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RECENT ADVANCES AND PROSPECTIVE RESEARCHES ON MOLECULAR EPIDEMIOLOGY OF DENGUE VIRUSES

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The determination of amino acid changes in the envelope protein by direct sequencing of either genomic RNA or PCR-amplified cDNA fragments provides useful informations for assessing the genetic variability and the geographic distribution of the actually most widespread dengue-2 serotype. The possible link of variations in the envelope protein-gene and virus virulence is discussed.

Key words: dengue virus – molecular epidemiology – sequencing – envelope protein

The four dengue viruses form a complex which belongs to the flavivirus genus in the family *Flaviviridae.* They are transmitted to man mainly by *Aedes aegypti* and *Aedes albopictus* in Asia, Africa, the Americas and South Pacific, and are responsible for a growing health problem in the tropical world (Fig. 1). They produce mild frebile flu-like illness (dengue fever -DF) in million of people each year and hemorrhagic manifestations and shocks (dengue hemorrhagic fever -DHF, dengue shock syndrome -DSS) due to bleeding diathesis, hemoconcentration and disorders of hemostasis. These abnormalities can progress to death, primarily among children. DHF occurs as an epidemic disease in Thailand, Burma, Indonesia and Vietnam. Over 300,000 DHF cases with more than 50,000 deaths were reported to WHO since the first epidemic which occured in Bangkok in 1958. DHF/DSS is now endemic in Southeast Asia with major outbreaks arising at 3-4 year intervals. The first cases of DHF/DSS have been recorded in the dengue endemic Carribean bassin since the early 1970's (Ehrenkranz et al., 1971) but emerged under endemic form during the dengue-2 outbreak in Cuba in 1981 (Guzman et al., 1984) and recently in Venezuela (Anonymous, 1990). In the absence of established vaccine against dengue, the uncontrolled spread of DHF/DSS from Southeast Asia to the Americas represents a threat for the New World. The advent of air travel leads to an increasing of the movement of dengue viruses between different geographic areas and to the introduction of new viruses by viremic human beings. The circulation of the four dengue serotypes of dengue in Africa has been demonstrated in the recent years with the presence of both a sylvatic cycle of dengue-2 and minor epidemic manifestations including serotypes 1 and 2 (M. Cornet, personnal communication). This discrepancy in the dengue epidemiology observed in the different continents is not well understood. The extend of the dengue disease and of its hemorrhagic manifestation incites numerous researches on its pathogeny. Up to now, attempts to define viral factors involved in the severity of the disease have only been embryonic. Nevertheless, major steps have been cleared to decode the genetic mechanisms of the viruses, to design the antigenic structure of their immunogenic proteins and to understand the immune response to these agents (see Henchal & Putnack, 1990).

Dengue viruses are characterized by a single-stranded positive-sense RNA associated with a core protein in a nucleocapsid surrounded by an envelope consisting of a lipidic bilayer including anchored viral envelope E and membrane M proteins. The four dengue serotypes were first identified by cross-neutralization data using polyclonal antibodies. Monoclonal antibodies and RNA oligonucleotide fingerprinting revealed that each serotype contained a number of geographic variants or topotypes (Trent et al., 1983; Repik et al., 1983; Monath et al., 1986). Two exhaustive analyses of Thai dengue-2 viruses demonstrated antigenic and genetic variations within a defined geographic area (Walker et al., 1988; Trent et al., 1989). Several genetic subgroups of a single topotype could be identified, resulting from a gradual microevolution of the genome. But no virulence marker could be iden-

