Genomic Heterogeneity of Simian Rotavirus SA11

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SUMMARY

A preparation of simian rotavirus SA11 was shown to contain, in addition to the normal 11 genome segments, an RNA species with electrophoretic mobility slightly higher than that of segment 4. Limiting dilution passages allowed the separation of two virus clones distinguishable from each other by the electrophoretic mobility of that genome segment. Possible implications of this finding in virus behaviour and in the comparison of rotaviruses by RNA electrophoresis are discussed.

Simian virus SA11 (Malherbe & Strickland-Cholmley, 1967) has been generally adopted as a representative strain of the genus rotavirus in numerous studies of the general properties of this group of viruses and as a standard for the comparison of the segmented virus genome by electrophoresis. In the course of our own studies we have observed that the SA11 strain used routinely as a standard showed an extra RNA band migrating slightly ahead of segment 4. The separation of two clones differing from each other in the mobility of segment 4 is described in the present paper.

The SA11 strain was kindly supplied by Dr G. W. Gary Jr, Center for Disease Control, Atlanta, Ga., U.S.A. and used in the present studies after two passages in the LLCMK2 and one passage in the MA104 cell lines. Two virus preparations derived from MA104 cultures infected with the same virus inoculum were concentrated and purified on separate occasions by isopycnic banding in CsCl as previously described (Pereira et al., 1983). Fractions with densities between 1.36 and 1.38 g/ml and showing a peak of absorbance at 260 nm were pooled, dialysed against 0.01 M-Tris-HCl pH 7.4 and tested by polyacrylamide gel electrophoresis (PAGE) following Laemmli's technique with slight modifications as described by Pereira et al. (1983). Gels stained with ethidium bromide (0.5 μg/ml for 1 h) and photographed in transmitted u.v. light revealed 11 characteristic bands, but when stained by silver impregnation as described by Boulikas & Hancock (1981), an extra faint band appeared slightly ahead of segment 4 as seen in Fig. 1 (a). This extra band will be referred to as 4F (fast-moving) in contrast to the 4S (slow-moving) band immediately above it. Inexplicably, the intensity of staining of 4F varied in the two different purified virus concentrates hereafter referred to as ‘4F faint’ and ‘4F intense’.

To test the hypothesis that the appearance of this extra band might be due to heterogeneity of the virus population, we attempted cloning experiments by limiting dilution passages. Serial tenfold dilutions of the two virus preparations referred to above as ‘4F faint’ and ‘4F intense’ were each inoculated into three tube cultures of MA104 cells. After 7 days incubation the cultures were frozen and thawed and the RNA from 400 μl of the contents of each tube was extracted with phenol–chloroform, precipitated with ethanol and analysed by PAGE. Fluids from tubes of the highest dilution of each titration giving positive RNA patterns were re-titrated as above, each dilution being inoculated into four MA104 tube cultures.

Results presented in Table 1 show the segregation of clones 4S and 4F after two successive limiting dilution passages of virus concentrates ‘4F faint’ and ‘4F intense’ respectively. Each of the two clones thus obtained underwent one low dilution (1/20) passage in an 8 oz bottle culture of MA104 cells maintained in 10 ml of medium and harvested 5 days after inoculation. Fig. 1 shows a comparison of the RNAs extracted from each of these two cultures by co-electrophoresis.
Table 1. Cloning of SA11 4S from virus preparations, '4F faint' and '4F intense'

<table>
<thead>
<tr>
<th>Dilution</th>
<th>4S</th>
<th>4F</th>
</tr>
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<tbody>
<tr>
<td>10^-6</td>
<td>3/3</td>
<td>1/3</td>
</tr>
<tr>
<td>10^-7</td>
<td>2/3</td>
<td>0/3</td>
</tr>
<tr>
<td>10^-8</td>
<td>0/3</td>
<td>0/3</td>
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'4F faint'
First passage (P1)

<table>
<thead>
<tr>
<th>Dilution</th>
<th>4S</th>
<th>4F</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^-6</td>
<td>4/4</td>
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<tr>
<td>10^-7</td>
<td>3/4</td>
<td>0/4</td>
</tr>
<tr>
<td>10^-8</td>
<td>1/4</td>
<td>0/4</td>
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Second passage (from P1 10^-7)

<table>
<thead>
<tr>
<th>Dilution</th>
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<th>4F</th>
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<tbody>
<tr>
<td>10^-7</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>10^-9</td>
<td>1/3</td>
<td>1/3†</td>
</tr>
<tr>
<td>10^-9</td>
<td>0/3</td>
<td>0/3</td>
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</table>

