Molecular Epidemiology of Tick-borne Encephalitis Virus: Peptide Mapping of Large Non-structural Proteins of European Isolates and Comparison with Other Flaviviruses

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SUMMARY

Nine virus-specified proteins were identified by SDS–polyacrylamide gel electrophoresis in [35S]methionine-labelled chick embryo cells infected with tick-borne encephalitis (TBE) virus by comparison with mock-infected cells. These proteins were designated P91, p74, p72, P67, GP53(E), P47, p25, P15(C) and P14.5 according to their molecular weights. Peptide mapping of P91, P67, GP53(E) and P47 from TBE virus-infected cells, as well as those of the corresponding proteins from West Nile virus (WNV)-infected cells (previously termed NV5, NV4, V3 and NV3), demonstrated the uniqueness of these proteins. Almost no subtype variability, with respect to the pattern of intracellular proteins, was found when isolates of TBE virus from Austria, Switzerland, Germany, Finland and Czechoslovakia were compared. Peptide mapping of NV5 (P91) and NV4 (P67) from all these isolates using limited proteolysis with α-chymotrypsin and V8 protease revealed completely identical patterns, thus extending our observations that TBE virus seems to represent a very stable member of the flavivirus genus, which was based on the lack of variation found with the structural glycoprotein. On the other hand, the Far Eastern subtype of TBE virus and the closely related louping-ill virus could not only be differentiated from the Western subtype by differences in the peptide maps of their structural glycoprotein but also in those of the non-structural protein NV5, i.e. subtype or subgroup variations are not confined to the virion surface glycoprotein. WNV and Murray Valley encephalitis virus (MVEV) revealed the expected heterogeneity of virus-specified proteins found in cells infected with different flaviviruses. It is especially interesting that also the largest non-structural protein, NV5, is subject to this heterogeneity, ranging in mol. wt. from 91000 for TBE virus to 98000 for MVEV and that also the peptide maps of NV5, as well as those of NV4, were unrelated. These proteins, therefore, revealed a variability between serologically distinct flaviviruses similar to that observed with the structural glycoprotein.

INTRODUCTION

Tick-borne encephalitis (TBE) virus is the causative agent of the most important arthropod-transmitted disease in Europe and the U.S.S.R. and, based on a cross-neutralization study (De Madrid & Porterfield, 1974), together with Langat, Negishi, Omsk haemorrhagic fever, Kyasanur Forest disease and louping-ill viruses forms the TBE complex of flaviviruses. An effective vaccine containing highly purified inactivated virus (Heinz et al., 1980) is available which represents a well-tolerated prophylactic means against the disease (Kunz et al., 1980).
In a recent study on the molecular epidemiology of TBE virus using peptide mapping and competitive radioimmunoassay (RIA) (Heinz & Kunz, 1981), we have found a remarkable lack of variation in the structural glycoprotein of strains isolated from all over Europe in different years from different hosts, pointing to a great stability of this protein. This is especially interesting since the envelope glycoprotein which also carries haemagglutinin (HA) activity (Heinz & Kunz, 1980) is the major and most likely single determinant for the induction of protective immunity (Heinz et al., 1981) and is expected to be subject to the highest selective pressure in the wild. These studies also confirmed, on a molecular level, the separation of TBE virus strains into a Western (European) subtype transmitted primarily by *Ixodes ricinus* and a Far Eastern (Russian) subtype with *Ixodes persulcatus* as its main vector, as previously proposed by Clarke (1964). If antigenic relationships are based on haemagglutination inhibition (Clarke, 1960) or neutralization tests (De Madrid & Porterfield, 1974), it is implied that these classifications reflect differences or similarities of the structural glycoprotein.

