New test may help to ensure that dengue vaccines do no harm

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As vaccines against a virus that infects 100 million people annually reach late-stage clinical trials this year, researchers have developed a test to better predict whether a given vaccine candidate should protect patients from the infection, or in some cases, make it more dangerous, according to an article just published in the journal *Clinical and Vaccine Immunology*.

Cases of tropical, mosquito-borne dengue fever have expanding globally for more than 50 years, with nearly a third of the human population in 100 countries now at risk of infection with the four types of dengue virus. Infection with the dengue flavivirus, which is related to West Nile Virus and Yellow Fever, results in an estimated 500,000 hospitalizations and 22,000 deaths, mostly among infants, each year, according to the World Health Organization. After decades of absence in the United States, experts say the disease is causing illness again along the Texas-Mexico border, and that widespread dengue infection in the continental United States is a real possibility.

A typical dengue infection confines a patient to bed for more than a week with fever and severe limb pains, but most recover. In less than five percent of cases, however, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), often deadly complications, develop just as the fever breaks. Mostly affecting babies between five and eight months of age, DHF causes victims to vomit and pass blood in their feces and urine. If diagnosed quickly, patients respond to intensive hospital treatment and fluids, but mortality can reach 15 percent when

undiagnosed. DSS comes when the infection has caused so much fluid to leak out of capillaries that there is not enough blood to supply organs. As of 2008, there were no antiviral drugs designed to treat dengue and no drug candidates in late-stage development.

"Aggressive health education and mosquito abatement programs have saved lives, but hopes for a true solution lie with vaccine design," said Xia Jin, M.D., Ph.D., associate professor in the Department of Medicine, Division of Infectious Diseases, at the University of Rochester Medical Center. "Our study shows that the new test is likely superior to the standard test in its ability to tell whether a patient's response to a vaccine is safe," said Jin, an author for the CVI paper.

Second Time Deadly

Most people, upon first exposure to any dengue virus, develop an immune response that protects them against that version of the virus for life. Unfortunately, the dengue virus, in its ancient relationship with humans, has evolved into four related but independent classes of virus called serotypes (DENV-1, DENV-2, DENV-3 and DENV-4). The frightening aspect of the disease comes with a person's second dengue infection with one of the other three dengue serotypes, which may place them at much greater risk for bleeding and shock.

In cases where simple dengue fever progresses to DHS, patients have about 100 times as much virus in their blood as seen in a mild infection. What makes the virus so much better at penetrating human cells and reproducing the second time around? Decades of research are just now providing the answer, which lies within the intricacies of the immune system designed to recognize and destroy invading organisms.

As patients attempt to fight off a dengue infection, their immune systems activate antibodies, immune proteins that lock onto certain

identifying pieces of the virus to form antibody-virus complexes that flag the virus for destruction. Humans produce a vast variety of antibodies, each with a unique "business end" shaped to recognize one specific viral protein, which enables the system to react to most invaders encountered. Ideally, an infected patient produces a large amount of the type of antibody that binds most strongly to the virus and that covers the greatest amount of the viral surface area to "neutralize" the virus (takes away its ability to reproduce).

Complicating matters is a second feature of antibodies, one which is the same across all antibodies: the crystalizable fragment (Fc). The Fc is designed to bind to proteins called the Fc receptors on the surfaces of macrophages, immune cells that roam the bloodstream seeking to engulf and "dissolve" viruses and bacteria. Coated with Fc receptors, macrophages constantly stick to the Fc end of antibodies, which brings whatever the antibody has locked onto into close contact with the cells capable of destroying it. In most people infected with their first dengue serotype, antibodies bind tightly to the viral surface and escort the virus via the Fc/Fc receptor link to macrophages where the virus is destroyed. The immune system then stores away a few of the successful antibodies in case that same virus is ever encountered again. When the system encounters a second dengue serotype, however, the antibodies from the first infection do not attach as securely to the new version in many cases, enabling the virus to break away from its antibody partner and begin copying itself. In this scenario, the antibody's Fc/Fc receptor interaction has served only to deliver the virus into cells that it could not otherwise penetrate.

The latter phenomenon, called antibody-dependent enhancement (ADE), has delayed the development of dengue vaccines for decades. The threat of enhancement dictates that any dengue vaccine must raise protective immunity against all four dengue serotypes simultaneously and equally, and several vaccine candidates have generated unequal responses across

serotypes. That creates the possibility that some of the antibodies created by such vaccines could raise the risk for hemorrhagic fever and shock, and calls for the development of tests that can precisely measure enhancement risk.

