VIROLOGY 230, 244–251 (1997)
ARTICLE NO. VY978504

Origins of Dengue Type 2 Viruses Associated with Increased Pathogenicity in the Americas

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Received September 24, 1996; returned to author for revision November 15, 1996; accepted February 17, 1996

The recent emergence and spread of dengue hemorrhagic fever in the Americas have been a major source of concern. Efforts to control this disease are dependent on understanding the pathogenicity of dengue viruses and their transmission dynamics. Pathogenicity studies have been hampered by the lack of *in vitro* or *in vivo* models of severe dengue disease. Alternatively, molecular epidemiologic studies which associate certain dengue virus genetic types with severe dengue outbreaks may point to strains with increased pathogenicity. The comparison of nucleotide sequences (240 bp) from the E/NS1 gene region of the dengue virus genome has been shown to reflect evolutionary relationships and geographic origins of dengue virus strains. This approach was used to demonstrate an association between the introduction of two distinct genotypes of dengue type 2 virus and the appearance of dengue hemorrhagic fever in the Americas. Phylogenetic analyses suggest that these genotypes originated in Southeast Asia and that they displaced the native, American genotype in at least four countries. Vaccination and other control efforts should therefore be directed at decreasing the transmission of these "virulent" genotypes.

INTRODUCTION

The importance of dengue virus as a disease-causing agent has been discussed in many recent publications (Centers for Disease Control, 1994a,b; Gubler and Clark, 1995; Institute of Medicine, 1992; Monath, 1994; National Institute of Allergy and Infectious Diseases, 1992); it is responsible for growing health problems in tropical and subtropical regions of the world. This mosquito-borne flavivirus causes illness varying from a mild, flu-like syndrome (dengue fever, DF) to a fulminating hemorrhagic disease with hypovolemic shock and death (dengue hemorrhagic fever, DHF). At present, as many as 50 million human cases occur annually, with an estimated 10,000 infant deaths due to the hemorrhagic form of dengue (World Health Organization, 1996).

The severe form of dengue is thought to be caused by several factors, including virus type and host immune status. Four closely related but antigenically distinct dengue virus serotypes (designated types 1–4) have been described; immunity to one serotype does not protect against infection by the others. In fact, sequential infection by another serotype substantially increases the probability of developing DHF; immune-enhancement of infection was first suggested (Halstead, 1970) and later demonstrated in infants who passively acquired dengue antibodies *in utero* (Kliks *et al.,* 1989). Prospective clinical studies have suggested that release of cytokines, interferon, and complement pathway components amplifies the pathological process begun by these nonneutralizing antibodies (Kurane *et al.,* 1994). However, the pathogenesis of DHF is poorly understood mainly due to the lack of both *in vitro* and *in vivo* models of disease.

The first indications of differences in virulence and/or transmission between dengue viruses came from descriptions of sylvatic cycles of virus transmission in forests in Western Africa (Robin *et al.,* 1980), Vietnam (World Health Organization, 1976), and Malaysia (Rudnick, 1965). Only recently have some of these serotype 2 viruses been analyzed genetically, and their distinct evolutionary origins were proven (Rico-Hesse, 1990). These viruses are transmitted by discrete vectors (canopy-inhabiting mosquitoes), among primates, and have not yet been implicated as causes of outbreaks in nearby human populations (Roche *et al.,* 1983). Other indications of virulence differences among dengue type 2 viruses come

The nucleotide sequence data reported in this paper have been submitted to the GenBank nucleotide sequence database and have been assigned the Accession Nos. U91859–U91891.

