GROWTH CHARACTERISTICS OF CHIMERIC VACCINE VIRUSES IN Aedes aegypti AND Aedes albopictus FROM THAILAND

STEPHEN HIGGS,* DANA L. VANLANDINGHAM, KIMBERLY A. KLINGLER, KATE L. MCELROY, CHARLES E. MCGEE, LAURA HARRINGTON, JEAN LANG, THOMAS P. MONATH, AND FARSHAD GUIRAKHOO
Department of Pathology, University of Texas Medical Branch, Galveston, Texas; Department of Entomology, Cornell University, Ithaca, New York; Sanofi Pasteur, Campus Mérieux, Marcy-L’etoile, France; Acambis, Inc., Cambridge, Massachusetts

Abstract. Four chimeric yellow fever (YF) 17D-dengue (DEN) candidate vaccine viruses (ChimeriVax™-DEN; Acambis, Cambridge, MA) were characterized in Aedes aegypti and Ae. albopictus mosquitoes collected from Thailand. The four vaccine viruses contained the relevant prM and E genes of wild-type dengue viruses (DENV, serotypes 1-4) substituted for the equivalent genes in the YF vaccine virus (17D) backbone. Each chimera conferred protection against the homologous DENV serotype; a tetravalent mix of all four chimeras stimulates an immune response against all serotypes. Field-collected mosquitoes from Thailand were fed on blood containing each of the viruses under study and held 21 days after infection. Infection and dissemination rates were based on antigen detection in the body or head tissues, respectively. All four wild-type DENV serotypes infected and disseminated, but the candidate vaccine viruses were highly attenuated in mosquitoes with respect to infection and especially with respect to dissemination. Considering the low level viremias anticipated in humans vaccinated with these viruses, it is predicted that the risks of infection and transmission by mosquitoes in nature is minimal.

INTRODUCTION

Dengue viruses (DENVs) are important arthropod-borne viruses that are emerging in many regions of the world. Coincident with the spread of different serotypes, the number of cases of dengue hemorrhagic fever (DHF) has increased dramatically throughout Asia and more recently in Latin America, with ~50-100 million cases annually and > 2.5 billion people at risk of infection.1 Thailand suffers from one of the highest rates of dengue fever and DHF in the world, primarily affecting children < 15 years of age.2 DHF first occurred in the Americas in 1981, and by 1998, had spread to 24 countries, resulting in ~10,000 cases.1 Currently, vector control is the only option available to limit the number of dengue cases; thus, the addition of an effective DENV vaccine would aid in combating this disease. A DENV vaccine would be the third mosquito-borne live attenuated flavivirus vaccine licensed for human use; the other two flavivirus vaccines are for protection against yellow fever virus (YFV) and Japanese encephalitis virus (JEV).

The ChimeriVax™ platform developed by Acambis, Inc. uses the live attenuated 17D vaccine strain of YFV, which has a long history of safe and efficacious use. Vaccines for JEV, West Nile virus (WNV), and DENV have been developed based on this system and have been characterized both in vitro and in vivo.2-15 A YFV/St. Louis encephalitis chimeric virus has been produced as a diagnostic tool.17 Previous vector-competence studies of ChimeriVax™ viruses have used both established colonies of mosquitoes and up to the fifth generation after collection of wild mosquito populations.18-20 Bhatt and others20 reported that ChimeriVax™-JE (YF/JE SA14-14-2) virus was not orally infectious for any of the species tested (Culex triaeniorhynchus, Aedes aegypti, Ae. albopictus) despite the high titer of virus to which the mosquitoes were exposed (8.9 log10 pfu/mL). Although the virus replicated in Ae. aegypti and Ae. albopictus after intra-thoracic inoculation, it did not replicate in the natural vector Cx. triaeniorhynchus. Replication in the vector did not result in genetic change. The ChimeriVax™-WNV is similarly attenuated in mosquitoes, with restricted replication and no dissemination in Cx. tritaeniorhynchus, Cx. nigripalpus, Cx. quinquefasciatus, Ae. aegypti, and Ae. albopictus.20 Vector competence studies with ChimeriVax™-DEN2 virus focused on Ae. aegypti and Ae. albopictus.21 When 6.3-7.7 log10 pfu/mL of virus was fed to mosquitoes, 17% of Ae. aegypti and 24% of Ae. albopictus were infected after 14 days; however, no dissemination occurred. Field-collected Ae. aegypti from Puerto Rico, DeQuincy, and the Republic of Vanautu were 100% susceptible to infection with DENV-2. However, ChimeriVax™-DEN2 virus replication was highly restricted in these populations, with infection rates ranging from 6% to 32% and no dissemination of virus in any population. A subsequent study19 used a highly sensitive real-time reverse transcriptase-polymerase chain reaction (RT-PCR) to determine the replication kinetics of the ChimeriVax™-DEN 1, 2, 3, 4 tetravalent virus mixture in Ae. aegypti. Growth rates of each chimeric virus in intrathoracically inoculated mosquitoes were similar regardless of whether it was used in the tetrav lent mix or as a single serotype infection. However, the viruses were highly attenuated when presented to mosquitoes in a blood meal, and the authors concluded that there is very little transmission potential for these viruses.