Different versions of dengue move around the globe, sometimes displacing each other. Asian serotype DENV-2 strains, for example, have been taking the place of relatively more benign American DENV-2. One important example of this was seen in 1981, when Asian DENV-2 struck Cuba with nearly 900 people hospitalized following an uneventful DENV-1 outbreak four years earlier. While the Cuba outbreak followed the standard pattern, with a spike in serious cases accompanying a second infection, another outbreak, in Iquitos, Peru, in 1995, was unusual. In an area infected with DENV-1 four years previously, the second infection in 1995 with DENV-2 outbreak did not cause fatal complications. The reasons why one DENV 2 strain caused fatal second infections, and another did not, remained a mystery for years.

The current study may have helped solve the mystery, while pointing out a weakness of the standard test of antibody responses. The assay used originally to analyze the blood of patients in Iquitos was the plaque-reduction neutralization test (PRNT), the recognized gold standard for determining how effectively the human immune system responds to dengue infection. PRNT starts with a sheet of cells chosen because they can be invaded by the virus, and because they share some qualities with the kind of cell targeted by the virus in the body, the macrophage.

When the viral strain being studied is introduced to this cell culture, it begins invading and killing the cells, and making copies of itself. By diluting these mixtures, scientists can identify and count "islands" (or plaques) in the culture where the virus has destroyed cells.

When serum (which contains antibodies) from an infected patient's blood is added to this mix, the number of spots over time reveals the degree to which the patient's antibodies can effectively neutralize the virus. In the case of dengue research, PRNT tests are used to measure how efficiently the antibodies from a natural infection protect the cultured cells from the experimental infection with a second dengue serotype.

According to past experiments on the standard PRNT test, it took on average about seven times as many antibodies created by DENV-1 infection to neutralize Asian DENV-2 vs. American DENV-2. Jin and colleagues added an important element to the PRNT test, and then retested the Iquitos samples. The new, more sensitive test found that it took up to 100 times as many DENV-1 antibodies to neutralize the Asian DENV-2 virus as it did to the American DENV-2 infection. The results suggest that, in the harmless Iquitos outbreak, the second dengue serotype to hit the region was the American version of DENV-2, was cross-neutralized with relative ease by antibodies created by the first infection. In Cuba, however, the people were unfortunate to be hit by the Asian DENV-2 upon second infection, which their antibodies from DENV-1 infection could not shut it down, and only helped to deliver the virus into their cells. By magnifying the differences in the ability of an antibody for a given dengue serotype to neutralize other serotypes, researchers believe the new test will capture enhancement that the older test misses.

To construct the new test of cross-neutralization, researchers took CV-1 fibroblast cells, which share some traits with macrophages, and genetically engineered them to include a gene that directs for the building of an FC receptor on their surfaces. They also constructed a CV-1 cell line for culture without Fc receptors for use as a control group that resembles standard PRNT cultures used in the past. Both sets of cultures were then subjected the blood taken from patients in the 1995

Peru outbreak, and the new test captured for the first time the contribution of antibodies to more severe disease via fc/fc receptor delivery of virus to target cells.

Along with Jin, the work in Rochester was led by corresponding author Jacob Schlesinger, M.D., and Robert Rose, Ph.D., as well as by graduate student W. W. Shanaka I. Rodrigo, who conducted the genetic engineering experiments on CV-1 cells. Also contributing in Rochester were Danielle Alcena and Zhihua Kou. Tadeusz Kochel contributed from at the Naval Medical Research Center Detachment, Lima, Peru, as did Kevin Porter at the US Naval Medical Research Center, Silver Spring, Maryland. Guillermo Comach led a team as well at the Laboratorio Regional de Diagnostico e Investigacion del Dengue y otras Enfermedades Virales in Maracay Estado Aragua, Venezuela.

"Beyond the Peruvian case, our test promises to have a profound effect on the design of vaccines because we can take the antibodies generated by two different candidate vaccines, and better compare which strongly neutralizes virus without threat of enhancement across all four serotypes," Jin said. "With experimental vaccines from companies like GlaxoSmithKline and Sanofi Aventis entering Phase II and Phase III clinical trials this year, we hope the new test will be adopted widely and soon because it is more likely to catch enhancement."

Source: University of Rochester Medical Center