Genetic Relatedness among Structural Protein Genes of Dengue 1 Virus Strains

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SUMMARY

The structural protein-coding genomic regions of dengue virus type 1 (DEN-1) strains representing three distinct topotypes (Thailand, Philippines and Caribbean) were cloned and sequenced. In addition the envelope (E) nucleotide sequences of two recent Caribbean topotype DEN-1 isolates were obtained by direct RNA sequencing. The nucleotide sequence of the DEN-1 viruses in the structural gene region was found to be highly conserved with greater than 95% nucleotide sequence homology and with less than 4% change in the amino acid sequence. Although there was a less than 2% change in the nucleotide sequence of DEN-1 E proteins, strains could be differentiated by the clusters of nucleotide changes. Furthermore, the deduced amino acid changes in the E protein were clustered primarily within the proposed immunologically reactive regions. Genomic nucleotide sequence comparisons did not define geographical or virulence markers but located unique clusters of nucleotide/amino acid changes for each of the three topotypes of DEN-1 viruses examined.

INTRODUCTION

The genomic organization and RNA sequence of many flaviviruses have now been determined. The publication of the genomic sequence of yellow fever (YF) 17D vaccine virus (Rice \textit{et al.}, 1985) was followed by that of either partial or entire sequences of Asibi YF (Hahn \textit{et al.}, 1987), Japanese encephalitis (JE) (McAda \textit{et al.}, 1987), Murray Valley encephalitis (MVE) (Dalgarro \textit{et al.}, 1986), West Nile virus (WN) (Wengler \textit{et al.}, 1987), Kunjin (KUN) (Coia \textit{et al.}, 1988), St Louis encephalitis (SLE) (Trent \textit{et al.}, 1987), dengue (DEN) viruses type 1 (Nauru Island) (Mason \textit{et al.}, 1987), DEN-2 (Jamaica) (Deubel \textit{et al.}, 1988), DEN-2 (PR-159 vaccine candidate, S1) (Hahn \textit{et al.}, 1988) and DEN-4 (Dominica) (Zhao \textit{et al.}, 1986). The flavivirus genome is composed of a single open reading frame approximately 10.5 kb in length, with the structural protein genes [capsid (C), premembrane (prM), membrane (M) and envelope (E) proteins] localized at the 5' end of the genome. The structural protein genes precede the nonstructural protein genes encoded in the 3' three-quarters of the genomic RNA (Rice \textit{et al.}, 1985).

Mosquito-borne flaviviruses are divided into three major serological subgroups (Porterfield, 1980): yellow fever, Japanese encephalitis (JE, MVE, WN, KUN and SLE) and dengue (DEN serotypes 1 to 4). Comparison of the nucleotide sequence encoding the structural genes of viruses between each of the subgroups has shown that YF shares a 45% and 32% similarity with JE (McAda \textit{et al.}, 1987) and DEN-2 (Deubel \textit{et al.}, 1986), respectively. Within the JE subcomplex, members share 60% to 80% of their nucleotide sequence (McAda \textit{et al.}, 1987). The nucleotide sequences of DEN-1, DEN-2 and DEN-4 viruses share 62% to 69% similarity over the entire genome (Deubel \textit{et al.}, 1988). Strains of the same virus diverge much less; YF Asibi and the 17D vaccine virus diverge by 0-63% in their nucleotide sequences (Hahn \textit{et al.}, 1987). DEN-2 Jamaica and DEN-2 PR-159 (S1) share more than 95% nucleotide sequence similarity (Deubel \textit{et}
The relatedness of these sequences supports the classification of these viruses into their own separate family, Flaviviridae (Westaway et al., 1985) and its serological subgroups.

Specific genomic nucleotide sequence changes have yet to be directly associated with alterations in the biological activity of flaviviruses. There were 68 nucleotide sequence differences between YF 17D and Asibi, resulting in 32 amino acid changes (Hahn et al., 1987). Since the 17D YF virus E protein contained 12 amino acid changes, it was suggested that the differences in virulence between these two strains were due to the changes in E protein which affect virus binding to cell receptors (Hahn et al., 1987). Similarly, sequence differences between the Jamaica and PR-159(S1) strains of DEN-2 virus result in a 3% amino acid divergence in the E glycoprotein with regions of high and low sequence homology (Deubel et al., 1988; Hahn et al., 1988). These observations suggest that regions which code for protein domains that define cross-reactive group epitopes and virus-specific epitopes may be highly conserved.