Relatively few species of mosquito have been implicated in the DENV transmission cycle. In Asia, Ae. aegypti, Ae. albopictus, Ae. malaysensis, Ae. scutellaris group, and the Ochlerotatus niveus-group mosquitoes have been identified as potential vectors, but of these, most are probably of minor importance. For human epidemics, only Ae. aegypti and Ae. albopictus have played a significant role. In the South Pacific, Ae. polynesiensis may potentially be an important vector.

Recent concerns expressed about the potential for vaccine viruses to revert to a virulent phenotype may be unwarranted.22 However, because of, for example, the potential risks of inadvertently administering live vaccines to immunocompromised individuals (who may develop higher viremias), including those with HIV/AIDS living in areas at high risk for dengue, it is important to determine the transmission potential of live attenuated chimeric viral vaccines. The purpose of

* Address correspondence to Stephen Higgs, Department of Pathology, Keiller 2.104 301 University Boulevard, Galveston, TX 77555-0609. E-mail: sthiggs@utmb.edu

Copyright © 2006 by The American Society of Tropical Medicine and Hygiene
this research was therefore to determine the susceptibility of mosquitoes from natural populations in Asia, where DENV vaccines are most needed, to infection with ChimeriVax viruses. Mosquitoes were obtained from Thailand and reared at University of Texas Medical Branch (UTMB), Galveston, TX. The field-collected mosquitoes were expanded in the laboratory, and second-generation adults were orally challenged with the viruses to determine infection and dissemination rates. Genetically these mosquitoes should be representative of the natural populations with respect to their susceptibility to infection.

MATERIALS AND METHODS

Viruses. The wild-type viruses and ChimeriVax™-DEN1, 2, 3, and 4 viruses were obtained as frozen stocks from Acambis (Cambridge, MA). YF vaccine YF-VAX (17D) (Aventis Pasteur, lot no. UE263AA) was reconstituted according to the manufacturer’s instructions. The wild-type parent viruses used for construction of ChimeriVax™-DEN viruses were DENV-1 (strain PUO359), DENV-2 (strain PUO218), DENV-3 (strain PaH 881/88), and DENV-4 (strain 1288). Parental wild-type viruses and the ChimeriVax™ vaccine candidates were thawed once to prepare aliquots and stored at −80°C.

Mosquitoes. Aedes aegypti eggs were collected in the spring of 2004 from Mae Sot Province, Thailand (16°45’N, 98°33’E), and Aedes albopictus eggs were collected in the summer 2004 from Surat Thani (ST) Province, Thailand (9°34’N, 90°07’E). Both species were reared for several generations, and F2 eggs were collected and stored for use in infectious feeds.