Flaviviruses characteristically induce several non-structural proteins in infected cells (for reviews, see Westaway, 1980; Trent & Naeve, 1980), and tryptic peptide mapping revealed unique sequences for most of these proteins from Kunjin virus (Wright et al., 1977; Wright & Westaway, 1977) and West Nile virus (WNV) (Wengler et al., 1979). In a comparison of seven flaviviruses from five different subgroups Westaway et al. (1977) found a remarkable uniformity in migration of the largest non-structural proteins, NV5 and NV4, in phosphate-buffered gels by SDS–polyacrylamide gel electrophoresis (SDS–PAGE) as opposed to heterogeneity of the other proteins, especially the envelope glycoprotein. Qureshi & Trent (1973a, b) purified intracellular virus antigens from cells infected with St. Louis encephalitis virus, Japanese B encephalitis virus, WNV and dengue-2 virus and demonstrated the type-specificity of NV5 in complement fixation and immunodiffusion tests, which was confirmed by Trent et al. (1976) using a solid-phase RIA. Using RNase T1 oligonucleotide fingerprinting of the virus RNA, Trent et al. (1981) have demonstrated genetic heterogeneity among St. Louis encephalitis virus isolates, which may reflect differences in genes coding for structural as well as non-structural proteins. In the present study, we extend our investigations on the molecular epidemiology of TBE virus to the non-structural proteins found in cells infected with different isolates and compare these patterns, as well as the peptide maps of the largest non-structural proteins (NV5 and NV4), with those from the corresponding proteins of serologically distant flaviviruses.

**METHODS**

**Virus strains.** All the viruses and strains investigated in the present study are listed in Table 1. The sources of these viruses have been described in detail previously (Heinz & Kunz, 1981). From these viruses, stocks were prepared as 10% (w/w) suspensions of infected suckling mouse brain in minimum essential medium (MEM) buffered with 15 mM-N-2-hydroxyethyl piperazine-N'-2-ethane sulphonic acid (HEPES) and 15 mM-N-2-hydroxyethyl piperazine-N'-2-propane sulphonic acid (EPPS) at pH 7.6 (MEM-HEPES/EPPS).

**Preparation and analysis of [35S]methionine-labelled cell extracts.** Primary chick embryo cells (CEC) were grown in plastic Petri dishes (3·5 cm diam.) and, after removal of growth medium (MEM containing 10% foetal calf serum), infected by the addition of 1 ml of the respective stock virus diluted in MEM-HEPES/EPPS containing 0·1% bovine serum albumin (MEM-HEPES/EPPS-BSA) at a titre of 10⁷ mouse intracerebral LD₅₀/0·02 ml. The virus was allowed to adsorb for 90 min, and then the inoculum was removed and 2 ml MEM-HEPES/EPPS-BSA were added to the cells. After 24 h incubation at 37°C the medium was
Table 1. *Strains of tick-borne encephalitis virus and other flaviviruses analysed*

<table>
<thead>
<tr>
<th>Geographic origin</th>
<th>Strain designation</th>
<th>Source of isolation</th>
<th>Year of isolation</th>
<th>No. of mouse brain passages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austria: Burgenland</td>
<td>Neudörfl</td>
<td><em>Ixodes ricinus</em></td>
<td>1971</td>
<td>3</td>
</tr>
<tr>
<td>Lower Austria</td>
<td>Scharl</td>
<td>Human brain</td>
<td>1956</td>
<td>3</td>
</tr>
<tr>
<td>Switzerland</td>
<td>I-40</td>
<td><em>Ixodes ricinus</em></td>
<td>1972</td>
<td>12</td>
</tr>
<tr>
<td>Germany: Karlsruhe</td>
<td>K-23</td>
<td><em>Ixodes ricinus</em></td>
<td>1975</td>
<td>4</td>
</tr>
<tr>
<td>Finland: Kumlinge island</td>
<td>A-52</td>
<td><em>Ixodes ricinus</em></td>
<td>1959</td>
<td>Unknown</td>
</tr>
<tr>
<td>Czechoslovakia</td>
<td>Hypr</td>
<td>Human blood</td>
<td>1953</td>
<td>&gt; 60</td>
</tr>
<tr>
<td><strong>TBE virus: Far Eastern subtype</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U.S.S.R.</td>
<td>Sofyn</td>
<td>Human brain</td>
<td>1937</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

**Other flaviviruses**
- Louping-ill virus (strain Moredun: Scotland)
- West Nile virus (isolate from Nigeria; see Wengler *et al.*, 1979)
- Murray Valley encephalitis (prototype of Yale Arbovirus Research Unit, New Haven, Conn., U.S.A.)

Molecular weight estimations of larger proteins, including the structural glycoprotein, were performed on 10% gels and those of smaller proteins on 17% gels using 14C-methylated lysozyme (14300), carbonic anhydrase (30000), ovalbumin (46000), BSA (69000), phosphorylase b (92 500) and myosin (200 000) as markers.