In tropical and subtropical regions dengue viruses are mosquito-transmitted flaviviruses that cause a spectrum of disease manifestations (Halstead, 1981). Most patients with classical dengue fever (DF) have fever, myalgia and general malaise. Infection with any of the dengue serotypes provides long term immunity to the infecting type but not to infection with a heterologous dengue serotype. Theories to explain serious dengue disease, designated dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) have suggested that the disease may be caused by specific DEN virus strains or immunological mechanisms (Halstead, 1988). It is as yet unclear whether DHF/DSS is solely the manifestation of increased virulence or whether host factors permit DEN virus infection to progress to a more serious disease (Halstead, 1981, 1988). The four DEN virus serotypes can be antigenically differentiated by serological tests (Burke, 1988) or by oligonucleotide fingerprint analysis (Vezza et al., 1980). Viruses within a DEN serotype can be separated by oligonucleotide fingerprint analysis into geographical varieties or topotypes (Repik et al., 1983; Trent et al., 1983). These topotypes can also be distinguished by signature analysis using specific monoclonal antibodies that differentiate E glycoprotein antigenic epitopes (Monath et al., 1986). Comparison of DEN-1 fingerprints from DF or DHF/DSS patients has not resolved whether the disease outcome is associated with virus strain variation.

We have analysed the nucleotide and amino acid sequences of structural genes of DEN-1 strains that represent distinct genetic topotypes to define the nucleotide sequence similarities of strains having unique fingerprints. This information has permitted us to understand sequence variation and evolution of DEN strains over geographical and temporal distances.

**METHODS**

**Virus strains.** Four DEN-1 virus strains were isolated from humans with classical DF: AHF82-80 (Thailand 1980), 836-1 (Philippines 1984, strain 162, AP2), 924-1 (Mexico 1983, strain 1378) and CV1636/77 (Jamaica 1977). One DEN-1 virus strain was isolated from a human case of DHF grade IV, 495-1 (Aruba 1985, strain 1566).

**Virus propagation, RNA preparation and fingerprinting.** Viruses were propagated in C6/36 Aedes albopictus cells infected at an m.o.i. of 0.1 and the supernantant fluids were harvested 9 days after infection. Virus titrations and plaque reduction neutralization were done in BHK-21 clone 15 cells (Morens et al., 1985). Indirect fluorescent antibody staining of infected C6/36 cells was done with type-specific monoclonal antibodies (Henchel et al., 1982).

**RNA purification, extraction and RNase T1-resistant oligonucleotide fingerprinting.** We have sequenced by reverse transcriptase in the presence of specific oligonucleotide primers (Kinney et al., 1986). Two specific oligonucleotide primers were used to initiate first strand cDNA synthesis: MC1, complementary to the 5' end of the DEN-2 non-structural NS1 protein (5' GCCACATTTACGTTCTT 3') (Deubel et al., 1986) and primer E1 (5' ATGCACAGTTGCGCAGGAC 3') based on the DEN-1 AHF82-80 sequence starting from nucleotide 1003 near the 5' end of E protein. Appropriate restriction fragments of cDNA were subcloned into the filamentous coliphage M13 mp18/19 (Yanisch-Perron et al., 1985) for sequencing by the dideoxynucleotide chain termination method (Sanger et al., 1977).

**DEN-1 cloning and sequencing.** Complementary DNA to DEN-1 RNA was synthesized using reverse transcriptase in the presence of specific oligonucleotide primers (Kinney et al., 1986). Two specific oligonucleotide primers were used to initiate first strand cDNA synthesis: MC1, complementary to the 5' end of the DEN-2 non-structural NS1 protein (5' GCCACATTTACGTTCTT 3') (Deubel et al., 1986) and primer E1 (5' ATGCACAGTTGCGCAGGAC 3') based on the DEN-1 AHF82-80 sequence starting from nucleotide 1003 near the 5' end of E protein. Appropriate restriction fragments of cDNA were subcloned into the filamentous coliphage M13 mp18/19 (Yanisch-Perron et al., 1985) for sequencing by the dideoxynucleotide chain termination method (Sanger et al., 1977). Complementary DNA inserts were identified by hybridization and sequencing by generating a series of overlapping deletion clones (Dale et al., 1985).