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0042-6822/97 $25.00

from recent outbreaks of DHF in the Caribbean and South America. Although several serotypes of dengue had been circulating in Cuba and the rest of the Americas, it was not until 1981 that an epidemic with severe disease occurred in Cuba (Gubler and Clark, 1995). During this epidemic associated with dengue type 2 virus, a total of almost 350,000 cases of dengue were reported, which included over 10,000 cases of DHF and 158 deaths (Kouri *et al.,* 1986). A recent report by Cuban researchers (Guzman *et al.,* 1995) described a genetic study of four dengue type 2 isolates from the 1981 epidemic, but provided insufficient data for direct comparison to phylogenies published previously (Blok *et al.,* 1991; Lewis *et al.,* 1993; Rico-Hesse, 1990). Partial nucleotide sequences from the E (201 bp) gene placed the Cuban viruses in a genetic group containing other isolates from Southeast Asia (Guzman *et al.,* 1995). The virus isolations made during this epidemic have not been made available for study, although isolates from neighboring areas have (Jamaica); genetic analyses of these contemporaneous samples revealed their probable origin to be somewhere in Southeast Asia, presumably Vietnam, Malaysia, or Thailand (Deubel *et al.,* 1993; Lewis *et al.,* 1993; Rico-Hesse, 1990).

Only since 1981, after the Cuban epidemic, have many other countries in the Americas reported DHF cases on an annual basis (Gubler and Clark, 1995; World Health Organization, 1994). The second outbreak of DHF took place in Venezuela, in 1989; isolates of serotypes 1, 2, and 4 were obtained during the epidemic, but severe cases were associated with type 2 virus (Pan American Health Organization, 1990). Colombia reported its first confirmed DHF cases in 1990, presumably as spillover from the outbreak in Venezuela (Pan American Health Organization, 1990, 1992). A third outbreak of DHF began in Brazil, in 1990, and was also associated with type 2 virus (Pan American Health Organization, 1992) (Nogueira *et al.,* 1993). For Mexico, only sporadic DHF cases were reported for the period 1984–1993; however, in 1995, there were 358 confirmed cases of DHF, with a vast majority of virus isolates belonging to serotype 2 (Briceno-Garcia *et al.,* 1996). By 1995, 14 countries in the Americas had reported confirmed DHF cases, and DHF is endemic in many of these areas (Gubler and Clark, 1995).

In this report we demonstrate the association between the introduction of a Southeast Asian genotype of dengue type 2 virus and the appearance of DHF in four American countries (Venezuela, Brazil, Colombia, and Mexico). This imported dengue genotype seems to have displaced the native, American genotype which had only been associated with less severe disease (DF).

MATERIALS AND METHODS

Viruses

Dengue viruses used in this study were selected from stocks or serum kept at our laboratories; aliquots were used to infect C6/36 mosquito cells and viruses were identified as dengue serotype 2 by indirect fluorescent antibody tests with type-specific monoclonal antibodies (Gubler *et al.,* 1984). Virus isolation and passage histories are given in Table 1; viruses described here were isolated or amplified from human serum, except strain PM33974, which was isolated from a mosquito pool.

RNA extraction and RT-PCR

Nucleotide sequences for 18 of the 62 dengue type 2 viruses described here were obtained previously by primer-extension sequencing from viral-extracted RNA (Rico-Hesse, 1990); the remainder were amplified by RT-PCR. In a preliminary test comparing nucleotide sequences obtained by these two methods (direct RNA sequencing vs RT-PCR), no differences were seen in the 240-nucleotide-long E/NS1 region used for phylogenies; 6 viruses were compared after two to three cell culture (C6/36) passages and RNA sequencing to those sequences obtained after RT-PCR and no cell passage (i.e., from serum).

