Electrophoretic Analysis of RNA Segments of Human Rotaviruses Cultivated in Cell Culture

(Accepted 4 January 1982)

SUMMARY

Human rotaviruses (HRVs) derived from stools were cultivated to high titres in an established cell line (MA-104) using the rotary culture system. Analysis of the 11 double-stranded RNA segments of the culture-adapted HRVs was carried out by polyacrylamide gel electrophoresis. Tissue culture-adapted HRVs, uncloned or cloned, had the same RNA gel patterns as those of the original HRVs from the individual stool specimens. The migrations of the RNA segments from the culture-adapted HRVs were markedly different from that of the RNA segments from calf rotavirus (Lincoln strain). Considerable heterogeneity in electrophoretic migration of the RNA was found among eight strains of the HRVs grown in cell culture. These results confirmed that viruses isolated from stool specimens were indeed of human origin and were not the result of laboratory contamination with tissue culture-adapted calf rotavirus or other viruses.

Rotavirus is now known to be a common cause of human and animal gastroenteritis in many parts of the world (Flewett & Woode, 1978). It has not been possible to cultivate the human rotaviruses (HRVs) in vitro by ordinary cell culture techniques, and only one strain (Wa strain) has been adapted to grow to high titre in cell culture after 11 serial passages in newborn gnotobiotic piglets (Wyatt et al., 1980). Quite recently, however, a number of HRVs from stool specimens have been successfully propagated in rotary cell cultures in the presence of trypsin (Sato et al., 1981; Urasawa et al., 1981). In addition, a plaque assay was developed by adapting the roller-cultured HRVs to a stationary culture system (Urasawa et al., 1981). In this communication, we describe the analysis by gel electrophoresis of RNA segments from the HRVs propagated in cell culture.

The faecal specimens used as a source of HRV were obtained from two schoolchildren (K8 and 44, aged 14 and 7 years respectively) involved in epidemics of gastroenteritis in 1977 and 1980, and from six sporadic cases (FU, KU, YO, $2, S 11 and S 12) of patients aged between 6 months and 10 years admitted to two hospitals between 1977 and 1980.

Propagation of the HRVs in cell culture was carried out as described previously (Urasawa et al., 1981). HRV-positive filtrates, pretreated with acetylated trypsin (final concn. 10 μg/ml, Sigma) for 30 min at 37 °C, were inoculated on to an MA-104 cell monolayer in culture tubes. The culture was maintained in Eagle’s minimum essential medium with 1 μg/ml trypsin in a roller drum (Hirasawa Co., Japan) at 37 °C for 3 to 5 days. After 6 to 9 passages carried out in a similar manner, the viruses were cultivated with Rollacell (New Brunswick Scientific Co.) to obtain large volumes of virus preparation. Clones of the HRVs were obtained by triple plaque-purifications after 7 to 13 passages (Urasawa et al., 1981).

The cultures were harvested after two freeze–thaw cycles, clarified by low-speed centrifugation, and concentrated by polyethylene glycol (PEG) precipitation. The concentrated virus was pelleted through 3 ml 45% (w/v) sucrose by ultracentrifugation. The virus was further purified by CsCl banding, and the fractions containing HRV were dialysed against 50 mM-tris–HCl-buffered saline (pH 7.5). Stool suspensions were purified by
trifluorotrichloroethane treatment, PEG precipitation and CsCl density-gradient centrifugation.

Purified virus was disrupted with SDS and 2-mercaptoethanol, and deproteinized with phenol as described by Espejo et al. (1979). Slab gels of 7.5% (w/v) acrylamide and 0.2% (w/v) bisacrylamide were prepared by the method of Laemmli (1970) but without a stacking gel. Electrophoresis was conducted at 20 mA for 12 to 17 h at 4 °C and the gels were
Table 1. Segment variations among eight isolates of human rotaviruses grown in cell culture as determined by co-electrophoresis of genome RNA