**Propagation and purification of [35S]methionine-labelled TBE virus.** TBE virus was pelleted from the supernatant of [35S]methionine-labelled cells and then purified by rate-zonal density-gradient centrifugation exactly as described by Heinz & Kunz (1981).

**Peptide mapping of intracellular proteins by limited proteolysis.** Peptide mapping was carried out according to the method of Cleveland *et al.* (1977). Protein bands to be analysed were excised from dried gels, cut into small pieces and eluted with 1 ml TAN buffer (0-02 M-triethanolamine, 0-13 M-NaCl, pH 8) containing 0-1% SDS and 0-1 mg/ml BSA by shaking overnight at 60 °C. The gel pieces were then centrifuged off, and the supernatant adjusted to 10% final concentration of trichloroacetic acid and left in an ice-bath for 30 min. The precipitate was collected by centrifugation at 3000 g at 4 °C for 5 min, washed twice in ice-cold acetone, dried and solubilized by boiling for 3 min in 0-125 M-Tris pH 6-8 containing 0-5% SDS and 10% glycerol. Proteolysis was performed with a-chymotrypsin (from bovine pancreas, 45 units/mg; Serva, Heidelberg, F.R.G.) or V8 protease from *Staphylococcus aureus* (Miles Laboratories, Stoke Poges, Bucks., U.K.) by incubation for 30 min at 37 °C at final concentrations of 100 μg/ml. Then, 2% SDS and 4% 2-mercaptoethanol were added, and the samples were subjected to SDS–PAGE in 17% gels according to Laemmli & Favre (1973). After the run, gels were fixed in 10% acetic acid/50% methanol and fluorographed according to Bonner & Laskey (1974) with the exception that acetic acid was used instead of dimethyl sulphoxide.
RESULTS

Analysis of virus-induced proteins in CEC infected with different TBE virus strains and other flaviviruses

A previous study on the molecular epidemiology of TBE virus has shown a remarkable homogeneity and stability of the structural glycoprotein (Heinz & Kunz, 1981), and we were interested as to whether this also holds true for virus-specified non-structural proteins found in infected cells. Six TBE virus isolates from different European countries and/or derived from different hosts (Table 1) were analysed with respect to the proteins they induce in infected CEC. In addition, a member of the Far Eastern subtype of TBE virus (Sofyn) and the closely related louping-ill virus, as well as two other flaviviruses, WNV and Murray Valley encephalitis virus (MVEV), were included in this comparison. As can be seen from Fig. 1 (a, b) several virus-induced proteins not present in mock-infected cells could be identified by SDS–PAGE. To get good resolution over the whole mol. wt. range two gel concentrations (10% and 17%) were used.

Designations shown on Fig. 1 have been reconciled with the recently proposed new nomenclature of flavivirus proteins (Westaway et al., 1980) but the previously used terms were also applied, thus enabling a comparison of presumably analogous proteins induced by different viruses. In accordance with previous studies on the synthesis of virus-specified proteins in flavivirus-infected cells (for review, see Westaway, 1980), NV5 is the largest TBE virus-specific protein with a mol. wt. of 91000 (P91), which is significantly smaller than the corresponding protein in WNV-infected cells (P95) and MVEV-infected cells (P98).