For 44 of the viruses shown here, a 200-μl aliquot of cell culture supernatant or human serum was treated with 1.0 ml of Trizol (GIBCO-BRL); RNA extractions were done according to the manufacturer's protocol. The RNA was resuspended in DEPC-treated water and $\frac{1}{5}$ of this sample was added to Superscript II RT buffer (50 m*M* Tris–HCl, 75 m*M* KCl, 3 m*M* $MgCl_2$, pH 8.3) (GIBCO-BRL), 10 m*M* DTT, and 50 p*M* antisense primer (D2/2578: 5′-TTACTGAGCGGATTCCACAGATGCC-3′) which hybridizes to all known dengue serotype 2 viruses (Rico-Hesse, 1990). The solution was heated to 90° for 90 sec and cooled on ice. Each of the four deoxynucleotides was added (1 m*M*) along with 40 U Rnasin (Promega) and 200 U reverse transcriptase enzyme (Superscript II; GIBCO-BRL). The mixture was incubated for 1 hr at 42° and cooled on ice. To this mix were added both PCR primers (D2/2578 and D2/2170V: 5′-ATGGCCATTTTAGGTGACACAGCCTGGGA-3′, sense) (150 p*M*), PCR buffer at pH 10.0 (60 m*M* Tris–HCl, 15 m*M* $(NH_4)_2SO_4$, 1.5 m*M* $MgCl_2$), and deoxynucleotides at 0.2 m*M,* and the volume brought up to 50 μl with DEPC water. AmpliTaq enzyme (Perkin–Elmer) was added last (5 U) and the solution overlayed with mineral oil. The samples, including positive PCR control (Perkin–Elmer), negative control (DEPC water), and dengue type 2 RNA control, were placed in a thermal cycler for 30 cycles of 94° for 60 sec, 55° for 2 min, and 72° for 3 min. The samples were kept at 4° after amplification. The entire sample was electrophoresed in 2% NuSieve GTG agarose (FMC) and stained with 1 μg/ml ethidium bromide for visualization of bands. This protocol produced a 408-bp product for sequencing; the 240-nucleotide fragment for genetic comparison is included within this product (Rico-Hesse, 1990). Specific amplification of RNA from virus strains belonging to five

TABLE 1

Dengue Type 2 Viruses Compared by Sequence Analysis

Strain	Passage history[a]	Location[b]	Year	Clinical[c]
NGC	monk.1,mosq.1,C6/36 1	New Guinea	1944	DF
TR1751	smb. 57	Trinidad	1954	DF
16681	MK2 1, C6/36 5	Bangkok, Thailand	1964	DHF
PR159	PGMK 6	Puerto Rico	1969	DF
D79-014	MK2 2, C6/36 1	Bangkok, Thailand	1979	DF
D80-141	MK2 3, C6/36 1	Bangkok, Thailand	1980	DHF
1318	mosq.2,C6/36 1	Puerto Rico	1981	DF
8110827	None	Jamaica	1981	DF
PM33974	mosq.1,C6/36 1	Republic of Guinea	1981	*Ae.africanus*
D81-081	C6/36 1	Bangkok, Thailand	1981	DHF
D82-137	C6/36 1	Bangkok, Thailand	1982	DF
1329	mosq.1, C6/36 2	Jamaica	1982	DF
JAH	C6/36 2	Jamaica	1982	DF
044	C6/36 2	Tapachula, Chiapas, Mexico	1983	DF
D83-516	Ts1, C6/36 2	Bangkok, Thailand	1983	DF
975	C6/36 3	Sri Lanka	1985	DF
348600	C6/36 3	Tumaco, Nariño, Colombia	1986	DF
D86-004	Ts 1, C6/36 1	Bangkok, Thailand	1986	DHF
D87-1372	Ts 1, C6/36 1	Bangkok, thailand	1987	DF
24H	C6/36 1	Hanoi, Vietnam	1987	DF
57S	C6/36 1	Saigon, Vietnam	1987	DF
VEN2	AP61 2	Maracay, Aragua, Venezuela	1987	DF
350447	C6/36 3	Tolima, Colombia	1987	DF
351863	C6/36 3	Tolima, Colombia	1988	DF
D89-1092	Ts 1, C6/36 1	Bangkok, Thailand	1989	DHF
D90-276	Ts 1, C6/36 1	Bangkok, Thailand	1990	DHF
MARA3	None	Maracay, Aragua, Venezuela	1990	DF
95524	C6/36 1	Guaribe, Guárico, Venezuela	1990	DF
95623	C6/36 1	Lara, Venezuela	1990	DF
96771	C6/36 1	Caracas, Dto. Federal, Venezuela	1990	DF
38998	C6/36 1	Niteroi, Rio de Janeiro, Brazil	1990	DF
39056	C6/36 1	Niteroi, Rio de Janeiro, Brazil	1990	DHF
40247	C6/36 2	Niteroi, Rio de Janeiro, Brazil	1990	DHF
41464	C6/36 2	Niteroi, Rio de Janeiro, Brazil	1990	DF
41576	C6/36 2	Niteroi, Rio de Janeiro, Brazil	1990	DF