Mosquito infection. Experimental mosquitoes, 7–10 days after emergence, were deprived of sugar for 24 hours and of water for 12 hours before being presented with the artificial infectious blood meal. The artificial blood meal contained equal parts of defibrinated sheep blood and freshly harvested virus, presented through a Hemotek feeder. Mosquitoes were allowed to feed for 30 minutes. To obtain freshly harvested virus, C6/36 (Ae. albopictus) cells were infected with wild-type dengue viruses at a multiplicity of infection (MOI) of 0.01. Supernatant medium was harvested at 6 (DENV-1) or 7 days (DENV-2, -3, -4) post-infection (p.i.) for immediate incorporation into the infectious blood meal. Vero (green monkey kidney) cells were infected with YF-VAX and ChimeriVax™-DEN1, 2, 3, and 4 viruses, also at an MOI of 0.01, and the supernatant was harvested after 6 days. For the tetravalent mix, 1.5 mL each of ChimeriVax™-DEN1, 2, 3, and 4, and 5 mL of this mixture was added to 5 mL of defibrinated sheep blood. Each blood meal was heated to 37°C just before feeding. Mosquitoes engorged to ≥ 70% capacity were kept and incubated at 28°C. Three mosquitoes were collected on days 0 and 1. All remaining mosquitoes were collected on day 21. Mosquitoes were stored at −80°C until assayed for infection. Oral infection was divided into two sets. In the first set, Ae. albopictus and Ae. aegypti were exposed to wild-type DENV-1, 2, 3, and 4 and the tetravalent mix. In the second set, Ae. albopictus and Ae. aegypti were exposed to ChimeriVax™-DEN1, 2, 3, and 4 and YF-VAX.

As negative controls, Ae. albopictus F2, Thailand were fed a non-infectious meal containing equal parts of culture supernatant collected from uninfected cells. One negative control mosquito was titrated with all sets of infected mosquitoes. To produce positive control mosquitoes, aliquots of wild-type DENV-1, 2, 3, and 4; ChimeriVax™-DEN1, 2, 3, and 4; and YF-VAX viruses were thawed and immediately used for intrathoracic inoculation of 7- to 10-day-old mosquitoes25 (0.5 μL/mosquito). Inoculated mosquitoes were maintained for 21 days at 28°C and collected and stored at −80°C pending analysis. Positive control mosquitoes were titrated with all sets of orally infected mosquitoes.

Determination of virus titer. Mosquito bodies and heads were assayed for infectious virus separately to determine overall infection rate and disseminated infection rate, respectively. Mosquito bodies were triturated in 1 mL of L-15 media (10% fetal bovine serum (FBS) + 10% tryptophosphate broth (TPB) + 100 units/mL penicillin + 100 μg/mL streptomycin + 1 μg/mL fungizone). One hundred microliters of each sample was loaded in duplicate and titrated in serial 10-fold dilutions in Vero cell culture in the first 8 rows of a 96-well plate. Mosquito heads were triturated in 150 μL of L-15 medium (same as above) and titrated in serial 10-fold dilutions in Vero cells in the last 4 wells of the same rows as corresponding bodies. Titration plates were incubated at 37°C for 10 days and fixed with 3:1 acetone-phosphate-buffered saline (PBS) for 10 minutes, dried, and stored at −20°C until analyzed by immunofluorescence assay (IFA). Mosquitoes that had ingested the tetravalent mix of chimeric viruses were titrated in quadruplicate, so that samples could be assayed for each of the four ChimeriVax™ vaccine serotypes. Therefore, only 10 μL of both bodies and heads were loaded into plates to ensure there would be enough sample for each assay.

Immunofluorescence assay. Viruses differed in their capacity to produce cytopathic effect (cpe) on Vero cells. While all of the ChimeriVax™ viruses consistently caused cpe, wild-type viruses were variable. In preliminary tests using titrations of stock ChimeriVax™ viruses, it was determined that wells without cpe were always negative when tested for IFA. All titration plates containing wild-type DENV were therefore tested by IFA26 to determine the endpoint (i.e., the highest dilution at which antigen was detected). Because the ChimeriVax™-DEN1, 2, 3, and 4 and YF-VAX viruses caused cpe in Vero cells, only plates in which cpe were observed were tested by IFA.