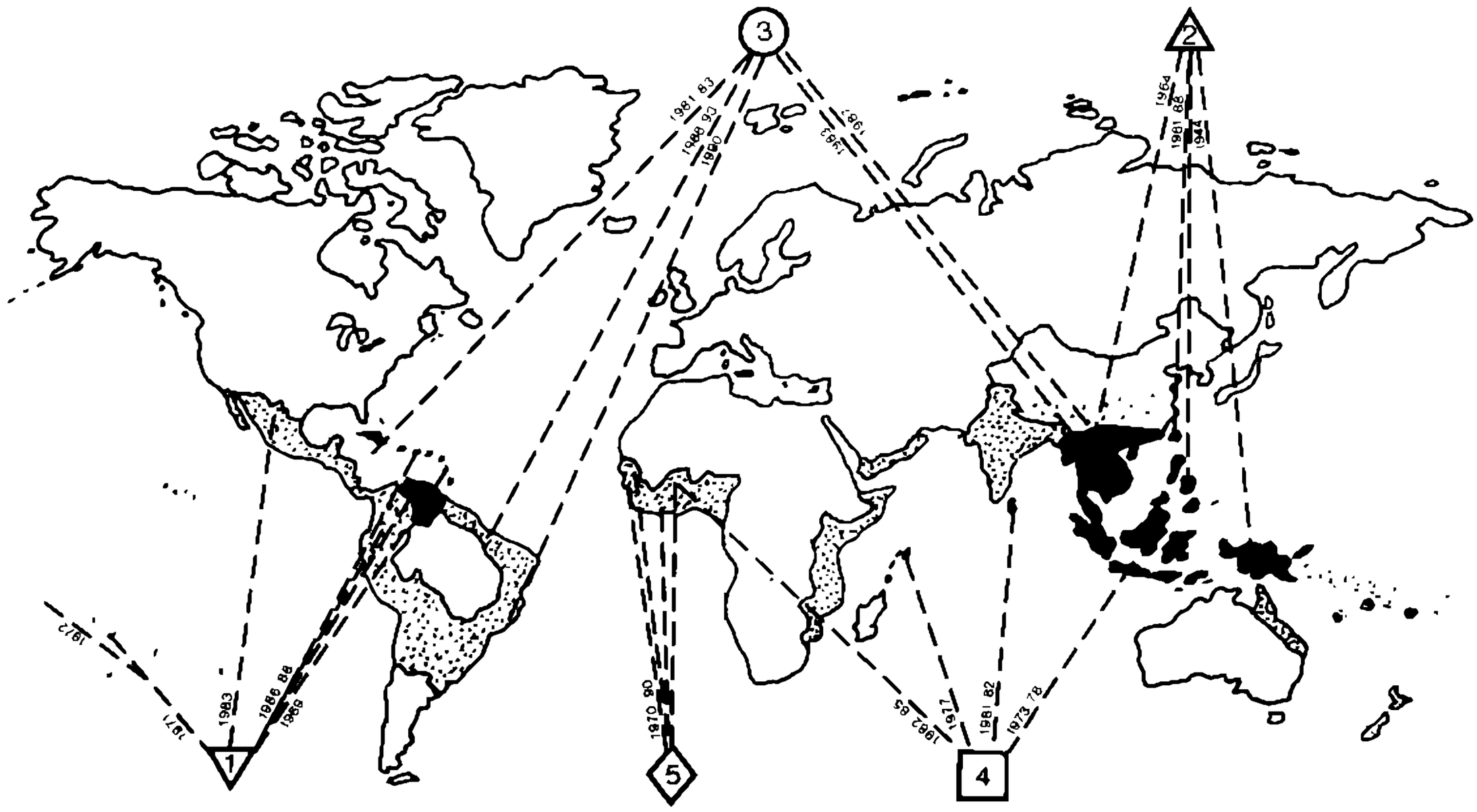

Fig. 1: geographic distribution of five dengue-2 virus genotypes. Virus classification was established according to Rico-Hesse (1990). The areas where the dengue fever syndrome is predominant are dotted and areas with epidemic DHF/DSS are blacked. The year or period of virus isolations is mentioned.

tified since similar T1-oligonucleotide patterns were observed among isolates from mild and DHF/DSS cases (Trent et al., 1989). Moreover, this approach failed to identify variation in phenotypic markers which can only be observed by nucleotide sequence comparison. The E-protein contributes to the biological functions of the virus such as recognition of cellular receptors, fusion to endosomal membranes, induction of neutralizing antibodies and of cellular immunity, and hemagglutination. The immunological pressure carried out on this protein leads to the emergence of viral variants. Variations in the E-protein sequence within a genotype have been observed (Blok et al., 1989; Samuel et al., 1989a, b, c; Chu et al., 1990). Although no genetic markers have been characterized which may be correlated to severe manifestations of the disease, some of them might be expressed on the structural proteins involved in the viral virulence (Samuel et al.,1989a, b, c; Bray et al., 1992).