previously described dengue 2 genotypes (Rico-Hesse, 1990) was obtained in this manner.

PCR fragment sequencing

Bands of the correct size were cut from agarose gels and equilibrated in β-agarase buffer (10 m*M* Tris–HCl, 1 m*M* EDTA, pH 6.5) (NE Biolabs). Agarose was digested with 6 U of β-agarase enzyme (NE Biolabs) and the DNA fragments were precipitated and washed in ethanol, according to the manufacturer's instructions. The DNA was resuspended in DEPC water and $\frac{1}{4}$ of this sample was used in dideoxynucleotide sequencing reactions. Sequencing was done manually, using the Sequenase 2.0 Kit (U.S. Biochemical, Amersham), according to the manufacturer's instructions, including pyrophosphatase and [α-^{35}S]dATP. Two primer-extension reactions, using the D2/2578 primer and D2/2452 (5′-CCACATTTCAGTTC-TTT-3′), provided clear results and overlap at the 240-nucleotide site used for comparisons (map sites 2311–2550) (Hahn *et al.,* 1988).

Sequence analysis and phylogenies

Nucleotide sequences for 18 of the strains shown here were reported previously (Accession Nos. M32932–M32971) (Rico-Hesse, 1990). The remainder of dengue type 2 sequences were submitted to GenBank. Sequences for the homologous region of serotype 1 virus (strain D81-135, Thailand, 1981), serotype 3 virus (strain H87, Philippines, 1956), and serotype 4 virus (strain 814669, Dominica, 1981) were obtained from GenBank (Accession Nos. M32925, M93130, and M14931, respectively). Alignments were done using encoded amino acids; none of the viruses compared here had additions or deletions in this region of the genome. Phylogenetic analyses were done using the PAUP program, with uniform character weights, TBR branch-swapping, and a heuristic search for the most par-

TABLE 1—*Continued*

Strain	Passage history[a]	Location[b]	Year	Clinical[c]
102091	C6/36 1	Táchira, Venezuela	1991	DHF
102586	C6/36 1	Caracas, Dto. Federal, Venezuela	1991	DF
102692	None	Aragua, Venezuela	1991	DHF
102781	C6/36 1	Apure, Venezuela	1991	DF
H506525	C6/36 2	Araguaina, Tocantins, Brazil	1991	DF
D91-157	Ts 1, C6/36 1	Bangkok, Thailand	1991	DHF
D92-287	Ts 1, C6/36 1	Bangkok, Thailand	1992	DF
360236	None	Bucaramanga, Santander, Colombia	1992	DF
360281	None	Ibagué, Tolima, Colombia	1992	DF
131	C6/36 1	Navojoa, Sonora, Mexico	1992	DF
132	C6/36 1	Navojoa, Sonora, Mexico	1992	DF
147	C6/36 1	Chínipas, Chihuahua, Mexico	1992	DF
125270	C6/36 1	Táchira, Venezuela	1993	DF
KD93-439	Ts 1, C6/36 1	Bangkok, Thailand	1993	DHF
E725	None	Aragua, Venezuela	1994	DF
1380	None	Portuguesa, Venezuela	1994	DF
1452	None	Miranda, Venezuela	1994	DHF
2722	None	Aragua, Venezuela	1995	DF
3602	None	Bolívar, Venezuela	1995	DF
4203	None	Táchira, Venezuela	1995	DF
8697	None	Mérida, Venezuela	1995	DHF
210097	None	Cali, Valle del Cauca, Colombia	1995	DF
195	None	Suchiapa, Chiapas, Mexico	1995	DF
203	None	Comalapa, Chiapas, Mexico	1995	DF
204	None	Tapachula, Chiapas, Mexico	1995	DF
382	None	Tapachula, Chiapas, Mexico	1995	DHF
383	None	Tamazunchale, S. L. Potosí, Mexico	1995	DHF