A variety of primary antibodies were used. Selection of the antibody and optimal dilution to give consistently bright and readable IFA data was based on visual evaluation using Vero cells infected with each of the viruses, grown on glass coverslips,27 and using positive and negative control mosquitoes titrated on Vero cells in 96-well plates. ChimeriVax™-DEN1, 2, 3, and 4 and the tetravalent mix plates were stained using serotype-specific antibodies provided by Acambis. Acambis DENV-1, -2, or -4 antibodies were used at a 1:200 dilution, and the Acambis DENV-3 antibody was used at a 1:100 dilution. Plates assayed for YF-VAX were stained using a hyperimmune mouse serum (designated MA93, produced by S. Higgs in 1993) at 1:500 dilution. All of the plates assayed for the viruses mentioned above were incubated overnight at 4°C, before the application of a secondary antibody, anti-mouse Ig, biotinylated, species-specific whole antibody from sheep (Amersham Biosciences, Piscataway, NJ) at 1:200 for 40 minutes at 37°C, followed by streptavidin fluorescein (1:200) for 10 minutes at 37°C. DENV-1, 2, 3, and 4 plates were stained with a rabbit-anti-DENV hyperimmune serum at 1:
100 (produced by S. Higgs in 1994) for 40 minutes at 37°C followed by a secondary antibody, anti-rabbit, Ig, biotinylated, species-specific whole antibody from donkey (Amersham Biosciences) at 1:100 for 40 minutes at 37°C and streptavidin fluorescein (1:200) for 10 minutes at 37°C. 1.4
Diazobicyclo(2.2.2)Octane (DABCO)-glycerol mounting solution was added to all plates, which were scored using an Olympus IX70 inverted epifluorescence microscope. Infection and dissemination rates were compared with Fisher exact test. 

RESULTS

Viral titers in triturates of whole bodies were obtained on days 0 and 21 p.i. for wild-type DEN 1-4, for ChimeriVaxDEN1-4 individually and as part of the tetravalent mix, and for YF-VAX in both Ae. albopictus and Ae. aegypti mosquitoes (Table 1). Mean titers with SEs were calculated based on titers from all positive mosquitoes. Mosquito bodies and heads were assayed separately to determine infection and dissemination rates in both mosquito species (Figure 1). Infection and dissemination rates were expressed as the percent of the total number of mosquitoes tested. The results of previous experiments have shown that the presence of antigen in the head is invariably indicative of salivary gland infection and the ability of a mosquito to transmit DENV. Positive and negative control mosquitoes were included in the analysis of each virus set.

Ae. albopictus, Thailand. The infection rate of wild-type DENV-1 (20/20) was significantly higher than the rates of ChimeriVaxDEN1-4 (3/20), ChimeriVaxDEN1 in the tetravalent mix (0/20), and YF-VAX (2/20) (Fisher’s exact test, P < 0.05; Table 2). The infection rate of wild-type DENV-2 (12/13) was also found to be significantly higher than those of ChimeriVaxDEN2, (0/20) ChimeriVaxDEN2 in the tetravalent mix (1/20), and YF-VAX (2/20) (P < 0.05; Table 2). However, the rate of infection of DENV-3 (6/20) was significantly above that of only ChimeriVaxDEN3 in the tetravalent mix (0/20) (Table 2). The infection rate of DENV-4 (6/20) was not significantly different (P > 0.05) from ChimeriVaxDEN4 in the tetravalent mix (2/20), or from YF-VAX (2/20) (Figure 1A; Table 2).

The ChimeriVaxDEN1 infection rate (3/20) was not significantly different from the other ChimeriVaxDEN vaccines either individually or in the tetravalent mixture (P > 0.05). The infection rate for ChimeriVaxDEN2 (0/20) was significantly lower (P < 0.05) than those for ChimeriVaxDEN3 (5/20) and ChimeriVaxDEN4 (8/20) alone. ChimeriVaxDEN3 (5/20) was significantly higher (P < 0.05) than ChimeriVaxDEN3 in the mixture (0/20). No significant difference (P > 0.05) was found between the ChimeriVaxDEN1-4 in the tetravalent mixture (0–2/20) compared with each other in the tetravalent mix or compared with YF-VAX (2/20) (Table 2).

Comparison of total dissemination rates, defined as the number of positive heads per total number mosquitoes tested, revealed differences between wild-type DENV-2 (77%) and ChimeriVaxDEN2 in the tetravalent mix (0), between wild-type DENV-4 (25%) and ChimeriVaxDEN4 in the tetravalent mix (0), and between YF-VAX (5%) and DENV-1 (100%) and DENV-2 (77%) (Table 2).

Infection and dissemination rates of the ChimeriVax vaccines, both individually and in the tetravalent mix, were generally lower than those of wild-type DENV viruses and close to that of YF-VAX (Figure 1A; Table 2). The infection rates of ChimeriVaxDEN3 (5/20 mosquitoes) and DENV-3 (6/20 mosquitoes) were similar, but the dissemination rate of ChimeriVaxDEN3 was considerably lower than that of its wild-type counterpart (5% versus 30%, respectively; Figure 1A).

