RESEARCH NOTE

Ultrastructural Aspects of the Replication of Dengue Virus Type 2 Isolated in Brazil

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The first outbreak of dengue fever in Brazil, confirmed by laboratory in Rio de Janeiro area, was registered in 1986. The virus isolates were characterized as dengue virus serotype 1 (DEN-1) (HG Schatzmayr et al. 1986 *Mem Inst Oswaldo Cruz 81*: 245-246). Four years later, an outbreak of dengue virus serotype 2 (DEN-2) ocurred in the same area (RMR Nogueira et al. 1990 *Mem Inst Oswaldo Cruz 85*: 253); enhancement of virulence was observed in only a few cases (Nogueira et al. 1991 *Mem Inst Oswaldo Cruz 86*: 269). Recently a DEN-2 outbreak in Fortaleza, State of Ceará, was followed by another one in the southern coast of State of Bahia (data from Fundação Nacional de Saúde, Ministério da Saúde, Brasília, 1995). Many thousand of cases were notified during all these outbreaks, and some dengue virus isolated were studied by electron microscopy.

We have studied the virus replication in the C6/36 *Aedes albopictus* cell line at the ultrastructural level of the DEN-1 virus strains isolated in Rio de Janeiro (OM Barth, HG Schatzmayr 1992 *Mem Inst Oswaldo Cruz 87*: 1-7), as well as the DEN-2 virus later obtained in the same area, comparing with the New Guinea C DEN-2 reference strain (OM Barth 1991 *Mem Inst Oswaldo Cruz 86*: 123-124, Barth 1992 *Mem Inst Oswaldo Cruz 87*: 565-574, Barth et al. 1994 *Mem Inst Oswaldo Cruz 89*: 21-24).

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Our purpose here is to describe and compare ultrastructural aspects of DEN-2 virus replication in the C6/36 mosquito cell line of the two otbreaks in the states of Ceará and Bahia. Sera from four patients from Fortaleza (Ceará) and two from Eunápolis (Bahia) were isolated and typed previously as DEN-2 at the Laboratory of Flaviviruses, Department of Virology, Instituto Oswaldo Cruz (Nogueira et al. 1995 *Rev Inst Med Trop São Paulo* submitted) by indirect immmunofluorescence test with type specific monoclonal antibodies. Cells were inoculated with 50 µl of the original human sera containing virus and maintained at 28°C in Leibovitz-15 medium. Showing 30-50% of cytopathic effect, the cell cultures were fixed in glutaraldehyde and processed as described previously (Barth et al. 1994 *loc. cit.*). Ultrathin sections were observed with a Zeiss EM-900 electron microscope. At least 50 cells were analyzed for virus replication in each sample.

The Brazilian DEN-2 virus replication in mosquito cell cultures of all examined sera present some peculiar morphological aspects, when compared with the reference New Guinea C strain. Typical featured virus particles, that occur in great number inside the rough endoplasmatic reticulum (RER) and derived vesicles after about three days of inoculation, are observed at high magnification in ultrathin sections and present always a smooth surface (Fig. 1); small granules on the virus particle surfaces can be observed by the negative staining technique at higher magnifications, corresponding to the virus membrane and envelope proteins (Fig. 2 arrow).

Beside these classical structured DEN-2 virus particles, virus-like particles occur in the RER and derived vesicles, also with the isolates of patient sera from State of Rio de Janeiro, but not in the cells infected with the reference New Guinea C strain. These particles, characterized by a fuzzy coated surface, are less numerous but persistent just to the 5th passage level in cells (Barth et al. 1994 *loc. cit.*). Cell controls were always free from these particles, as well as the same cell line infected by DEN-1 viruses. No antibodies, that may be engulfed by the cells during endocytosis, were found inside the infected cells; this means that the last described particles are not virus particles covered by antibodies.

Human sera from Fortaleza and Eunápolis cities, show the same morphological aspects of the virus particles as described for DEN-2 from Rio de Janeiro (Fig. 3). Classical structured virus particles occur beside the fuzzy coated ones in the same RER vesicles. Smooth membrane structures,

as rounded vesicles and tubules of unknown function, are also present inside the RER and appear when virus replication is starting in the infected cell.