'4F intense'
First passage (P1)

<table>
<thead>
<tr>
<th>Dilution</th>
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<th>4F</th>
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<tbody>
<tr>
<td>10^-7</td>
<td>0/4</td>
<td>4/4</td>
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<tr>
<td>10^-8</td>
<td>0/4</td>
<td>2/4</td>
</tr>
<tr>
<td>10^-9</td>
<td>0/4</td>
<td>1/4</td>
</tr>
</tbody>
</table>

Second passage (from P1 10^-9)

* Number of tubes showing indicated pattern/number of tubes inoculated.
† RNA from one of three tubes showing 4S faint and 4F intense.

alongside the profile given by one of the starting virus concentrates ('4F faint'). It is clear that the two limit-dilution-derived clones differ from each other only in respect to band 4.

The above results support the hypothesis that the original SA11 preparation used in this study contained two virus subpopulations in which RNA segment 4 exhibits different electrophoretic mobilities. To test the stability and homogeneity of these two clones, each was propagated through four low dilution (1/20) passages in MA104 cell cultures. Electrophoretic profiles characteristic of clones 4S and 4F were maintained throughout these passages and there was no indication of band splitting, even in 12.5% polyacrylamide gels shown by Sabara et al. (1982) to have maximal resolving power.

The coding assignment of SA11 genome segment 4 has been studied by several workers (Smith et al., 1980; Arias et al., 1982; Mason et al., 1983) and although some of the conclusions have been discrepant, the most recent results (Mason et al., 1983) demonstrate that the gene product of this segment is the trypsin-sensitive polypeptide present in the outer virion shell. An outer shell polypeptide has been assigned to genome segment 4 of a bovine rotavirus by McCrae & McCorquodale (1982). This observation, together with the finding by Greenberg et al. (1981) and Kalica et al. (1981) that the capacity of human/bovine rotavirus reassortants to grow in vitro is dependent on the acquisition of segment 4 from the cultivable bovine parent virus, suggests that this genome segment plays an important role in virus growth.

Our results extend to SA11 previous findings obtained with bovine rotaviruses by Sabara et al. (1982) who were able to separate several electrophoretically distinct clones from each of six virus isolates. Heterogeneity of simian rotavirus SA11 has also been described in relation to its structural outer shell glycoprotein, VP7, by Estes et al. (1982) who suggested that the cause of this heterogeneity was a spontaneous mutation of the corresponding gene subsequently shown by Mason et al. (1983) to be located in genome segment 9.

Analysis of genomic RNA of rotaviruses by PAGE has been extensively used for the differentiation of strains causing human and animal infections (e.g. Kalica et al., 1976; Espeto et al., 1977, 1980; Verly & Cohen, 1977; Todd et al., 1980; Rodger et al., 1981; Lourenço et al., 1981; Pereira et al., 1983). These and subsequent studies have revealed considerable variation in migration patterns of rotavirus genome segments, allowing the distinction of many electrophoretic types. Although this variation may reflect only minor changes in nucleotide sequence (Clarke & McCrae, 1982) and is not necessarily associated with altered antigenic behaviour (Beards, 1982), it may be of value in epidemiological investigations. The method has also been used to demonstrate the occurrence of mixed infections (Lourenço et al., 1981; Spencer et al., 1983). Genomic heterogeneity of the type described here has been suggested as one of
Fig. 1. Comparison of clones 4S and 4F with original sample '4F faint'. Electrophoresis of RNA extracted from (a) original sample '4F faint', (b) clone 4S, (c) clone 4F, and (d) a mixture of clones 4S and 4F in a 4% to 10% polyacrylamide gel gradient. The gel was stained with silver nitrate (Boulikas & Hancock, 1981) and dried under negative pressure. The apparent splitting of band 11 in (a) is a drying artefact.

several possible explanations for the occurrence of mixed electrophoretic profiles obtained with certain faecal samples (Spencer et al., 1983).

The biological significance of the genomic change observed in our study deserves investigation. It will be of interest to find out if it results in changes of the gene product. It has been reported by Rubin & Fields (1980) that the sensitivity of a reovirus outer capsid protein to proteolytic enzymes is correlated with virus virulence. A change in the SA11 gene coding for the trypsin-sensitive outer capsid protein might be expected to alter virus behaviour in important ways.

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REFERENCES


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