**Primer-directed RNA sequencing.** DEN-1 virus RNA was sequenced by the dideoxynucleotide method adapted by Dr B. J. B. Johnson (personal communication) from the methods of Biggin et al. (1983) and Sanger et al. (1977). For direct RNA sequencing of the E glycoprotein gene, six nucleotide primers were synthesized complementary to the E protein sequence of AHF82-80. The primers were identified as M2 (nucleotides 1144 to 1173), M3
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(nucleotides 1327 to 1358), M4 (nucleotides 1474 to 1503), M5 (nucleotides 1681 to 1710), M6 (nucleotides 1886 to 1914) and M7 (nucleotides 2107 to 2136). Two micrograms of virion RNA was hybridized to 100 ng of synthetic oligonucleotide primer for 2 min at 80 °C in 12.5 μl hybridization buffer (0.05 M-Tris-HCl pH 8.1 and 0.1 M-KCl). The sequencing reaction contained 5 μl of hybridized RNA-primer mixture, 2 μl of 10 × reverse transcriptase buffer (0.05 M-Tris-HCl pH 8.1, 0.6 M-KCl, 0.1 M-MgCl2, 0.1 M-dithiothreitol), 30 units of RNasin (Promega Biotec), 20 μCi [α-35S]dCTP (New England Nuclear), 20 units reverse transcriptase (RAV-2, 20 units/μl, Amersham) and stocks of appropriate ddNTP/dNTP mixes as recommended (Biggin et al., 1983). DNA fragments were separated by electrophoresis on 6% acrylamide–urea gels in Tris-borate-EDTA buffer at 1800 V, 24 mA for 3 to 4 h. The gels were transferred onto Ephortec film (Transfilm, Haakbuchler Instruments), soaked in 10% acetic acid, dried and autoradiographed on Kodak XAR-5 film.

RESULTS
RNA fingerprint analysis
The genetic similarity of each of the DEN-1 RNAs to each other was examined by T1-resistant oligonucleotide fingerprint analysis. Thailand, Philippines and Caribbean DEN-1 isolates each presented a distinct RNA fingerprint (Fig. 1). Strains CV1636/77, 495-1 and 924-1 belong to the Caribbean topotype described by Repik et al. (1983); however, the two recent Caribbean isolates, 495-1 (1985) and 924-1 (1983), contained several unique oligonucleotides. DEN-1 virus AHF82-80, Thailand 1980, shares many oligonucleotides with the Thailand strains isolated during the 1980s yet is quite distinct from the other Thai isolates (M. C. Chu, unpublished observations). The fingerprint of strain AHF82-80 is not similar to those of the other four DEN-1 strains examined in this study. The RNA fingerprint of strain 836-1 is representative of one of the two distinct DEN-1 topotypes cocirculating in the Philippines in 1984 (M. C. Chu, unpublished observations).

(a) (b) (c)

Fig. 1. RNase T1-resistant oligonucleotide fingerprints of DEN-1 strains: (a) Caribbean CV1636/77, (b) Thailand AHF82-80 and (c) Philippines 836-1.
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
</tbody>
</table>

...
Variation among dengue 1 viruses

Fig. 2. Nucleotide sequences coding for the structural gene regions of DEN-1 strains Caribbean CV1636/77, Thailand AHF82-80 and Philippines 836-1.

Nucleotide sequence of DEN-1 structural protein genes

The nucleotide sequences encoding the structural proteins of Caribbean CV1636/77, Thailand AHF82-80 and Philippines 836-1 are shown in Fig. 2. The 5' ends of the genomes have 101, 97 and 91 nucleotides, respectively, before the first AUG codon. Nucleotides coding for the DEN-1 C proteins are contained in the first 342 base pairs of the coding region, followed by 273 and 225 nucleotides that encode prM and M proteins, respectively. The C proteins are composed of 117 amino acids that have an approximate Mr of 12800. The prM-M coding regions are located between putative signalase cleavage sites that appear to be conserved among all three DEN-1 strains and among deduced flavivirus protein sequences (Rice et al., 1985; Deubel et al., 1986; Mason et al., 1987). Cleavage of prM-M to produce mature M proteins results in the formation of an M protein with an Mr of 8000.

The E glycoprotein is encoded by nucleotides 841 to 2325, i.e. the last 1485 nucleotides of the structural gene region. The N terminus of the deduced DEN-1 495 amino acid E glycoprotein begins at a signalase cleavage site which is identical to those suggested for other DEN viruses (Hahn et al., 1988). Two likely glycosylation sites at amino acids 347 and 433 are conserved among the DEN-1 virus amino acid sequences. The C-terminal segments of prM, M and E contain hydrophobic regions that are thought to be membrane anchoring regions for each of the respective structural proteins.