[a] monk., rhesus monkey; mosq., whole mosquito; C6/36, *Aedes albopictus* cell line; smb., suckling mouse brain; MK2, LLC-MK2 monkey kidney cell line; PGMK, primary green monkey kidney cell line; Ts, *Toxorynchites splendens* mosquito; none, acute serum of patient; AP61, *Aedes pseudoscutellaris* cell line.

[b] City and/or state, country.

[c] DF, dengue fever; DHF, dengue hemorrhagic fever; *Ae. africanus* mosquito.

simonious trees (Swofford, 1993); sequences from representatives of the other three serotypes (1, 3, and 4) were used to root the trees. The reliability of the inferred trees was estimated using the bootstrap method, with 100 replications (Felsenstein, 1985).

RESULTS

Nucleotide sequences from the E/NS1 gene junction were identical for several viruses from the same country and general time period. All isolates from the 1990 Brazil epidemic (38998, 39056, 40247, 41464, and 41576) were identical, as were isolates from Tolima, Colombia, in 1987 and 1988 (350447, 351863); some isolates from Venezuela in 1990 (95524, 96771), from Colombia in 1992 (360236, 360281), and from Mexico in 1995 (195, 382), although from different locations in those countries, were also identical. This may reflect the wide transmission (stability in time) of a given genotype of dengue virus or its transport to a more distant geographic area (in the latter cases). Most identical sequences were excluded from subsequent phylogenetic analyses; only those from Tolima, Colombia (1987 and 1988) were included, because of the small sample size from that country and time period. Thus, a total of 55 dengue type 2 viruses were included in the resulting phylogenetic tree.

Comparison of these 240-nucleotide-long fragments using maximum parsimony analysis generated phylogenetic or evolutionary trees (Fig. 1) that closely resemble those published previously (Rico-Hesse, 1990). The five genotypic groups described before are represented in Fig. 1: one isolate from Guinea, 1981 (African sylvatic cycle); one from Sri Lanka, 1985 (originally containing samples from Indonesia, the Seychelles, Burkina Faso, and Sri Lanka); a group from the Caribbean, Mexico, and South America (originally containing samples from the South Pacific also); a group containing isolates from Jamaica, Vietnam, Thailand, and others from the Americas; and a group containing the New Guinea prototype virus along with Thai and American samples (originally containing isolates from Taiwan and the Philippines also). Bootstrap values among the five genotype groups ranged from 100 to 57%, thus supporting the tree branching pattern shown in Fig. 1. The position of strain TR1751 (Trinidad 1954) relative to the American genotype group and some of the strains within the two Southeast Asia geno-

