

Correspondence

Association of ovarian proteins with transovarial transmission of dengue viruses by *Aedes* mosquitoes in Rajasthan, India

Sir,

Transovarial or vertical transmission of dengue viruses across mosquito generations¹⁻³ may play a crucial role in retention of viral pathogen during inter epidemic periods of dengue in an endemic setting. Significance of persistence of this phenomenon across mosquito generations as maintenance mechanism of virus has already been reported^{4,5}. Recently we have reported that transovarial transmission precedes appearance of human infection of dengue⁶. This necessitates that vertical transmission of dengue virus needs to be studied as host-virus interaction in mosquitoes to understand the molecular epidemiology of dengue.

Our earlier studies have shown that all the progeny of the mosquitoes experimentally infected by dengue virus do not show vertical infection of virus⁷. These observations further indicate towards the need for studies on the role of host factors of mosquitoes which facilitate transovarial transmission through their generations. Intracellular internalization of 50 nm dengue virus particle into a human leucocyte has been described⁸. The pathway involved in the process includes entrance of dengue virion through process of penetration and its further multiplication in the cytoplasm of host cells by dissolving invaginated cell membrane through the action of HCl and proteases in the host cells. Although molecular basis of entrance and subsequent replication of dengue virus in mosquito cells, especially during transovarial transmission, is not yet reported, based on its reported invaginating mode of entrance and subsequent dissolution of host cell membrane by HCl and proteases, as reported for human cells, membranes of ovaries of mosquitoes appear to be target sites of internalization of virus into ovarian cells of mosquitoes. Proteomic composition of

ovarian cells thus could play a role in virus entry and its subsequent intracellular replication. We report here an association of ovarian proteins of mosquitoes with transovarial transmission of dengue virus.

Larvae of *Ae. aegypti* and *Ae. vittatus* were collected from the domestic and peri-domestic containers in the urban and rural areas of Jodhpur, Kota and Jaipur districts of Rajasthan, India from December 2006 to September 2007. Larvae of *Ae. albopictus* were collected from peri-urban foci, mainly from the tree holes of urban areas. Larvae were reared into adults in the laboratory of Desert Medicine Research Centre, (DMRC) Jodhpur. The adult mosquitoes were kept in laboratory in Barraud cages, on 4 per cent glucose solution. Indirect fluorescence antibody test (IFAT)⁹ was employed on head squashes of mosquitoes for detecting dengue viral antigen. The virus detected in laboratory reared mosquitoes which did not feed on any blood meal, was treated as vertically transmitted virus. Polyclonal antibodies supplied by National Institute of Virology, Pune, India, were used for IFA test. To confirm the results of IFA test, mosquito remnants of samples showing positive IFA test were pooled to prepare viral suspension and it was injected intracerebrally into mice. Mice developing sickness after 4-6 days were sacrificed and their brains were centrifuged at 25982 g. The supernatant was subjected to IFA test to confirm the present of virus. The study protocol was approved by ethics committee.

Before pooling all the remnants of IFA test positive mosquitoes, the ovaries of individual mosquitoes subjected to IFA test, were dissected out. To ensure assay of membrane proteins, ovarian tissues were teased in cell lysis buffer and centrifuged at 25982 g for 20 min. Supernatant was taken and sediment was again

Pooled data suggest that in urban areas of all the three districts, 3 (3.4%) out of 31 *Ae. aegypti*, 1 (1.5%) out of 29 *Ae. albopictus* and only 1 (1.5%) out of 63 *Ae. vittatus* showing 200 kDa in their ovaries were positive for presence of dengue virus. On other hand, 6 (24%) out of 20 mosquitoes not showing 200 kDa were positive for dengue virus. Similarly, in rural areas, only 4 (18.7%) out of 47 *Ae. aegypti*, and 1 (3.2%) out of 31 *Ae. vittatus* with 200 kDa protein showed presence of virus, and 6 (24%) out of 25 *Ae. aegypti* and 11 (31.4%) out of 35 *Ae. vittatus* not having 200 kDa were virus positive (Table).

Our observations suggest that when 200 kDa ovarian proteins was present, less number of mosquitoes showed transovarial transmission of dengue virus whereas in absence of this protein in ovaries the presence of corresponding virus in the samples was more.

Transovarial transmission of dengue viruses ensures presence of viral pathogen in mosquitoes independent of their feeding upon an infective human blood carrying dengue virus^{3,4}. This vertical mode of virus retention across mosquito generations may carry crucial aetiological importance for amplifying an ongoing disease outbreak as well as for the re-emergence of disease in an endemic setting. In dengue endemic areas of Jodhpur, Rajasthan, when a protein of 200 kDa molecular weight was present, majority of mosquitoes did not show the presence of dengue virus whereas, samples from same areas without 200 kDa were found infected¹⁰.

The present observations made with respect to three *Aedes* species in urban areas of Jodhpur district indicated an association of 200 kDa protein with transovarial transmission of dengue virus. Our data emerged in the present study also highlights that *Ae. aegypti* which is most common vector for dengue transmission, had shown least presence of 200 kDa and corresponding maximum presence of virus. Similarly, *Ae. albopictus* known to be a maintenance species of virus¹¹ has exhibited relatively more number of specimens with 200 kDa and without dengue virus and the same was true in the case of *Ae. vittatus*.

Internalization of dengue virions into mosquito cells involve physical rather than biochemical interaction of virus with cell membranes¹². The virus particle enters the host cells through endocytosis, where invaginating flexibility of cell membranes determines the whole

engulfing of virus into its cytoplasm. Since virus particle after entering into cytoplasm remains encircled by a part of the cell membrane, this membrane needs to be digested by HCl and proteolytic enzymes of cell and again protein composition of the membrane plays a role in replication of virus after entry into cell. Our observations suggested that instead of presence of a protein being responsible for virus internalization, absence of it appeared to render the cell susceptible for virus entry.

In conclusion, the present study showed an association of ovarian proteins with the ability of individual mosquitoes to allow transovarial transmission of dengue virus. The exact cause and effect relationship between specific protein and vertical transmission of virus needs further study.

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