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ABSTRACT

With epidemics of dengue fever throughout the world, and the steady arrival of infected travelers arriving in Florida, local transmission is a major public health concern. However, public health consequences may be mitigated through targeted technological developments. The purpose of this project was to characterize transmission efficiency of *Aedes aegypti* using live-attenuated candidate dengue vaccines. This research contributes to an assessment of the potential for use of novel dengue vaccines for disease control in Florida.

The Co-PIs have developed live-attenuated vaccine candidates for dengue virus by incorporating a single highly conserved, broadly neutralizing epitope from dengue virus into the backbone of the yellow fever virus (YFV) vaccine 17D. These vaccine candidates are neutralized by a panel of anti-dengue human monoclonal antibodies that represent the primary protective antibody response in naturally infected dengue survivors. The potential use of a live-attenuated dengue vaccine for Florida’s public would also necessitate an appraisal of transmission efficiency in the vector *Ae. aegypti*. The success of the YFV vaccine (17D) is that *Ae. aegypti* is unable to transmit this live-attenuated virus. Because living virus is recovered from the blood stream (viremia) of vaccinated humans at very low levels, there is minimal risk that blood feeding mosquitoes may become infected with YFV (17D). The latter attribute is particularly important because it prevents the initiation of a transmission cycle and so the virus is unable to become virulent.

We evaluated susceptibility to infection and transmission potential of live attenuated dengue vaccine candidates 2-1 and 2-2 in Florida *Ae. aegypti*. For comparison, we also included the parental YFV vaccine (17D) as a benchmark as well as the virulent YFV strain Asibi. Viral infection rates were highest in YFV strain Asibi and 17D, whereas *Ae. aegypti* showed lowest infection rates to both dengue vaccine candidates 2-1 and 2-2. Viral dissemination rates were performed on a subsample of mosquitoes and were highest in YFV strain Asibi and not detected in 17D, 2-1, and 2-2, suggesting that the live attenuated vaccines encounter midgut escape barriers in *Ae. aegypti*. We found no evidence for detection of infectious viruses in the saliva of
mosquitoes from any of the treatment groups suggesting limited ability of transmission potential in *Ae. aegypti*. Our observations suggest that there is a very low probability that these dengue vaccine candidates would be capable of infecting and being transmitted by the primary dengue vector *Ae. aegypti*. Therefore, the potential risk of reversion to become more virulent is a highly unlikely scenario for these two dengue vaccine candidates.

**INTRODUCTION**

Dengue viruses are associated with the most numerous human infections of all the mosquito-borne viruses (WHO 2012). Locally transmitted dengue viruses have occurred in recent years in Florida posing a threat to public health. There have been substantial challenges to develop a vaccine for this pathogen because of the need to elicit a life-long protective antibody response against the four serotypes of dengue virus associated with human illness. Infection by any of the four dengue serotypes elicits a life-long protective antibody response against that serotype. However, these same antibodies do not protect against the other three serotypes. Instead, they often recognize and bind the viral particles of other serotypes without neutralizing them, and direct them to immune system cells such as macrophages and dendrocytes that express antibody receptors on their cell surfaces. These cells, which are not normally infected by dengue, become infected and increase the viral load and enhance disease severity. Thus, any dengue vaccine that does not induce broad protection against all four serotypes is potentially able to cause this enhancement effect.

One approach to developing an effective attenuated vaccine is through the use of chimeric vaccines that include components of more than one virus. The yellow fever virus (YFV) 17D vaccine has served as a model system to the development of other vaccines by replacing the entire surface protein region of yellow fever with the surface protein region from other flaviviruses (McGee et al. 2008, Chambers et al. 1999, Monath et al. 1999). This approach has been used to develop the Sanofi tetravalent Dengvaxia vaccine against dengue. However, this vaccine shows low levels of protection, and has recently been linked to more severe clinical disease outcomes, suggestive of enhancement (Halstead and Russell 2016).