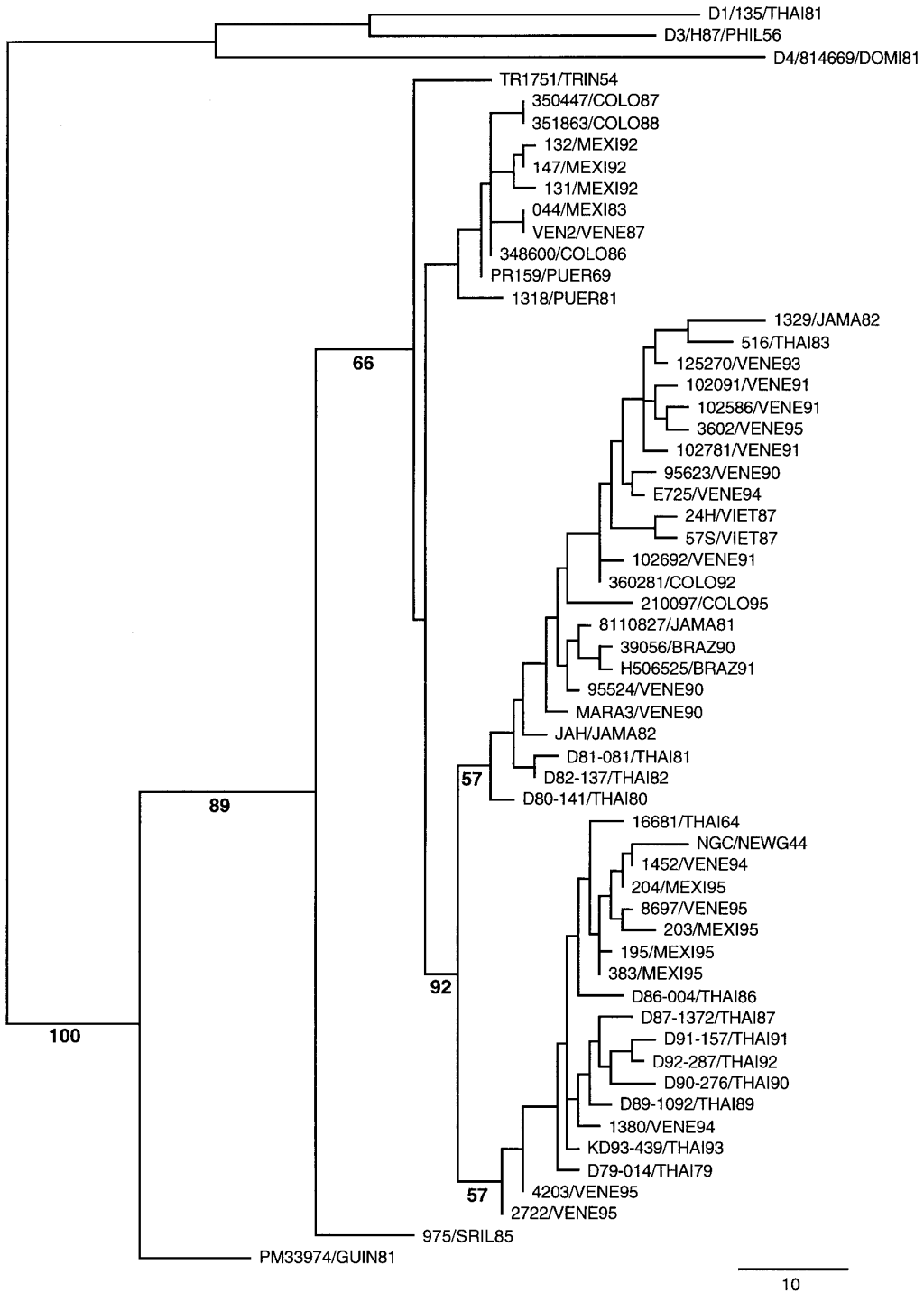

FIG. 1. Phylogenetic tree generated by PAUP analysis of nucleotide sequences from the E/NS1 junction of 55 dengue type 2 viruses and representatives of types 1, 3, and 4. Viruses are listed by strain number followed by abbreviation for country and year (see Table 1). Bootstrap values are in boldface below the branches that connect each of the five genotypic groups of dengue type 2 and the outgroup (three type representatives). Branch length is proportional to bar representing 10 nucleotide substitutions.

typic groups differed among the most parsimonious trees. These evolutionary relationships among dengue type 2 viruses have been shown to be similar when other regions of the viral genome were used to generate phylogenetic trees. The genetic relationships were similar whether the compared nucleotide sequences were from an entire gene (e.g., E or NS1) (Blok *et al.,* 1989, 1991; Lewis *et al.,* 1993) or portions of genes (e.g., 198 nucleo-

tides of the E gene) (Deubel *et al.,* 1993), and they were independent of the type of phylogenetic analysis algorithms used (e.g., UPMGA, NJ, or PAUP) (Blok *et al.,* 1989, 1991; Deubel *et al.,* 1993; Lewis *et al.,* 1993).