Comparison of deduced structural proteins among DEN serotypes

Caribbean CV1636/77, Thailand AHF82-80 and Philippines 836-1 DEN-1 viruses have very similar nucleotide sequences throughout the structural gene region with less than 5% (within a range of 68 to 108 nucleotides out of 2325) sequence changes between the strains. These nucleotide differences translate to 2% overall amino acid changes between the reference Caribbean CV1636/77 strain and Thailand AHF82-80 and Philippines 836-1 DEN-1 viruses (Fig. 2 and 3).

The deduced amino acid sequences throughout the structural protein regions of the three DEN-1 virus strains were compared with the published sequences of similar proteins of DEN-1 Nauru Island (Mason et al., 1987), DEN-2 Jamaica (Deubel et al., 1988), DEN-2 PR-159(S1) (Hahn et al., 1988) and DEN-4 Dominica (Zhao et al., 1986) (Fig. 3). The relatedness of the deduced amino acid sequences for C, prM, M and E are summarized in Table 1. Portions of the nucleotides in the 5' non-coding region preceding the first AUG codon of the open reading frame appear to be conserved among all the DEN virus sequences (Fig. 3) (Deubel et al., 1988; Zhao et al., 1986; Mason et al., 1987; Hahn et al., 1988). A consensus sequence of six nucleotides, CUACGU, between the DEN-1 viruses and DEN-2 Jamaica is located near the 5' cap, the region that Brinton & Dispoto (1988) reported to be highly conserved in all flaviviruses. In comparison with other DEN-1 strains, the structural protein sequence of Thailand AHF82-80 has the most changes in the C protein. Five of the nine amino acid differences in the AHF82-80 C protein sequence are similar to those of the DEN-2 Jamaica and Puerto Rico 159-S1 virus C proteins (Fig. 3).
Fig. 3. Comparison of deduced amino acid sequences for structural proteins of DEN-1, DEN-2 and DEN-4 virus strains.
Table 1. Comparison of the structural protein sequences of DEN virus strains using Caribbean CV1636/77 as the reference strain

<table>
<thead>
<tr>
<th>Type</th>
<th>Strain</th>
<th>C</th>
<th>prM</th>
<th>M</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEN-1</td>
<td>Thailand AHF82-80</td>
<td>9 (8)*</td>
<td>3 (3)</td>
<td>1 (1)</td>
<td>5 (1)</td>
</tr>
<tr>
<td></td>
<td>Philippines 836-1</td>
<td>3 (3)</td>
<td>3 (3)</td>
<td>1 (1)</td>
<td>9 (2)</td>
</tr>
<tr>
<td></td>
<td>Nauru Island†</td>
<td>5 (4)</td>
<td>2 (2)</td>
<td>1 (1)</td>
<td>10 (2)</td>
</tr>
<tr>
<td>DEN-2</td>
<td>Jamaica‡</td>
<td>32 (28)</td>
<td>23 (25)</td>
<td>23 (31)</td>
<td>161 (33)</td>
</tr>
<tr>
<td></td>
<td>PR-159(S1)§</td>
<td>33 (29)</td>
<td>21 (23)</td>
<td>23 (31)</td>
<td>155 (31)</td>
</tr>
<tr>
<td>DEN-4</td>
<td>Dominica¶</td>
<td>36 (32)</td>
<td>30 (33)</td>
<td>31 (41)</td>
<td>191 (38)</td>
</tr>
</tbody>
</table>

* Number of amino acid changes. Numbers in parentheses represent percentage of amino acid changes in reference to Caribbean CV1636/77.
† Mason et al. (1987).
‡ Deubel et al. (1986).
§ Hahn et al. (1987).
¶ Zhao et al. (1986).

Table 2. Nucleotide substitutions in DEN-1 envelope glycoprotein sequences: comparisons with Caribbean CV1636/77 reference strain

<table>
<thead>
<tr>
<th></th>
<th>Philippines 836-1</th>
<th>Thailand AHF82-80</th>
<th>Caribbean 495-1</th>
<th>924-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transitions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A → G</td>
<td>9</td>
<td>5</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>G → A</td>
<td>10</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C → U</td>
<td>6</td>
<td>3</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>U → C</td>
<td>13</td>
<td>5</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>Transversions*</td>
<td></td>
<td>8</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>23</td>
<td>27</td>
<td>29</td>
</tr>
</tbody>
</table>

* Transversions: purine to pyrimidine and vice versa.

