Hybridization Studies with Subtypes and Mutants of Foot-and-Mouth Disease Virus Type O

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Seven serologically and immunologically distinct types of foot-and-mouth disease virus (FMDV) are known; these types can be subdivided into numerous subtypes. Recently, we reported on polynucleotide sequence homologies among the ribonucleic acids (RNA's) of FMDV types A, O and C (Dietzschold et al. 1971). Making use of saturation and competition experiments various degrees (44 to 65 %) of base sequence homologies between different types were obtained. This report is concerned with similar studies but using subtypes and mutants of FMDV, type O. We felt that there is a need especially for quantitative data on genetic relationships between different subtypes, because the serological classification is not always congruent with a classification based on immunological properties (Wittmann, 1964). Furthermore, it appeared interesting to compare in cross-annealing experiments wild-type virus with mutants reacting identically in serological tests.

The experiments were performed with radioactive labelled FMDV RNA of five O-subtypes and five mutants of subtype O₁ which were hybridized with denatured unlabelled FMDV-specific double-stranded RNA of the respective virus-strains.

FMDV O-subtypes adapted to grow in BHK-21 cells were used for virus propagation. These strains were O₁ LOMBARDY (O₁L), O₁ KAUFBEUREN (O₁K), O₃ BRESCIA (O₃B), O₃ VENEZUELA (O₃V) and O₇ ITALY (O₇). For exact determination of the subtypes the technique of Rouminantzeff, Stellmann & Dubouclard (1965) was followed. The following mutants were used: a temperature-sensitive O₁L-strain (O₁L-ts) (Wittmann & Ahl, 1964); an interferon sensitive O₁L-strain (O₁L-if); a myotropic O₁K-strain; a O₁K carrier virus; and a pH- and thermostable O₁K-strain. The myotropic O₁K-strain was adapted to muscles of guinea pigs weighing 280 to 320 g. It had undergone 26 passages in guinea pigs, and showed a reduced pathogenicity in cattle after intradermal lingual infection. The carrier strain of subtype O₁K was isolated from cattle 7 months after exposure to FMD. This carrier virus had undergone two passages in calf thyroid cells and was propagated in roller bottles of BHK-21 cells. The pH- and thermostable mutant was obtained after repeated partial inactivation of O₁K virus in acid buffer at 50°.

[³²P]-virus RNA was extracted from [³²P]-labelled purified FMDV by the method of Bachrach (1960). Double-stranded RNA was prepared from FMDV infected cells as reported by Colby & Duesberg (1969) for cells infected with vaccinia virus. [³²P]-Labelled RNA from the different O-subtypes was cross-hybridized with denatured unlabelled FMDV specific double-stranded RNA of the respective virus strains as previously described (Dietzschold et al. 1971). For determination of the saturating concentration of double-stranded RNA standard amounts of [³²P]-labelled virus RNA were annealed with increasing amounts of unlabelled double-stranded RNA.

Fig. 1. shows one of the saturation experiments using labelled O₁, O₃B, O₁L and O₃V RNA's and unlabelled O₁L double-stranded RNA. A plateau was reached with 8 μg. double-stranded RNA. Furthermore, different degrees of hybridization occurred with the four RNA's tested.

Table 1 summarizes the data for all O-subtypes tested, and in Table 2 the results of the
Fig. 1. Hybridization of unlabelled denatured O:L double-stranded RNA with parental RNA from different subtypes of FMDV type O. ○—○, O:L; ●—●, O:V; △—△, O:2B; □—□, O:2V. A high excess of [32P]-BHK cell RNA did not hybridize significantly.

Table 1. Hybridization percentage between unlabelled denatured double-stranded RNAs at saturation and labelled parental RNAs of five different subtypes of FMDV type O.

Homologous hybridization was taken as 100%. —, not tested.

<table>
<thead>
<tr>
<th>Strain of parental [32P]-RNA</th>
<th>Strain of unlabelled, denatured double-stranded RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O:L</td>
</tr>
<tr>
<td>O:L</td>
<td>100</td>
</tr>
<tr>
<td>O:2B</td>
<td>70</td>
</tr>
<tr>
<td>O:2V</td>
<td>48</td>
</tr>
<tr>
<td>O:V</td>
<td>73</td>
</tr>
<tr>
<td>O:K</td>
<td>71</td>
</tr>
</tbody>
</table>

Hybridization as % of that in homologous reaction

Cross-annealing experiments with mutants and the respective wild types are shown. In each case the percentage of hybridization was calculated from the mean value out of 10 determinations of the degree of hybridization between [32P]-FMDV RNA and a saturating quantity of unlabelled denatured double-stranded RNA. The extent of heterologous hybridization was related to that in the homologous reaction which was regarded as 100%. The deviation from the mean was less than 3% for all O-subtypes and mutants tested. [32P]-labelled RNA isolated from infected BHK cells did not hybridize with the FMDV specific double-stranded...
Table 2. Hybridization percentage between unlabelled denatured double-stranded RNAs of wild types O_1K and O_1L at saturation and labelled parental RNAs of five different mutants of strain O_1L or O_1K respectively.

Homologous hybridization was taken as 100%. A high excess of [32P]-BHK cell RNA did not hybridize significantly. —, not tested.

<table>
<thead>
<tr>
<th>Strain of unlabelled, denatured double-stranded RNA</th>
<th>O_1L wild type</th>
<th>O_1L temperature sensitive</th>
<th>O_1L i.f. wild type</th>
<th>O_1K myotrop. (guinea pig)</th>
<th>O_1K carrier virus (cattle)</th>
<th>O_1K pH- and thermostable</th>
</tr>
</thead>
<tbody>
<tr>
<td>O_1L wild type</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>O_1K wild type</td>
<td>—</td>
<td>—</td>
<td>100</td>
<td>100</td>
<td>93</td>
<td>98</td>
</tr>
</tbody>
</table>

RNA. The experiments show that considerable differences exist between the polynucleotide sequences of the RNA's from O-subtypes, corresponding to their well-known different immunological properties.

Our results concerning the particular behaviour of subtype O_3V agree with the observations of Wittmann (1964), that the subtype O_3V differs essentially from other O-subtypes. Recently, Reda & Wittmann (personal communication) again demonstrated this particular behaviour of subtype O_3V in the passive haemagglutination test.

Table 2 shows that in general significant differences of wild types and mutants do not exist except in the case of O_1K carrier virus, which differs significantly from its wild type because each of the 10 determinations on which the calculated mean value of 93% is based is lower than each of the 10 wild-type determinations. The O_1K carrier virus showed an increased production of and susceptibility to interferon, a reduced pathogenicity for cattle and suckling mice, and formed smaller plaques in primary calf kidney monolayer cultures (Kaaden, Eissner & Böhm, 1970). Our results concerning the hybridization experiments with mutants agree with the results of Hobson & Scholtissek (1970), who could not find any significant differences between influenza virus strain ws and the mutants ws-dh 1 derived therefrom. The technique employed does not exclude, however, the possibility of small deletions or point mutations of the RNA. We intend to obtain more data concerning the molecular differences of the virus genome between mutants and wild types from the melting profiles of the heterologous hybrids.

REFERENCES


Short communications


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