The approach taken in the laboratory of the Co-PIs is based on investigation of the human antibody response to dengue infection. We were the first investigators to study individual human monoclonal antibodies against dengue and to show that different human antibodies could have either neutralizing or non-neutralizing activity (Schieffelin et al. 2010). We, and others, found that one epitope in particular is also nearly identical (highly conserved) across all four serotypes of dengue, and antibodies that recognize this epitope have broadly neutralizing activity against all four serotypes of dengue (Costin et al. 2013, Dejnirattisai et al. 2014, Rouvinski et al. 2015). We used this information to create chimeric virus candidates with the conserved dengue neutralizing epitope exchanged into the approved YFV 17D vaccine.

Attenuated vaccines are live vaccines that will interact with the target host population as well as vectors that transmit the virus. The YFV vaccine 17D can be recovered from the blood stream of vaccinated humans (i.e., viremia). However, the quantity of virus in the blood is not sufficiently high enough to enable blood feeding mosquitoes to become infected and transmit
the virus (Whitman 1939). While there have been some instances of mosquito infection with the vaccine, those mosquitoes were incapable of transmission (Whitman 1939). Thus, the vaccine has proven to be very safe and effective because the live vaccine does not establish a transmission cycle between humans and mosquitoes which may allow for the possibility of subsequent evolution of more virulent forms.

One concern in the development of live attenuated vaccines for dengue viruses is whether they may revert to become more virulent. An often stated hypothetical risk associated with the use of live attenuated virus vaccines is that there is the potential for recombination-driven reversion to become more virulent if an arthropod was infected with both a wild type and vaccine virus (McGee et al. 2008). However, there is no experimental or field data to suggest that this has occurred and so it is a highly unlikely scenario (Hahn et al. 1987). Regardless of whether this scenario could occur, it would be a nonissue if the mosquitoes were not competent for virus transmission.

Here we evaluated the transmission efficiency of *Ae. aegypti* for live-attenuated dengue vaccines. Susceptibility to infection and transmission of the live attenuated vaccines were compared to the parental YFV vaccine (17D) as a benchmark as well as the virulent YFV strain Asibi. The candidate dengue vaccines are based on the YFV vaccine (17D) which was originally derived from the Asibi strain and so are appropriate comparisons.

**METHODOLOGY**

**PLAN OF RESEARCH**

*Virus isolates and mosquitoes.* Virus strains, including yellow fever 17D vaccine strain, pathogenic Asibi strain, and two chimeric dengue/yellow fever variants (2-1 and 2-2) were grown in African green monkey kidney (Vero) cells, according to standard procedures used in our laboratories. All personnel working with these viruses were vaccinated with yellow fever 17D through the department of health as a precaution. We used F3 generation of *Ae. aegypti* from field collections in Key West, FL to initiate this study. Mosquitoes were reared to adulthood on a diet of brewer’s yeast and lactalbumin at 26-28°C. Adults were housed together and provided with 10% sucrose by cotton wicks. Adults aged 11-13 days were used for the virus infection studies in the biosafety level-3 virology facility at the Florida Medical Entomology Laboratory.

*Comparison of the growth of the viruses.* Initial tests compared virus growth over time in cultured Vero cells for the live attenuated dengue and yellow fever (17D) vaccines as well as the virulent Asibi strain of yellow fever virus using standard procedures used in our laboratories (Shin et al. 2013). Monolayers of Vero cells in T-175 cm² flasks were inoculated with 200 μl of test virus and incubated for 1 hr at 37°C and 5% CO2 atmosphere after which 24 ml media (Medium 199 supplemented with 10% fetal bovine serum, penicillin/streptomycin, mycostatin) were added to each flask and incubated for an additional six days. Cell culture supernatant samples were collected daily, and replaced with fresh media, from infected cell cultures, and the concentration of virus was determined by plaque forming tests (Costin et al. 2013). The comparative growth studies provided necessary information on virus kinetics as well as
informed us on the appropriate timing to collect virus cultures for the preparation of infectious blood meals for mosquitoes, controlling that mosquitoes are exposed to similar viral titers among the viruses.