The next well-established protein (NV4) has an apparent mol. wt. of 67000 and 69000 in TBE virus- and WNV-infected cells respectively (P67 and P69). However, there are two additional bands (p74 and p72) between NV4 and NV5 in TBE virus-infected cells only, which have also been found by Svitkin et al. (1981). The structural glycoprotein (V3 or E) of TBE virus co-migrates with its intracellular counterpart at an estimated mol. wt. of 53000 in 10% gels [GP53(E)] which is slightly smaller than our previous estimate of 55000 derived from 17% gels (Heinz & Kunz, 1981). It should be stressed that mol. wt. estimations in SDS–polyacrylamide gels can vary from gel to gel especially when different acrylamide concentrations or different mol. wt. markers are used. A reliable statement on the mol. wt. homogeneity or heterogeneity of closely related proteins therefore has to be based upon a comparison performed on the same gel. As shown previously by the analysis of purified virions (Heinz & Kunz, 1981) and in accordance with Wengler et al. (1979), the structural glycoprotein of WNV is definitely smaller than that of TBE virus, with an estimated mol. wt. in 10% gels of approx. 48000 [GP48(E)]. The band termed P47 found in TBE virus-infected cells may correspond to the previously designated NV3 which has been described for other flaviviruses (Westaway et al., 1980). Between the mol. wt. range 15000 to 40000 (Fig. 1 b) only one virus-specified protein can be detected in TBE virus-infected cells (p25), whereas three proteins are clearly discernible in those infected with WNV probably corresponding to NVX, NV2 1/2 and NV2 (Westaway et al., 1980). Migrating slightly faster than the core protein (V2 or C) of purified virions (mol. wt. 15000 for both TBE and WNV: Heinz & Kunz, 1981; Wengler et al., 1979), another strongly labelled band is found in TBE virus- and WNV-infected cells termed P14–5. As shown by tryptic peptide mapping for Kunjin virus by Wright & Westaway (1977) and for WNV by Wengler et al. (1979), this protein represents an intracellular form of C probably derived by post-translational cleavage. However, using 17% gels, we could clearly resolve a second, though fainter band [P15(C)] exactly co-migrating with C in TBE virus-infected cells as well as in WNV-infected cells. Although the structural proteins of WNV have not been included as a control in this analysis, we know from previous studies (Heinz & Kunz, 1981) that the core proteins of WNV and TBE virus
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Fig. 1. SDS-PAGE analysis in (a) 10% and (b) 17% gels of [14C]methionine-labelled CEC infected with different TBE virus isolates and other flaviviruses (see Table 1). Purified TBE virus and uninfected cells were used as controls.
have identical migration rates in SDS–polyacrylamide gels. Another virus-specified protein, P10 (previously designated NV1) found in flavivirus-infected cells was, however, undetectable in both our system and in WNV-infected BHK-21 cells (Wengler et al., 1979).

All the TBE virus isolates, including the Far Eastern strain Sofyn, yielded a very similar pattern with almost identical mol. wt. for the most prominent proteins (Table 2). However, due to the high resolution obtained, even very slight variations lying beyond 1000 daltons could be detected and concerned NV3, NVX and V2 (for details, see Table 2). The p74 protein was not found in Hypr-infected cells nor in Sofyn-infected cells (Fig. 1a). Louping-ill virus could be distinguished from TBE virus strains by a slightly slower migration of NV5 (P93) as well as the intracellular form of the structural glycoprotein [GP54(E)] and the core protein [P16(C)]. The patterns of WNV and MVEV exemplify the heterogeneity of virus-induced proteins between different flaviviruses even belonging to the same serological subgroup.

Comparison of NV5, NV4, V3 and NV3 by peptide mapping

To analyse possible relationships between the large well-defined virus-specified proteins found in cells infected with TBE virus (strain Neudörfl) the corresponding bands were excised from SDS–polyacrylamide gels and subjected to peptide mapping by limited proteolysis as described in Methods using both α-chymotrypsin as well as V8 protease (Fig. 2). Parallel analysis of the same gel region derived from mock-infected cells was an effective means to differentiate between cleavage products derived from virus-specified proteins versus host proteins, the synthesis of which is not shut off in flavivirus-infected cells (Westaway, 1980). As a control, WNV-specified proteins, which have previously been shown by tryptic fingerprinting to be unrelated to each other, were subjected to the same procedure (Fig. 3). As can be seen from Fig. 2 and 3 a completely different set of cleavage products was obtained from all the virus proteins analysed, thus confirming their uniqueness. The comparison of the intracellular and the virion-associated glycoprotein of TBE virus [GP53(E)] also showed that they were almost identical. Slight differences, however, were observed in the maps obtained with V8 protease (double band at mol. wt. approx. 45 000 only present in virion-derived glycoprotein). Indistinguishable maps were obtained for the structural glycoprotein of WNV and its intracellular counterpart GP48(E).