Divergence in the sequence coding for prM proteins of different DEN-1 strains represented a 3% change. These amino acid changes at positions 56 to 60 are clustered and indicate a possible secondary structure change in Thailand AHF82-80 and Philippines 836-1 compared to Caribbean CV1636/77. The only protein sequence change in the M protein occurred at amino acid position 5 for the DEN-1 strains. The protein sequence similarity was greatest between 836-1 and Nauru Island (Mason et al., 1987) which shared 99.7% similarity.

Comparison of the E glycoprotein genomic sequences of DEN-1 viruses

To evaluate genetic stability of the E protein nucleic acid sequence variations within a single topotype, the genomes of two recently isolated DEN-1 Caribbean strains (Aruba 495-1, 1985; Mexico 924-1, 1983) were sequenced throughout the E glycoprotein region. Comparison of the E protein nucleotide sequences for the five DEN-1 viruses is presented in Fig. 4 and Table 2. There were 23 to 46 nucleotide changes in the 1485 nucleotides coding the E protein region. This represented a change of 1.55% to 3% with five to nine amino acid changes, giving an at least 97% sequence similarity over the entire E glycoprotein region. These results show less divergence than those observed between DEN-2 Jamaica and PR-159(S1) E sequences (Hahn et al., 1988). The similarity within the E sequence is maintained when comparing the sequence of the DEN-1 Nauru Island (Mason et al., 1987) with our results.

The recent Caribbean strains, 495-1 and 924-1, differed from the earlier Caribbean CV1636/77 virus by 27 and 29 nucleotide transitions, respectively, and by one additional transversion in 924-1. Shared nucleotide differences in the Caribbean DEN-1 strains were primarily located at nucleotides 321 to 339. Three nucleotide differences occurred between 417 and 429, three changes between 482 and 547, a region of change between 626 and 720 and four
Changes between 1076 and 1336. There were 22 nucleotide differences common to DEN-1 viruses 495-1 and 924-1, with amino acid changes occurring at identical genome positions.

Nucleotide differences are located in clusters for the Philippines and Thailand DEN-1 topotypes (Fig. 4). There were 23 nucleotide differences between Thailand AHF82-80 and Caribbean CV1636/77 virus strains consisting of 20 transitions and three transversions, leading to five amino acid substitutions. Nucleotide differences do not always reflect protein structural changes; the positions at which differences were observed for Thailand AHF82-80 as compared to Caribbean CV1636/77 were clustered at E protein nucleotides 217 to 288, 339 to 390, 444, 447, 738, 798 to 891, 1206 and at 1336. The Philippines strain 836-1 differed from Caribbean CV1636/77 by 46 nucleotide changes, with a total of nine amino acid changes in the protein sequence (Table 3). The first 20 nucleotide differences occurred within the E protein gene between nucleotides 6 and 288, followed by six changes between nucleotides 339 and 444, five changes between positions 482 and 611, and finally a series of changes that are clustered around nucleotides 780, 1000 and 1336. Three amino acid differences located at amino acid positions 359, 382 and 446 appear to be unique for Caribbean CV1636/77.

**DISCUSSION**

An unanswered question in fingerprint analyses has been whether geographical variants of DEN can be associated with genomic markers for virus virulence. To analyse diversity and variation among DEN-1 virus strains with different geographical origins and from cases with different clinical manifestations, we have sequenced and compared the genomic regions that encode the structural proteins of different DEN-1 virus strains. RNase T1 fingerprinting

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**Table 3. Deduced amino acid changes in the DEN-1 envelope glycoprotein: comparisons with Caribbean CV1636/77 reference strain**

<table>
<thead>
<tr>
<th>Amino acid position</th>
<th>Philippines 836-1</th>
<th>Thailand AHF82-80</th>
<th>Caribbean 495-1</th>
<th>Caribbean 924-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>N → D</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>88</td>
<td>A → T</td>
<td>A → T</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>114</td>
<td>L → I</td>
<td>L → I</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>160</td>
<td>I → T</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>198</td>
<td>V → M</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>203</td>
<td>K → E</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>297</td>
<td>-</td>
<td>M → V</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>359</td>
<td>I → T</td>
<td>I → T</td>
<td>I → T</td>
<td>I → T</td>
</tr>
<tr>
<td>382</td>
<td>S → A</td>
<td>S → A</td>
<td>S → A</td>
<td>S → A</td>
</tr>
<tr>
<td>446</td>
<td>I → V</td>
<td>I → V</td>
<td>I → V</td>
<td>I → V</td>
</tr>
</tbody>
</table>

