Genetic Relationships of the Dengue Virus Serotypes

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SUMMARY

Previous studies have compared RNA genomes from the different dengue virus serotypes by cDNA-RNA hybridization using dengue-1 virus- and dengue-2 virus-specific cDNA probes. These probes revealed that there is a close genetic relationship between dengue virus serotypes 1 and 4. In this communication, the cDNA–RNA hybridization results using dengue-3- and dengue-4-specific cDNA probes to determine the genetic relatedness of all four dengue virus serotypes are reported. The results indicate that serotypes 1 and 4 are genetically very closely related (sharing about 70% of their genomes as detected by both the dengue-1 and dengue-4 cDNA probes), as are serotypes 3 and 4 (sharing about 50% of their genomes as detected by both the dengue-3 and dengue-4 cDNA probes). Serotype 2 does not seem to be very closely related to the other dengue virus serotypes by cDNA–RNA hybridization analysis.

Flaviviruses are a group of related viruses within the family Togaviridae, which replicate in arthropod and vertebrate cells. Some of these viruses cause major diseases such as Japanese encephalitis, yellow fever and dengue haemorrhagic fever in humans. Flaviviruses are subdivided by their reactions in serological tests. Each virus contains antigenic determinants common to the group, others which specify a complex of viruses and those which determine serotype specificity (Trent, 1977). There are four dengue virus serotypes which form a discrete complex of flaviviruses (Porterfield, 1980), and each of these serotypes is associated with a wide range of clinical manifestations.

Dengue viruses consist of a single-stranded RNA genome (about 12000 nucleotides long), a capsid protein (C), a low mol. wt. membrane protein (M) and an envelope glycoprotein (E) (for review, see Schlesinger, 1977). To determine the relatedness of dengue virus RNA genomes from each of the four serotypes, near full-length serotype-specific cDNA probes were synthesized and used in cDNA–RNA hybridization experiments. Studies with dengue-1- and dengue-2-specific cDNA probes were reported previously (Blok et al., 1984) and results with dengue-3- and dengue-4-specific probes are described below.

Prototype viruses of the four dengue serotypes (dengue-1, Haw; dengue-2, NGC; dengue-3, H87; dengue-4, H241) were grown in the C6/36 clone of Aedes albopictus cells, and virus particles extruded into the medium were purified by polyethylene glycol precipitation. Viral RNA was extracted from the virus pellets and used either as a template for cDNA synthesis in vitro using an oligo(dT) primer and the enzyme reverse transcriptase, or in cDNA–RNA hybridization experiments as described earlier (Blok et al., 1984).

In order to synthesize the amount of radioactively labelled cDNA needed for cDNA–RNA hybridization analyses, the concentrations of dengue-3- and dengue-4-specific RNA, of oligo (dT)12–18 primer, [32P]dCTP and avian myeloblastosis virus reverse transcriptase were increased tenfold over that used in previous studies with RNA from dengue-1 and dengue-2 serotypes (Blok et al., 1984). One possible explanation for the necessity to increase the concentrations of reactants is that the poly(A) or adenosine-rich region at or near the 3' end of the RNA genomes of dengue-3 and dengue-4 prototype viruses is shorter than that found in the other two serotypes. The 3' terminal sequences of dengue virus RNA genomes have not yet been determined but published sequence data from the West Nile and Japanese encephalitis.
Table 1. Estimated sequence homology among dengue viruses*

<table>
<thead>
<tr>
<th>RNA used for cDNA synthesis</th>
<th>RNA used in hybridization reaction</th>
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<tbody>
<tr>
<td></td>
<td>Dengue-4</td>
</tr>
<tr>
<td>Dengue-4 (H241)</td>
<td>100</td>
</tr>
<tr>
<td>Dengue-3 (H87)</td>
<td>52</td>
</tr>
<tr>
<td>Dengue-2 (NGC)†</td>
<td>23</td>
</tr>
<tr>
<td>Dengue-1 (Haw)†</td>
<td>73</td>
</tr>
</tbody>
</table>

* cDNA–RNA hybrids were formed and assayed with S1 nuclease as described in the text and the estimated percentage sequence homology was calculated as described (Gould & Symons, 1977; Gonda & Symons, 1978).
† From Blok et al. (1984).
‡ Each percentage represents an average of at least five experiments.

flaviviruses suggest that they do not contain a poly(A) tail (Wengler & Wengler, 1981; Takegami et al., 1984). However, there is a purine-rich region near the 3' end of the yellow fever virus genome (C. M. Rice, personal communication) and if this occurs in dengue viruses, it may be long enough to bind an oligo(dT) primer with variable efficiency.

By optimizing the in vitro cDNA synthesis conditions, a sufficient amount of dengue-3- and dengue-4-specific cDNA probes was produced to study the genetic relationship of all dengue virus serotypes by cDNA–RNA hybridization. These cDNA hybridization probes were similar in electrophoretic mobilities to viral RNA on polyacrylamide gels, and therefore represented near full-length genome copies. The probes were purified by electroeluting the cDNA from the region of the gel corresponding to the viral RNA.

The cDNA–RNA hybrids were formed under stringent conditions in buffer containing 0.18 M-NaCl at 65 °C for 20 h, essentially as described by Gould & Symons (1977). The hybrids formed were treated with 100 units/ml S1 nuclease at 45 °C for 30 min in buffer containing 0.08 M-NaCl. R0,t curves were determined for dengue-3 and dengue-4 cDNA probes and a log10 R0,t value of 1 was used in all hybridization experiments. The results of these hybridizations have been combined with those obtained with the dengue-1- and dengue-2-specific cDNA probes (Table 1) and reveal that (i) the background of S1 nuclease-resistant hybrids formed with non-homologous RNA (either yeast or C6/36 cellular nucleic acid) is about 10%, (ii) there is a very close genetic relationship between dengue-1 and dengue-4 since a 73% sequence is detected by both dengue-1- and dengue-4-specific cDNA probes and (iii) there is an average sequence homology of 54% between dengue-3 and dengue-4.

There is some evidence of a serological subcomplex of dengue virus serotypes 1 and 3 (Russell & Nisalak, 1967; Henchal et al., 1982), as well as a subcomplex of serotypes 2 and 4 as detected by monoclonal antibodies (E. A. Henchal, personal communication). However, there does not need to be a correlation between serology and nucleic acid hybridization since the antigenic determinants are located on the major virus glycoprotein (E), which is encoded by about 15% of the viral RNA genome. The close genetic relationship between serotypes 1 and 4, and 3 and 4, and the lack of a close sequence homology of serotype 2 with any other dengue virus serotype suggest that the serotypes may differ in their evolutionary origins.

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REFERENCES


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