Peptide mapping of NV5 and NV4 from TBE virus strains and other flaviviruses

These two proteins have consistently been found in cells infected with all flaviviruses so far analysed (for review, see Westaway, 1980). As identical migration rates in SDS–PAGE do not necessarily mean identity, we subjected these proteins to peptide mapping by limited proteolysis. As shown previously (Heinz & Kunz, 1981), minor differences may be missed if such analyses are performed with only one protease, and we therefore used two proteases with different cleavage specificities, α-chymotrypsin and V8 protease. The results for NV5 are shown in Fig. 4 (a, b). It is striking that NV5 from all European TBE virus isolates analysed yielded completely identical cleavage patterns, whereas those from the Far Eastern strain (Sofyn) and louping-ill virus showed slight deviations from an otherwise very similar pattern. In Fig. 4 (a) the double band at mol. wt. 32 000 (arrow) definitely migrates faster in NV5 from strain Sofyn, and the lowest band of the cluster at mol. wt. 20 000 (arrow) is lacking in Sofyn as well as in louping-ill virus. The upper band of this cluster also migrates more slowly in the case of louping-ill virus than its counterpart from the TBE virus strains. In Fig. 4 (b) a strongly labelled band at mol. wt. 35 000 (arrow) is only found in strain Sofyn which, however, lacks a band at mol. wt. 32 000 (arrow) present in the European TBE virus strains.
Table 2. **Virus-specified proteins in CEC infected with strains of tick-borne encephalitis virus and other flaviviruses**

<table>
<thead>
<tr>
<th></th>
<th>Austria</th>
<th>Switzerland</th>
<th>Germany</th>
<th>Finland</th>
<th>Czechoslovakia</th>
<th>U.S.S.R.</th>
<th>louping-ill</th>
<th>WNV</th>
<th>MVEV</th>
</tr>
</thead>
<tbody>
<tr>
<td>NV5</td>
<td>Neudorfl</td>
<td>Scharl</td>
<td>I-40</td>
<td>K-23</td>
<td>A-52</td>
<td>Hypr</td>
<td>Sofyn</td>
<td>p74</td>
<td>p74</td>
</tr>
<tr>
<td>-</td>
<td>P91</td>
<td>P91</td>
<td>P91</td>
<td>P91</td>
<td>P91</td>
<td>P91</td>
<td>P93</td>
<td>P95</td>
<td>P98</td>
</tr>
<tr>
<td>NV4</td>
<td>P67</td>
<td>P67</td>
<td>P67</td>
<td>P67</td>
<td>P67</td>
<td>P67</td>
<td>P69</td>
<td>P67</td>
<td>-</td>
</tr>
<tr>
<td>V3</td>
<td>GP53(E)</td>
<td>GP53(E)</td>
<td>GP53(E)</td>
<td>GP53(E)</td>
<td>GP53(E)</td>
<td>GP53(E)</td>
<td>GP54(E)</td>
<td>GP55(E)</td>
<td>-</td>
</tr>
<tr>
<td>V2</td>
<td>P15(C)</td>
<td>P15(C)</td>
<td>P15(C)</td>
<td>P15(C)</td>
<td>P15(C)</td>
<td>P15-2(C)</td>
<td>P15(C)</td>
<td>P16(C)</td>
<td>P15(C)</td>
</tr>
<tr>
<td>NV1</td>
<td>P14-5</td>
<td>P14-5</td>
<td>P14-5</td>
<td>P14-5</td>
<td>P14-5</td>
<td>P14-5</td>
<td>P15</td>
<td>P14-3</td>
<td>P14-5</td>
</tr>
</tbody>
</table>

* Nomenclature is according to Westaway et al. (1980).
and louping ill virus. On the other hand, unique maps were obtained for NV5 from WNV and MVEV. The same also holds true for the analysis of NV4 (Fig. 5a, b); however, practically no differences were found between European TBE virus isolates on the one hand and the Far Eastern strain (Sofyn) and louping ill virus on the other.

**DISCUSSION**

Nine virus-specified proteins were detected in TBE virus-infected CEC, which we have designated by the previously used terminology for flavivirus proteins as well as the newly proposed nomenclature (Westaway et al., 1980). One of the interesting features of the protein pattern obtained was the presence of two additional virus-specified bands (p74 and p72) between NV5 and NV4 which have not been described in cells infected with 11 different flaviviruses (Westaway et al., 1977). Proteins of similar mol. wt. are also discernible on an electrophoretic profile of TBE virus-infected pig embryo kidney cells (Svitkin et al., 1981), although only the larger one (p79) has been designated NV4 1/2. Corresponding proteins have also been described by Trent & Naeve (1980) in St. Louis encephalitis virus-infected HeLa cells after subtracting the normal HeLa cell proteins from SDS–PAGE profiles. For
Fig. 3. Comparison of P95, P69 and GP48(E) from WNV-infected cells and E from purified WNV by peptide mapping as described in the legend to Fig. 2. (a) a-chymotrypsin; (b) V8 protease.