As can be seen at the top of Fig. 1, viruses obtained from the Caribbean and from South America previous to 1990 fell into the same group, including three samples from northwestern Mexico obtained in 1992. Dengue serotype 2 virus was first isolated in Brazil in 1990 so previous samples were not available for study (Pan American Health Organization, 1992). These samples from Puerto Rico, Colombia, Venezuela, and Mexico, from different years, represent the "native" American genotype; this group of viruses apparently had been evolving independently for some time and their closest relative (and possibly their progenitor) is the 1954 isolate from Trinidad.

Virus samples obtained from South America during and after 1990 and from Mexico in 1995 fell into either of two other genotype groups containing isolates from Southeast Asia or Jamaica. The latter group contains three isolates from Jamaica which were postulated to have been introduced to the Caribbean during the 1981 Cuban epidemic (Rico-Hesse, 1990). The inclusion of three isolates from Thailand (D80-141, D81-081, and D8-137) from the 1980–1982 period further support the view that virus from Southeast Asia was probably introduced to Cuba during the first major epidemic of DHF in the Americas. Samples from more recent (post-1989) dengue virus outbreaks in Brazil, Colombia, Venezuela, and Mexico, including those from patients with DHF, fell into the genotypic groups containing the most ancestral Southeast Asian viruses (see New Guinea 1944, Thailand 1964, and Thailand 1980s). Venezuelan viruses, from a wide range of locations and a long time period (1990–1995), were distributed among these two "Southeast Asian" genotypic groups, while samples from Brazil (1990 and 1991), Colombia (1992 and 1995), and Mexico (1995) were limited to either of the two groups. Because caution should be taken when interpreting the final branching patterns (right side of the tree) of these genotypic groups, it is impossible, using these data, to determine the direction of spread or transmission of these viruses within or among these American countries. However, it is clear from these data that the post-1989 samples from Brazil, Colombia, Mexico, and Venezuela have a common progenitor with isolates or samples from Southeast Asia; this suggests that the direction of transmission was from Southeast Asia to the Americas.

Epidemiologically, Venezuela has recently been considered a region of dengue virus hyperendemicity because of the very large number of cases of DF and DHF reported yearly since 1990 (Gubler and Clark, 1995; World Health Organization, 1996). This is reflected in the large number of genetic variants of dengue type 2 from this country (Fig. 1); this variability resembles the transmission characteristics described for Thailand in the past (Trent *et al.,* 1989; Walker *et al.,* 1988). The inclusion of more recent isolates from Thailand (through 1993) supports the conclusion that many genetic variants have circulated in this country. More detailed studies of dengue transmission in these countries might help elucidate the population dynamics of this virus in conditions of hyperendemicity.

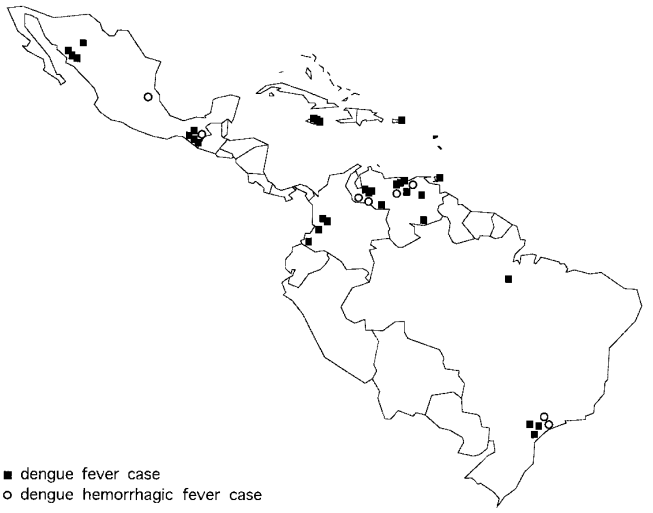

FIG. 2. Map of dengue type 2 samples from the Americas, according to case location and clinical classification.

