Reviewer's report

Title: Vaccine candidates derived from a novel infectious cDNA clone of an American genotype dengue virus type 2

Version: 3 Date: 4 September 2004

Reviewer: Duane Gubler

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This is another in a series of excellent papers on dengue vaccine development by the NIH group in Bethesda. With the exception of a few redundancies on what was done and how, in the discussion, the paper is well written and provides the proper amount of detail. The paper should be published after minor revision of the points outlined below.

Specific Comments and Essential Revisions

1. Page 3, Para 1. The authors should use standard terminology. Dengue hemorrhagic fever/dengue shock syndrome is abbreviated as DHF/DSS, not DHFS. Also, the virus serotypes are designated as DEN-1, DEN-2, DEN-3 and DEN-4. These should be changed throughout the manuscript.

   Lines 6 – 10. Risk factors for DHF/DSS include the strain of virus, age and genetic background of the host in addition to previous dengue experience. Whether or not a person develops DHF is not just due to immune enhancement.

2. Page 6, Para 2. The Tongan DEN-2 virus was not isolated in C6/36 cells; it was isolated by inoculation of human serum into Aedes albopictus mosquitoes, and subsequently passaged in C6/36 cells.

3. Page 12, Para 1. The level to which wild-type dengue viruses replicate in monkeys varies greatly with the strain of virus; they do not all replicate to 6 logs as indicated.

   Para 2, most likely, the reason the rDEN2 and rDen2?30 did not infect Aedes aegypti was the low dose. No doubt the strain and the attenuating mutations affected infectivity, but it is misleading the way it is written. Few dengue viruses will infect Ae aegypti orally at such a low dose. If they would have inoculated the virus into Ae aegypti, they would likely have found high infectivity like they did with Toxorynchites. This should be clarified because the way it is written, persons who do not understand mosquito infectivity will go away with the impression that the virus won’t replicate in Ae aegypti. The authors should specify oral infectivity.

4. The first 3 pages of the discussion have redundancies that could be eliminated, e.g., repeating the methods and results.

5. Page 19, Para 2. The statement about the co-circulation of DEN-1 and DEN-3 in the Americas in the 1960s and 1970s is misleading. In the 1960s DEN-2 and DEN-3 were the only dengue viruses known in the Caribbean; DEN-1 was not reported in the Americas from 1964-1977. In the 1970s, DEN-2 was still only known in the Caribbean and DEN-3 had moved to Colombia. DEN-1 was introduced in 1977 and caused widespread epidemics in the region in the late 1970s and 1980s. The authors are probably correct that DEN-2 was widespread in the region, but there are no data to prove that; thus the prevalence was low.

6. Page 20, Para 2. The statements that the American genotype DEN-2 viruses had low infectivity and thus low transmission is also misleading. Viruses of this genotype caused large explosive epidemics in the Americas. (Puerto Rico, 1969-1975) and the Pacific (Tahiti, 1971; Fiji, 1971; New Caledonia, 1972; Niue, 1972). Again, the strain of virus is all important, not just the genotype.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the
major compulsory revisions

**Level of interest:** An article of outstanding merit and interest in its field

**Quality of written English:** Acceptable

**Statistical review:** No

**Declaration of competing interests:**

None