LETTER TO THE EDITOR

FAILURE OF *AEDES AEGYPTI* TO BECOME INFECTED BY FEEDING ON DENGUE VIRUS-INFECTED IMMUNO-COMPROMISED NUDE MICE

D.T. MOURYA, C.G. RAUT, R.P. DEOLANKAR, M.K. GOVERDHAN, M.D. GOKHALE, C.B. LANDE

National Institute of Virology, 20-A, Dr. Ambedkar Road, Pune 411 001, India

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The studies on dengue (DEN) viruses employing immuno-compromised nude mice (BL6, nu/nu) have shown that nude mice are excellent animal model for dengue haemorrhagic fever (DHF) (*1*). The nude mice not only emulate the symptoms and signs of DHF but also manifest clinical features of DHF dengue shock syndrome (DSS) (DHF/DSS), when inoculated intraperitoneally (i.p.) or intracerebrally (i.c.). However, the viraemia in experimentally infected nude mice has not been observed employing mice and tissue cultures for virus detection, therefore further studies using xenodiagnosis were undertaken to detect whether mosquitoes when inoculated with the blood of nude mice infected intrathoracically (i.t.) or fed orally can pick up the infection.

In the present study, we have attempted to confirm whether nude mice infected with DEN virus by i.c. route have viraemia sufficient enough for *Aedes aegypti* mosquitoes to pick up the infection by i.t. or oral route.

Mosquitoes. The mosquitoes employed for the experiment were from a laboratory colony maintained at this institute since 1966. The mosquitoes were reared in an insectary maintained at 28 ± 2°C and 70 - 80% relative humidity.

Virus. DEN virus type 2 (TR 1751), a human isolate from Trinidad at its 25th mouse passage (i.c.), was employed to infect mice. The virus stock was titrated in infant mice inoculated by i.c. route.

Mice. BL6 (nu/nu) mice, maintained under barriered conditions, were used. Four- to six-week-old mice were inoculated i.c. with 10^4 LD_{50} of DEN virus in 0.03 ml of 10% brain-suspension.

Oral route of infection of mosquitoes. Four- to five-day-old female mosquitoes were fed on the infected mice on days 2,4,6,8,10 and 12 post infection (p.i.) and the fully engorged females were incubated for 10 days in the insectory. The protocol followed was similar to that described previously (*2*).

Intrathoracic route of infection of mosquitoes. Mice employed for feeding mosquitoes were bled and batches of four- to five-day-old female mosquitoes were inoculated i.t. with approximately 0.2 µl of serial 10-fold dilutions of the blood of the infected mice. The dilutions were made in Bovine albumin phosphate saline (BAPS). The method used for i.t. inoculation of mosquitoes was similar to that described by Rosen and Gubler (*3*). Inoculated mosquitoes were incubated as described above.

Detection of virus in mosquitoes. Detection of DEN viral antigen in the head squashes of the mosquitoes was done using an indirect immunofluorescence antibody (IFA) technique (*4,5*).

The mice infected with DEN virus by i.c. route showed haemorrhagic manifestation. In a series of experiments, nude mice were infected with DEN virus by i.c. route and a total of 1726 mosquitoes were fed on these mice from day 2 to 12 p.i. After 10 days of incubation period, head squashes of 1334 mosquitoes were tested by the IFA technique and all were found negative for DEN virus antigen. Similarly, a total of 1108 mosquitoes which were inoculated with dilutions (10^{-1} - 10^{-5}) of blood taken from the nude

Abbreviations: DEN = dengue; DHF = dengue haemorrhagic fever; DSS = dengue shock syndrome; i.c. = intracerebral(ly); i.p. = intraperitoneal(ly); i.t. = intrathoracical(ly); IFA = indirect immunofluorescence antibody; p.i. = post infection

mice on different days p.i. were also found negative. However, there was no noticeable mortality in the inoculated and orally infected mosquitoes.

We conclude from these experiments that the virus titer in the nude mice was either very low or transient, i.e. not allowing mosquitoes to be infected by oral or i.t. route.

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