In terms of clinical presentation of disease (DF vs DHF), there was no segregation of samples in the phylogenetic tree; this is probably due to the use of nucleotide sequences from the E/NS1 gene junction for constructing phylogenies. Other regions, such as those encoding antigenic domains (e.g., beginning and middle of E gene) (Megret *et al.,* 1992), might contain sites important in determining pathogenicity. Others have shown that a Puerto Rican 1969 isolate (PR159) and a Brazilian 1990 isolate (40247), both included here, differ by 14/495 (2.8%) amino acids in their E gene (Lewis *et al.,* 1993). However, biological markers of pathogenicity are required to identify probable virulence determinants, and only full genome sequencing (of closely related strains) would probably yield information on location of possible determinants. This study addressed only long-term evolutionary trends which could pinpoint the origin of more pathogenic dengue strains.

Figure 2 shows the geographic range of sampling in the Americas, with the clinical classification of cases according to WHO criteria (World Health Organization, 1986). All DHF cases included here occurred during the period 1990–1995; only samples from a total of eight cases were obtained because of the low rates of confirmation of disease etiology (i.e., virus isolation). Of the four American countries chosen for comparison, only Colombia lacked a DHF sample. Because samples from DF

and DHF cases from the other countries included here are not distinguishable by comparison of these 240-nucleotide-long fragments, we have assumed that Colombian DF samples are representative of those that might have been obtained from DHF cases. Further analysis of case samples from Colombia should clarify this observation.

DISCUSSION

One of the most important questions concerning dengue virus epidemiology has been the origin of strains associated with severe disease. The genetic study of natural field isolates and original DF and DHF case specimens has allowed us to determine the evolutionary origins of dengue viruses associated with outbreaks of severe disease in the Americas. The approach used here has previously been shown to reflect the geographic origin of dengue virus strains from four continents (Rico-Hesse, 1990); this has permitted the identification of Southeast Asia as the source of dengue type 2 genotypes which are new to the Americas. The native, American genotype seems to have been displaced by the imported, Southeast Asian genotypes, since there have been no isolations of the former in areas where the latter are circulating. In fact, Mexico may be the only country, of the four studied here, where this displacement phenomenon is still occurring (see samples from the northwest, 1992). It remains to be seen whether other countries in the Americas (e.g., Central America) may be importing the Southeast Asian virus genotypes from their neighbors.

Although there are no animal models of severe dengue disease to test the hypothesis that the Southeast Asian dengue type 2 viruses are directly responsible for some of the DHF cases in the Americas, this study has indicated that there is a direct association between the introduction of an imported strain and severe disease. This conclusion is based on epidemiologic and laboratory reports that have improved in number and reliability due to intensified surveillance in the region. Since the 1981 Cuban epidemic, public health authorities in the Americas and worldwide have been specifically monitoring the incidence of DHF in areas where it was previously unreported; this has recently led to the classification of DHF as an "emerging" disease. Further studies are currently directed at understanding host immune mechanisms that enhance dengue virus pathogenesis and the influence of the mosquito vector in selecting virus genetic variants which may be more pathogenic.

The efficient worldwide control of dengue virus requires the definition of sources of epidemic viruses and the precise identification of virus genotypes. This study suggests that recent efforts to produce a human vaccine against dengue, which uses a Thai-derived attenuated strain of dengue type 2 (Gubler and Clark, 1995), would probably be efficient at interrupting the transmission of the "virulent" type of virus. The genetic monitoring of virus transmission, using an approach such as that described here, would be very useful in future control efforts.

ACKNOWLEDGMENTS

We thank Hermann Schatzmayr, Marco F. Suarez, David Vaughn, Sharone Green, Alan Rothman, and Francisco Pinheiro for their help in shipping virus samples. This work was funded in part by grants from NIH (AI01124, AI34533) to R.R.H.

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