several flaviviruses, NV3 has been difficult to resolve due to a prominent host protein (presumably actin) of similar mol. wt. (Westaway et al., 1977), and for Kunjin virus the results of tryptic peptide maps did not provide evidence for a virus-coded component when the differentially labelled cellular protein was used as a control (Wright et al., 1977). In our gel system, NV3 (P47) was clearly resolved from host proteins, and peptide mapping by limited proteolysis using the corresponding region from uninfected cells as a control established NV3 as a virus-induced protein. This confirms the suggestion of Lyapustin et al. (1980) of the possible viral nature of P47 derived from the observation that the synthesis of this protein in TBE virus-infected (Far Eastern strain Sofyn) cells was considerably less inhibited by the combined action of hypertonic NaCl and cycloheximide than the corresponding cellular protein. Furthermore, comparison of the peptide maps of NV3 with those of NV5, NV4 and V3 showed that NV3 is unrelated to any of those proteins and, therefore, NV3 also does not represent a cleavage product of V3 (Shapiro et al., 1973). In
Fig. 4. Comparison of NV5 from cells infected with the different viruses listed in Table 1 by peptide mapping using limited proteolysis with (a) α-chymotrypsin and (b) V8 protease followed by SDS–PAGE in 17% gels.
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addition, the peptide maps obtained from NV5, NV4, V3 and NV3 were unique, which is in accordance with results obtained for Kunjin virus (Wright et al., 1977) and WNV (Wengler et al., 1979).

A very slight difference was observed in the map of V3 derived from purified virus compared to its intracellular form after V8 protease treatment, a finding which has also been described for Kunjin virus V3 by Wright et al. (1977), and probably reflects differences in the extent of glycosylation.
Fig. 5. Comparison of NV4 from cells infected with the different viruses listed in Table 1 by peptide mapping using limited proteolysis with (a) α-chymotrypsin and (b) V8 protease followed by SDS–PAGE in 17% gels.
In contrast to WNV and MVEV, which were used as controls, no proteins were found in cells infected with any of the TBE virus strains analysed which would correspond in mol. wt. to NV2 1/2 and NV2. Two very close bands were resolved in the mol. wt. region around 15000, one exactly migrating with V2 from purified virus [P15 (C)] and one with an estimated mol. wt. of 14 500 which might correspond to an intracellular form of the core protein also found in Kunjin virus-infected cells (Wright & Westaway, 1977) and WNV-infected cells.
WNV- or MVEV-infected cells. It must, however, be stressed that in Laemmli gels NV1 is more conserved than the structural glycoprotein, which revealed mol. wt. between 91,000 (TBE virus) and 98,000 (MVEV) and, therefore, seems to be almost as variable as the structural glycoprotein (mol. wt. range approx. 50,000 to 60,000). This is further substantiated by the completely unrelated peptide maps obtained for NV5 and NV4 of TBE viruses on the one hand, and WNV and MVEV on the other, and (at least in the case of NV5) even those of the very closely related Far Eastern subtype of TBE virus and lousing-ill virus revealed distinct differences of otherwise identical peptide maps, which is similar to the results obtained with the corresponding structural glycoproteins (Heinz & Kunz, 1981). We have, therefore, no evidence that the largest non-structural protein NV5 from serologically distinct flaviviruses is more conserved than the structural glycoprotein, even when these viruses belong to the same serological subgroup (Heinz & Kunz, 1981), a finding which correlates with the observed immunological type specificity of NV5 (Qureshi & Trent, 1973a, b; Trent et al., 1976). This heterogeneity is contrasted by an impressive identity of the peptide maps of NV5 and NV4 from European TBE virus isolates, thus extending our previous observation that TBE virus seems to represent a very stable member of the flavivirus genus, which has been deduced from the immunological and biochemical homogeneity of the structural glycoprotein from viruses isolated all over Europe in different years and from different hosts (Heinz & Kunz, 1981).
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