The circulation and the distribution of world-wide isolates of dengue-2 variants has recently been investigated by direct sequencing fractions of the E-gene (Rico-Hesse, 1990). Rico-Hesse performed a primer extension sequencing of 240 nucleotides across the E/NS1 gene junction directly on the purified genomic RNA. Alternatively, Deubel et al. (1993) were examining a nucleotide fragment encoding amino acids 29 to 94 in the E-protein by the direct sequencing of a PCR-amplified product (Deubel et al., 1990). A genetic relationship between dengue-2 virus isolates from various geographic areas and hosts allowed to establish five distinct genotypic groups having less than 5% nucleotide sequence divergence (Fig. 1). Caribbean dengue-2 viruses in particular (No. 3 in Fig. 1), isolated in Jamaica in 1981-83, showed a closer relationship with Thai and Vietnamese strains rather than with the preexisting Puerto Rican genotype (No. 1) prevalent in the Caribbean until the 1980's and in Central America until 1988. We have recently demonstrated the incursion of the 1981-83 Jamaican topotype in the East part of South America in 1988-90 (Deubel et al., 1993). Therefore, the appearance of an Asian genotype in this area may be correlated to more severe dengue epidemics (R. Nogueira and J.-M. Reynes, personnal communication). The topotype determination of the dengue-2 virus involved in the DHF/DSS epidemics in Cuba, 1981, and in the recent severe outbreak in Venezuela (Anonymous, 1990) should add data on this point. Rico-Hesse (1990) also demonstrated a genetic divergence in dengue-2 viruses in Africa. It has been suggested that the isolates from the epidemic in Burkina Faso 1982-86 were issued from Sri Lanka or India through