Only ChimeriVaxDEN4 had an infection rate higher than that of its wild-type counterpart (8/20 versus 6/20, respectively; Figure 1A; Table 2); however, the difference was not significant (P > 0.05). The infection rate of the individually fed ChimeriVaxDEN4 (8/20) was higher than that of ChimeriVaxDEN4 in the mix (2/20) (Table 2). Importantly, the dissemination rate of ChimeriVaxDEN4 was lower than that of DENV-4 (10% versus 25%; Figure 1A).

One difference between the ChimeriVaxDEN4 alone and the ChimeriVaxDEN4 in the mix that might have contributed to the observed difference in the infection rate was that the titer of the ChimeriVaxDEN4 blood meal was ~1.5 logs higher than that of ChimeriVaxDEN4 in the tetravalent mix blood meal (Table 1). However, this was also

<p>| Table 1 |
| Comparison of day 0 p.i. and day 21 p.i. viral titers analyzed by titration of the bodies of Ae. albopictus and Ae. aegypti mosquitoes |</p>
<table>
<thead>
<tr>
<th>Virus</th>
<th>Infectious body titer†</th>
<th>Ae. albopictus body titer†</th>
<th>Ae. aegypti body titer†</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT DENV-1</td>
<td>5.52</td>
<td>Negative</td>
<td>20</td>
</tr>
<tr>
<td>ChimeriVaxDEN1</td>
<td>6.95</td>
<td>2.62 ± 0.58</td>
<td>20</td>
</tr>
<tr>
<td>WT DENV-2</td>
<td>5.52</td>
<td>3.00 ± 0.50</td>
<td>13</td>
</tr>
<tr>
<td>ChimeriVaxDEN2</td>
<td>5.95</td>
<td>2.44 ± 0.14</td>
<td>20</td>
</tr>
<tr>
<td>WT DENV-3</td>
<td>3.95</td>
<td>Negative</td>
<td>20</td>
</tr>
<tr>
<td>ChimeriVaxDEN3</td>
<td>3.95</td>
<td>1.83 ± 1.26</td>
<td>20</td>
</tr>
<tr>
<td>WT DENV-4</td>
<td>4.52</td>
<td>1.50 ± 0.39</td>
<td>20</td>
</tr>
<tr>
<td>ChimeriVaxDEN4</td>
<td>4.52</td>
<td>3.44 ± 0.14</td>
<td>20</td>
</tr>
<tr>
<td>ChimeriVaxDEN tetravalent mix</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ChimeriVaxDEN1</td>
<td>4.52</td>
<td>1.27</td>
<td>20</td>
</tr>
<tr>
<td>ChimeriVaxDEN2</td>
<td>5.95</td>
<td>2.25 ± 0.87</td>
<td>20</td>
</tr>
<tr>
<td>ChimeriVaxDEN3</td>
<td>3.95</td>
<td>1.27</td>
<td>20</td>
</tr>
<tr>
<td>ChimeriVaxDEN4</td>
<td>4.52</td>
<td>2.95 ± 0.00</td>
<td>20</td>
</tr>
<tr>
<td>YF-VAX</td>
<td>4.95</td>
<td>3.66 ± 0.25</td>
<td>20</td>
</tr>
</tbody>
</table>

Tetravalent mixture data was obtained using monoclonal antibodies specific for each of the four DENV serotypes on four replicate 96-well plates.

* Blood-meal titers are reported as log_{10}TCID_{50}/mL.
† Titer of mosquito bodies are reported as log_{10}TCID_{50}/mosquito.
true of the blood meals containing ChimeriVax™-DEN1 and ChimeriVax™-DEN1 as part of the mix where the infection rates in *Ae. albopictus* did not differ as markedly (Table 1). Overall, 19/160 (11.8%) of *Ae. albopictus* that were fed the ChimeriVax™ vaccines became infected, but only 4/160 (2.5%) developed a disseminated infection.

