado-based InViragen.

Any vaccine made with a live, replicating virus poses risks. The virus could mutate and turn pathogenic. Viral products could trigger an adverse immune response. And in people lacking a strong immune system, even a weak virus could be a danger. This was highlighted during clinical trials of the first tetravalent live virus vaccine for dengue, developed by Mahidol University in Thailand. Some trial subjects became sick; it turned out that one of the dengue strains had been insufficiently weakened. "You sometimes don't discover the safety issues until late in development," says Innis.

To reduce these risks, some manufacturers are developing nonreplicating dengue vaccines designed to prime the immune system without exposing the body to an infection.

A vaccine from GenPhar, a biotechnology startup in Mount Pleasant, South Carolina, uses a pair of live but nonreplicating adenoviruses to carry dengue antigens. Protein-coding nucleotides from two serotypes are spliced symmetrically on either side of each adenovirus's genome; together, the two recombinant viruses carry antigens from all four dengue pathogens. Primate studies show that the vaccine is 100% effective in preventing viremia, according to John Dong, president and chief scientific officer of GenPhar. "We mimic a natural dengue infection without using a dengue virus," says Dong. "And we get the same type of immune response."

Hawaii Biotech's vaccine contains no viruses, but instead has truncated recombinant envelope proteins from each of the four dengue serotypes. Preliminary studies show that this "subunit" formulation evokes a strong immune response in primates, says Beth-Ann Coller, vice president for research and development at the Aiea, Hawaii-based company, which has 44 full-time employees. Hawaii Biotech's vaccine technology is "inherently safer than a live vaccine, particularly for immunocompromised individuals," according to Coller. A further advantage for their vaccine, she says, is that doses could be given 1 month apart, as opposed to the 6 month interval live vaccine might need.

Some experts say that nonreplicating dengue vaccines provide less immunity than those based on a live virus—an assertion that GenPhar and Hawaii Biotech contest, citing favorable animal data. Both companies say they hope to launch Phase I trials soon and catch up with their multinational competitors.

Dengue's unique features make it challenging to design a clinical trial. Since no animal model exists, "any clinical study has to start with humans being the first safety model," says Robin Levis of the U.S. Food and Drug Administration. To get good data, subjects with pre-existing immunity to one or more dengue strains should ideally be excluded from trials. Methods to identify such persons are not very reliable; furthermore, most adults in dengue-prone regions have already been exposed to the disease. "If you are looking for a naive population to test the immune response, you have your work cut out for you," Levis says.

Even with an ideal set of subjects, testing efficacy is tricky. Researchers can't reliably gauge a subject's level of protection from their immune response to the vaccine, says Levis. The only reliable test, therefore, is to observe how a vaccinated subject responds to a real dengue infection. In the Americas, where the disease breaks out at unpredictable intervals, this would be hard to do—vaccinees might need to be monitored for several years until the next epidemic hits. In parts of Asia, where epidemics are more frequent, a shorter period of monitoring might suffice, says Levis.

The final barrier to a dengue vaccine is not scientific, but economic. According to some estimates, it costs more than a billion dollars to bring a new vaccine to the market. Dengue strikes mostly in poor tropical nations that can't afford to pay more than a dollar or two per vaccine dose. Normally, this would discourage manufacturers, but a global consortium spearheaded by the WHO and the Pediatric Dengue Vaccine Initiative (PDVI) is working hard to make it a more attractive commercial proposition. Funded mainly by the Gates Foundation, PDVI supports many manufacturers in the research, development, evaluation, and—eventually—distribution of dengue vaccines. An unprecedented collaboration among vaccine manufacturers, research institutes, regulatory agencies, and other stakeholders is generating the momentum needed to make the dengue vaccine a reality, says PDVI director Harold Margolis. "But it is still going to take a number of years," he says. "You can't rush vaccine science."

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