* No amino acid change is denoted by -.
randomly resolves 10% to 15% of the genomic RNA into distinct oligonucleotides for analysis (Aaronson et al., 1982), whereas by genomic sequence analyses any region of the RNA can be examined. The total genomic data analysed in this study comprise less than 25% of the genome for any one isolate (2325 of 10269 nucleotides). Within the nucleotide sequences of the DEN-1 structural protein region there is a remarkable similarity among strains separated by geography and time. With the present data we cannot directly correlate genomic variations with specific oligonucleotide changes. However, DEN-1 strains obtained from different geographical regions can be differentiated generally by their pattern of nucleotide substitutions at specific loci in the E glycoprotein.

Studies by Monath et al. (1986) have shown that anti-E glycoprotein monoclonal antibodies differentiate DEN-2 topotypes. We observed that DEN-1 strains of the Caribbean topotype have E glycoprotein nucleotide sequences which are more similar to each other than to those of strains from Thailand or the Philippines. Caribbean strains (CV1636/77, 495-1, 924-1) which were isolated over a span of 8 years show high conservation in the nucleotide and amino acid sequences of the E glycoprotein. Sequence analysis of the E glycoproteins of Australian and Papua New Guinea isolates of MVE virus showed not more than 1.7% nucleotide sequence divergence in pairwise comparisons of the Australian isolates, 6.8% divergence in the two Papua New Guinea isolates and 9% to 10% between the prototype Australian prototype and Papua New Guinea strains (Lobigs et al., 1988). These isolates were from widely separated geographical regions over a 25 year period.

Oligonucleotide mapping suggests that annual rates of nucleotide substitution in regional isolates of enteroviruses and polioviruses may vary from 0.43% to 2% (Smith & Inglis, 1987). Although the annual rate of mutation and selection in the DEN-1 genome is unknown, oligonucleotide fingerprint results suggest that DEN-1 viruses in the Caribbean have remained genetically stable (Repik et al., 1983). Based on these observations we could predict a 3% change in the DEN-1 genome sequence between a 1977 and a 1985 isolate (0.43% x 8 years). The observed nucleotide differences between strains CV1636/77 and 924-1 or 495-1 were 1.9% and 1.8% respectively, suggesting that the number of nucleotide changes over time was infrequent. These observations may explain why the nucleotide sequences encoding the E protein of the Caribbean DEN-1 isolates from classical DF and fatal DHF/DSS cases are nearly identical although they were isolated over an 8 year period.

The JE virus E glycoprotein has two major immunoreactive domains (McAda et al., 1987); Domain I includes the amino-terminus up to amino acid residue 122 and Domain II is located between amino acid positions 293 and 401. Nowak & Wengler (1987) identified R1, R2 and R3 immunoreactive regions for the WNV E glycoprotein. R1 encompasses the same region as the JE Domain I and amino acid residues 184 to 332 and 367 to 446 make up the R2 and R3 regions, respectively. We have examined the amino acid sequence differences among DEN-1 virus E proteins in these regions to determine the genetic stability of the virus. Amino acid differences were observed for the Philippines strain 836-1 at amino acid positions 37, 88 and 114 and the Thai strain at position 88 within Domain I/R1. Two amino acid changes in DEN-1 Philippines strain 836-1 occur at the intra-loop regions between R1/R2 at position 160 and between the R2/R3 loop at position 359. A unique amino acid difference in Thailand strain AHF82-80 was located at residue 297, for Philippines strain 836-1 at residues 196 and 203; all of these are within R2. The final group of DEN-1 amino acid changes are located in Domain II/R3 at residues 382 and 446. The E glycoprotein amino acid changes of our sequenced DEN-1 strains appear to be located in the immunoreactive regions as reported for other flaviviruses.

Our results demonstrate that within the DEN-1 serotype the nucleotide sequences of the structural regions are very similar for viruses obtained from different geographical regions. Comparative analyses have not identified genomic markers to permit differentiation of geographical or virulence characteristics. However strains could be differentiated by nucleotide changes and subsequent deduced amino acid changes by examining the clustered pattern unique to each topotype. Assessment of the significance of these genetic changes will require molecular characterization of isolates obtained from different geographical regions and analysis of strains selected for virulence to locate genomic changes associated with alterations in biological characteristics.
REFERENCES


*(Received 12 December 1988)*