*Ae. aegypti*, Thailand. The infection rates of the wild-type DENV viruses were all significantly higher than their vaccine counterparts, either individually or as part of a tetravalent mix (Table 2). All of the wild-type DENV infection rates were also significantly higher than that of YF-VAX (Table 1; Figure 1B). No significant difference (*P* > 0.05) was found between the infection rates of the ChimeriVax™-DEN-1, 2, 3 vaccine viruses, when used singly (all 0) or in the tetravalent mix (all 0), compared with ChimeriVax™-DEN4 (1/17) (Figure 1B). There was a significant difference (*P* < 0.05) between ChimeriVax™-DEN-4 (1/17) and ChimeriVax™-DEN-4 in the tetravalent mix (0/14) and between ChimeriVax™-DEN4 (1/17) and YF VAX (0/14) (Table 2). No other significant differences were determined for infection rates. Among the

![Figure 1](image)

**Figure 1.** Infection and dissemination rates of wild-type and ChimeriVax™-infected mosquitoes. (A) *Ae. albopictus* Thailand. (B) *Ae. aegypti* Thailand.

<table>
<thead>
<tr>
<th>Virus</th>
<th><em>Ae. albopictus</em></th>
<th><em>Ae. aegypti</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. infected (%)</td>
<td>Disseminated</td>
</tr>
<tr>
<td>WT DENV-1</td>
<td>20/20 (100)</td>
<td>20/20 (100)</td>
</tr>
<tr>
<td>ChimeriVax™-DEN1</td>
<td>3/20 (15)</td>
<td>1/20 (5)</td>
</tr>
<tr>
<td>WT DENV-2</td>
<td>12/13 (92)</td>
<td>10/13 (77)</td>
</tr>
<tr>
<td>ChimeriVax™-DEN2</td>
<td>0/20 (0)</td>
<td>None</td>
</tr>
<tr>
<td>WT DENV-3</td>
<td>6/20 (30)</td>
<td>6/20 (30)</td>
</tr>
<tr>
<td>ChimeriVax™-DEN3</td>
<td>5/20 (25)</td>
<td>1/20 (5)</td>
</tr>
<tr>
<td>ChimeriVax™-DEN4</td>
<td>8/20 (40)</td>
<td>2/20 (10)</td>
</tr>
<tr>
<td>ChimeriVax™-DEN tetravalent mix</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ChimeriVax™-DEN1</td>
<td>0/20 (0)</td>
<td>None</td>
</tr>
<tr>
<td>ChimeriVax™-DEN2</td>
<td>1/20 (5)</td>
<td>None</td>
</tr>
<tr>
<td>ChimeriVax™-DEN3</td>
<td>0/20 (0)</td>
<td>None</td>
</tr>
<tr>
<td>ChimeriVax™-DEN4</td>
<td>2/20 (10)</td>
<td>None</td>
</tr>
<tr>
<td>YF-VAX</td>
<td>2/20 (10)</td>
<td>1/20 (5)</td>
</tr>
</tbody>
</table>
four ChimeriVax™ vaccines, ChimeriVax™-DEN4 had the highest rate of infection in Ae. aegypti and Ae. albopictus (1/17 and 8/20, respectively) and also had the highest dissemination rate in Ae. albopictus (10%) (Table 2).

Overall, the infection rates of the ChimeriVax™ vaccines in Ae. aegypti (Thailand) were extremely low (0–6%), compared with those of the wild-type viruses (47–77%) and similar to that of the vaccine strain YF-VAX (0%) (Table 2), which is known to have a safe track record and not to be transmitted by mosquitoes. Only 1 of 124 Ae. aegypti mosquitoes, which ingested the ChimeriVax™ vaccines either individually or as part of the mix, became infected (0.8%; Table 2), and none developed a disseminated infection (Figure 1B; Table 2).

**DISCUSSION**

Despite a relatively long history of research on arthropod-borne viruses and their vectors, knowledge about the interactions between viruses and vectors is often rudimentary and species specific. The reason for this vector specificity is unknown. Numerous studies have discussed intraspecific variation of Ae. aegypti with respect to susceptibility to infection with arboviruses. Vector competence studies reveal considerable variation between mosquito strains and often poor reproducibility. Susceptibility to YFV is different in strains collected from different geographic locations. Lourenço de Oliveira and others found that the ability of a Brazilian YFV isolate to disseminate in field-collected mosquitoes from YF endemic regions throughout Brazil ranged from 11.1% to 46.4%. Other studies have found that colonization affects susceptibility to oral infection and genetic selection has been used to produce resistant and susceptible phenotypes. For this reason, our study on chimeric DENV vaccine candidates used mosquitoes representing the two most important natural vectors of DENV, Ae. aegypti and Ae. albopictus, that were only second-generation progeny from eggs collected in Thailand where DENVs circulate endemically.