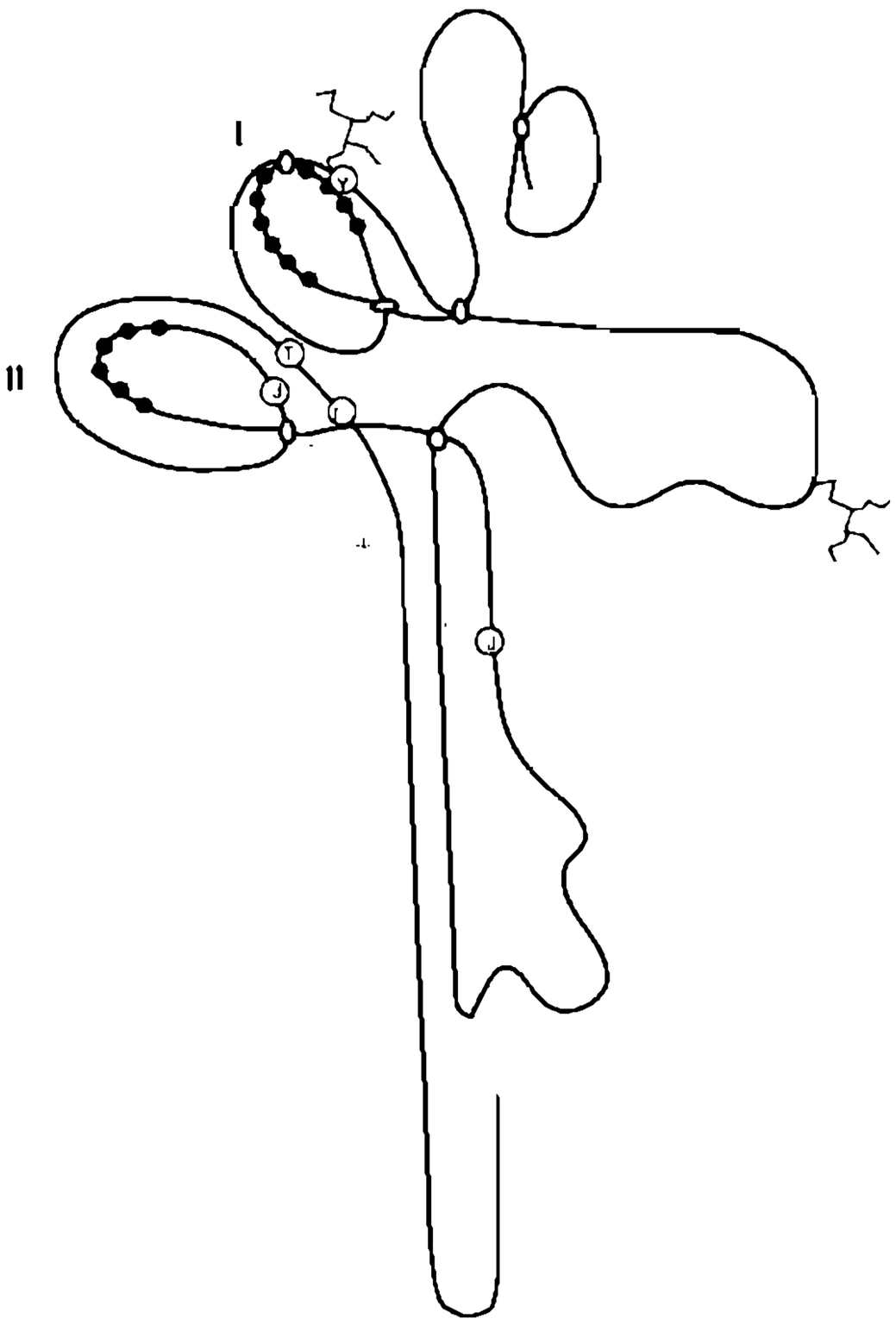

Fig. 2: model of dengue virus protein. Open ellipses represent cysteine residues forming disulfide bridges. Potential N-glycosylation sites are drawn. Position numbers are shown every 100 amino acids. Solid circles indicate hydrophobic sequences which may be involved in membrane fusion activity. The antigenic domains I and II are indicated. Labeled open circles represent an amino acid change observed in Japanese encephalitis (J), tick-borne encephalitis (T), Murray Valley encephalitis (M) and yellow fever (Y) antigenic mutants resistant to neutralization by anti-E monoclonal antibodies, and associated with attenuation of virulence.

the Seychelles (No. 4 in Fig. 1), while an endemic variant remained in a forest cycle in West Africa between 1970 and 1990 (No. 5).

Although genetic differences between the envelope proteins have been observed amongst various isolates from the mild and severe forms of dengue disease, the significance of these changes in not known. Failure to correlate specific amino acid changes to dengue virulence is partly due to the lack of animal model. Analyses of the sequences of some flavivirus antigenic mutants resistant to neutralization by anti-E monoclonal antibodies showed two clusters of mutations which seemed related to an attenuation of virulence (Lobigs et al., 1987, 1990; Heinz et al., 1990; Cecilia & Gould, 1991). Mutants affected in amino acids 71-72 in yellow fever virus, 382-384 in Murray Valley encephalitis virus, 333 in Japanese encephalitis virus and 384 in tick-borne encephalitis virus showed modified pathogenicity for mice. These major amino acid substitutions have been defined in two disulfide-stabilized antigenic domains I and II of the flavivirus E-protein (Fig. 2), corresponding to sequences 60-121 and 298-397, respectively, which were shown to be into vicinity in the tertiary structure of the molecule (Roehrig et al., 1990; Mégret et al., 1992). These regions were suggested to be implicated in the recognition of specific receptors and/or in fusion activities in the endosomal compartment (Lobigs et al., 1990; Roehrig et al., 1989). Therefore, the access to those two specific amino acid sequences in the E-protein of various isolates of different geographic and pathogenic potential for human might help to define virulent determinants. The development of infectious cDNA clones of flavivirus genomes (Rice et al., 1990; Lai et al., 1991) and their mutagenesis *in vitro* would also bring benefic informations in the significance of genome changes related to virulence.

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