Mosquito infection.

Freshly propagated virus was used for the mosquito infection studies. Media from the infected cell cultures and blood were combined on the day of the feeding trial. Ten to 13-day old adult females (F1-3 generation) will be provided with virus infected defibrinated bovine blood (Hemostat, Dixon, CA) using an artificial membrane feeding system (Hemotek, Lancashire, United Kingdom) as described previously (Alto et al. 2014a, Alto and Bettinardi 2013). Infectious blood meals will contain one of the following types of live viruses; candidate dengue vaccines, yellow fever vaccine (17D), or virulent Asibi strain of yellow fever virus. Mosquitoes were provided with a low and high dose of infectious blood. After the feeding trials, fully engorged females were maintained in cages in an incubator with a 14:10 hour light:dark photoperiod, 30°C, and access to a 10% sucrose solution. After the feeding trials, fully engorged females were held in 16 oz. cylindrical cages for 14 days and then tested for transmission potential by collecting saliva in capillary tubes with immersion oil using methods previously described (Alto et al. 2014b). Individual mosquito bodies, legs, and saliva were assayed by a plaque forming test to determine infection, disseminated infection, and transmission potential. These methodologies enable us to identify midgut and salivary gland barriers to viral transmission.

RESULTS AND DISCUSSION

Virus kinetics were compared in cell culture between dengue vaccine candidates (2-1 and 2-2), 17D, and the virulent Asibi strain of yellow fever virus. During the initial growth phase, both of the dengue vaccine candidates reached the highest titer by 2 days post infection (dpi) whereas 17D and the Asibi strain of yellow fever highest titers were reached by 3 dpi. These observations suggest a replicative advantage of the dengue vaccine candidates in cell culture relative to the other virus treatment groups. Titers were highest in both dengue vaccine candidates and Asibi strain of yellow fever, whereas the yellow fever vaccine 17D titers were approximately 33x lower than the other virus treatment groups. Overall, there were declines in virus titer for all virus treatment groups during the later growth phase.

We evaluated susceptibility to infection and transmission potential of live attenuated dengue vaccine candidates 2-1 and 2-2 in Florida Ae. aegypti. For comparison, we also included the parental YFV vaccine (17D) as a benchmark as well as the virulent YFV strain Asibi. Viral infection rates were highest in YFV strain Asibi (low dose, 66.7%; high dose 71.0%) and 17D (low dose, 52.8%; high dose 70.3%), whereas Ae. aegypti showed lowest infection rates to both dengue vaccine candidates 2-1 (low dose, 18.4%; high dose 23.9%) and 2-2 (low dose, 18.3%; high dose 24.5%). The low dose treatment resulted in similar or lower infection rates compared to the high dose treatment. Viral dissemination rates were performed on a subsample of mosquitoes and were highest in YFV strain Asibi (30%) and not detected in 17D (0%), 2-1 (0%), and 2-2 (0%), suggesting that the live attenuated vaccines encounter midgut escape barriers in Ae. aegypti. We found no evidence for detection of infectious viruses in the saliva (0%) of
mosquitoes from any of the treatment groups suggesting limited ability of transmission potential in *Ae. aegypti*. These results suggest substantial barriers to infection (midgut infection and escape barriers and possibly salivary gland barriers) in *Ae. aegypti* infected with dengue vaccine candidates 2-1 and 2-2. In comparison, *Ae. aegypti* had an inefficient midgut infection barrier towards YFV strain Asibi and 17D, but substantial midgut escape barriers and possibly salivary gland barriers. Our observations suggest that there is a very low probability that these dengue vaccine candidates would be capable of infecting and being transmitted by the primary dengue vector *Ae. aegypti*. Therefore, the potential risk of reversion to become more virulent is a highly unlikely scenario for these two dengue vaccine candidates.

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References