Vector competence studies have been an important component of previous studies to characterize candidate live attenuated vaccine viruses. Typically, these viruses are poorly infectious to mosquitoes, with low transmission rates. For the DENV-2 S-1 vaccine virus, an average of 16% of mosquitoes became infected, and none transmitted (versus 56% and 14%, respectively, for the parent). It was estimated that the vaccine strain was ~63 times less efficient than the parent strain in orally infecting Ae. aegypti. A few mosquitoes that were allowed to feed on humans infected with the S-1 strain became infected, but dissemination and transmission did not occur. By intra-thoracic inoculation, both viruses produced similar infection and dissemination rates (98–100%). In similar studies, a DENV-4 vaccine candidate (PDK35-TD3 FRhL p3) was attenuated for Ae. aegypti, but DENV-1 (45AZ5) and DENV-3 candidates (CH53489) were not. In comparison with its wild-type DENV-4 strain 814669 parent, a live attenuated DENV-4 vaccine (2AΔ30) produced by reverse genetics is slightly restricted in its ability to infect Ae. aegypti midguts and restricted in its ability to disseminate to the salivary glands. Importantly, the 2AΔ30 vaccine virus was not transmitted to Ae. albopictus mosquitoes fed on human vaccines. JEV vaccine candidate strain SA14-2-8 was attenuated in mosquitoes compared with the parent (SA14) and remained avirulent after mosquito passage.

Of particular relevance to this study, it has long been known that, whereas wild-type YFV can infect, disseminate, and be transmitted by Ae. aegypti, the 17D vaccine derivative can only infect midgut epithelial cells but cannot disseminate and be transmitted when mosquitoes are infected orally. The French neurotropic vaccine (FNV) is similarly attenuated in mosquitoes. In one study, when Ae. aegypti were presented with virus in a blood meal (viral titers 7.2–8.3 log10 PFU/mL), dissemination rates ranged from 90% to 100% for wild-type YFV but were only 3% for YFV 17D. Jenner and others reported that midguts became infected with 17D but also observed a lack of dissemination.

In this study, test viruses were propagated using the optimal conditions recommended by Acambis to maximize the titer presented in blood meals to mosquitoes. The highest titer was produced by YF-VAX virus, which as our gold standard control, is regarded as a safe and efficacious attenuated vaccine virus that is not transmissible by mosquitoes. Experimental mosquitoes imbibed relatively high viral titers compared with those titers typically observed in primate vaccinated with the ChimeriVax™ viruses and those that they would likely encounter if feeding on vaccinated humans. Despite this deliberate bias to maximize the potential for vector infection, the candidate vaccines were highly attenuated, although the development of disseminated infections with all of the wild-type DENV serotypes showed susceptibility. Lack of infection with ChimeriVax™ viruses is therefore indicative of viral attenuation and not of mosquito refractoriness. In the principle DENV vector, Ae. aegypti, no infections were observed with ChimeriVax™-DEN1, 2, and 3 viruses. A 6% infection rate of Ae. aegypti with ChimeriVax™-DEN4 failed to result in dissemination. In Ae. albopictus, ChimeriVax™-DEN4 infected 10% more mosquitoes than its wild-type counterpart, but disseminated in 15% fewer mosquitoes than the wild-type. These apparent differences between the DENV-4 serotype viruses and other serotypes seem to reflect the natural phenotype of the wild-type DENV-4 as reported by others. The ChimeriVax™-DEN4 virus does, however, replicate to lower titer than YF-VAX, and so at titers anticipated in vaccinees is highly unlikely to infect vectors. The transient relatively low viremias observed in humans after immunization with, for example, the ChimeriVax™-DEN2 vaccine provides an additional safety characteristic likely to prevent vector infection.

In an overall comparison of the two mosquito species, Ae. aegypti was generally less susceptible to infection than Ae. albopictus, with lower dissemination rates and typically with lower viral titers attained after 21 days of incubation. Importantly, Ae. aegypti, the predominant vector of DENV, had the lowest infection and dissemination rates for the ChimeriVax™ vaccines when fed individually and the lowest infection rate for all ChimeriVax™ vaccines when presented as a tetravalent mix.