These observations suggest that the DEN-2 strains isolated in Fortaleza and Eunápolis cities are derived from the Rio de Janeiro outbreak, first noticed in 1990. No additional modifications at the morphological level in the virus infected cells were detected.

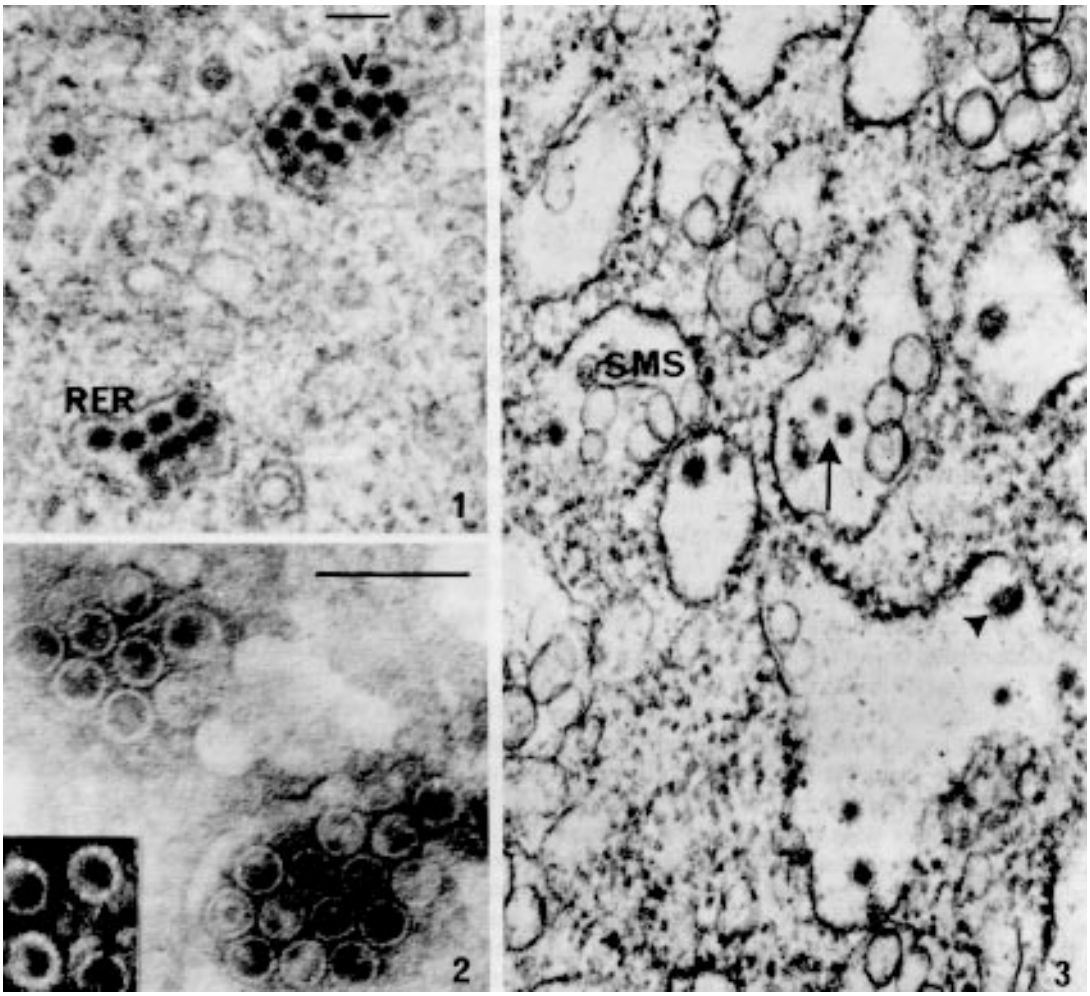

Fig. 1: typical structured dengue virus particles (V) inside vesicles originated from the rough endoplasmatic reticulum (RER), 80,000x. Fig. 2: DEN-2 virus particles from a gradient fraction; negative staining; inset: virus particles in underfocus to show surface granulations (arrow), 200,000x. Fig. 3: smooth (arrow) and fuzzy-coated (arrow head) virus particles and smooth membrane structures (SMS) inside RER vesicles from a Bahia isolate, 80,000x. (Bar = 100 nm).