ChimeriVax™-DEN1, ChimeriVax™-DEN2, ChimeriVax™-DEN3, and the ChimeriVax™-DENV tetravalent mix viruses were never detected in Ae. aegypti at 21 days p.i., despite the presence of up to 4 log10 TCID50 of virus immediately after feeding. When fed separately, ChimeriVax™-DEN4 virus was detected by whole body titration at 21 days p.i., but the titer was only 1.52 log10 TCID50, with the virus presumably restricted to the midgut because no dissemination was observed. YF-VAX failed to infect Ae. aegypti. In Ae. aegypti...
albopictus, disseminated infections were observed for ChimeriVax™-DEN1, ChimeriVax™-DEN3, and ChimeriVax™-DEN4 viruses; however, the rates were considerably lower than observed for the wild-type parent viruses. We hypothesize that if these mosquitoes were exposed to the low viral titers developed in vaccinated humans, these infection and dissemination rates would be lower, or zero, because the titers will likely be below the infection threshold. Although ChimeriVax™-DEN2 and ChimeriVax™-DEN4 viruses were detected in Ae. albopictus after feeding with the tetravalent mix, infection rates were only 5% and 10%, respectively, with no dissemination. This is important because it is likely that these viruses will be used in the context of a mixture to induce broad spectrum protection and minimize the risks of adverse cross-serotypic responses. The overall infection and dissemination rates for Ae. albopictus fed with the ChimeriVax™-DEN vaccines were 19/160 (11.8%) and 4/160 (2.5%), respectively, whereas those for YF-VAX were 10% (2/20) and 5% (1/20), suggesting that the Acambis strategy for chimerization to produce vaccine viruses may contribute further to restrict dissemination in the mosquito vector.

Although some mosquitoes were infected with the vaccine viruses, dissemination rates were very low, < 10% for the individual vaccine viruses and always 0% in the tetravalent mix. These low rates were despite experimental mosquitoes imbibing relatively high viral titers compared with the titers that they would encounter if feeding on vaccinated humans. Furthermore, the whole body viral titers never exceeded \(2.88 \pm 0.56 \log_{10} \text{TCID}_{50}/\text{mosquito}\). Such a low titer is unlikely to facilitate transmission, although this will be evaluated in future studies. Based on these data, it seems highly unlikely that mosquitoes from these Asian populations would be infected with the ChimeriVax™-DEN viruses if feeding on vaccinated humans and so do not represent an opportunity for viral reversion and transmission to naive persons. Although a low rate of dissemination did occur in Ae. albopictus, there was no dissemination in Ae. aegypti, which is considered to be the more important vector of DENV for humans. Moreover, the experiments were designed to exceed the likely conditions that would occur in the wild. This is therefore very reassuring.

Received May 1, 2006. Accepted for publication June 21, 2006.

Acknowledgments: The authors thank J. Huang for assistance in rearing mosquitoes.

Financial support: This work was supported by Sanofi-Pasteur, Marcy-L’Etoile, France. Kate McElroy and Charles McGee are recipients of CDC Fellowships for training in vector-borne infectious diseases (TO1/CCF622892).

Disclosure: Some of the authors wish to disclose that they have financial interest in Acambis, the company that sponsored the study. Some authors are current or former employees of Acambis. These statements are being made in the interest of full disclosure and not because the authors consider this to be a conflict of interest.

Authors’ addresses: Stephen Higgs, Dana Vanlandingham, Kimberly Klingler, Kate McElroy, and Charles E. McGee, Department of Pathology, University of Texas Medical Branch, Galveston, TX 77555-0609. Laura Harrington, Department of Entomology, 3138 Comstock Hall, Cornell University, Ithaca, NY 14853. Jean Lang, Sanofi Pasteur, Campus Mérieux, 1541 Avenue Marcel Mérieux, Marcy-L’Étoile F-69280, France. Thomas Monath and Farshad Guirakhoo, Acambis, Inc., 38 Sidney Street, Cambridge, MA 02139, Telephone: 617-761-4323, Fax: 617-494-1741. Current address for Thomas Monath, Kleiner Perkins Caufield & Byers, 21 Finn Road, Harvard MA 01451. Farshad Guirakhoo, Acambis, Inc., 38 Sidney Street, Cambridge, MA 02139, Telephone

REFERENCES

15. Pugachev KV, Guirakhoo F, Trent DW, Monath TP, 2003. Tra-