<table>
<thead>
<tr>
<th>Strains co-electrophoresed</th>
<th>Segment variations</th>
<th>Total no. of variations</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S_2 + K_8$</td>
<td>1 2 3 4 5 6 7 8 9 10 11</td>
<td>11</td>
</tr>
<tr>
<td>$S_2 + K_U$</td>
<td>1 2 3 4 5 6 7 8 9 10 11</td>
<td>11</td>
</tr>
<tr>
<td>$S_2 + S_{12}$</td>
<td>1 2 3 4 5 7 9 10 11</td>
<td>9</td>
</tr>
<tr>
<td>$S_2 + Y_O$</td>
<td>1 2 3 4 5 6 7 9 10 11</td>
<td>10</td>
</tr>
<tr>
<td>$K_8 + K_U$</td>
<td>1 2 3 4 5 6 7 8 10 11</td>
<td>10</td>
</tr>
<tr>
<td>$K_8 + S_{12}$</td>
<td>1 2 3 4 5 6 7 8 9 10 11</td>
<td>11</td>
</tr>
<tr>
<td>$K_8 + Y_O$</td>
<td>2 3 4 5 6 7 8 9 10 11</td>
<td>10</td>
</tr>
<tr>
<td>$S_{12} + Y_O$</td>
<td>1 2 3 4 6 9 10 11</td>
<td>9</td>
</tr>
<tr>
<td>$Y_O + K_U$</td>
<td>2 3 4 5 6 7 8 9 10 11</td>
<td>10</td>
</tr>
<tr>
<td>$K_U + S_{11}$</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$S_{12} + F_U$</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$Y_O + 44$</td>
<td>0</td>
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stained with 1 μg/ml ethidium bromide in 10 mM-tris–HCl buffer pH 7.5. Stained gels were examined on a shortwave u.v. transilluminator and were photographed.

The genomes of cell culture-adapted HRVs (KU and S12 strains) were compared by polyacrylamide gel electrophoresis (PAGE) with those of the original HRVs purified from the individual stool suspensions. As shown in Fig. 1 (a), co-electrophoresis showed that in both strains all the corresponding RNA segments migrated identically. Furthermore, a total of five clones (three clones of KU strain, and one clone each of YO and S12 strains), which were randomly selected using the plaque-forming technique, produced electrophoretic patterns that were identical to those seen for the individual uncloned HRV RNA preparations. Electrophoretic comparison between uncloned and cloned HRVs (YO strain) is shown in Fig. 1 (b).

Serological differences between the tissue culture-adapted HRVs and a calf rotavirus (Lincoln strain) have already been established (Urasawa et al., 1981). In the present study, the identity of the culture-adapted isolates was investigated by co-electrophoresing their virus RNA with those of the Lincoln strain of calf rotavirus and the Wa strain (originally obtained from Dr A. Z. Zapikian, NIH, Bethesda, Md., U.S.A.) of HRV, which had been adapted to cell culture and well characterized. Co-electrophoresis of the HRV RNA (KU strain) with the calf rotavirus RNA revealed marked differences in the electrophoretic mobility of segments 3, 4, 5, 6, 7, 9, 10 and 11. The RNA gel pattern of the KU strain differed from the RNA of the Wa strain in the mobility of segments 1, 2, 3, 4, 5, 7, 8 and 10 (Fig. 1 c). Comparisons of the seven other isolates of HRVs with the calf rotavirus and the Wa strain of HRV were similarly performed, and variations in the electrophoretic mobility of several RNA segments in each comparison were readily recognized (data not shown).

Polymorphism of the migration of the RNA segments of HRVs was found among the eight strains isolated in cell culture. The ‘short’ HRV genome, in which segments 10 and 11 migrated quite slowly (Espejo et al., 1980; Rodger et al., 1981), was detected in one strain, while the remainder showed the ‘long’ electropherotype. Comparative studies of the eight isolates by co-electrophoresis allowed the electropherotypes of the eight isolates to be grouped into five classes (Fig. 1 d). Typical co-electrophoretic comparisons among the isolates are shown in Fig. 1 (e). Table 1 summarizes the results of co-electrophoretic comparisons among the five different electropherotypes. The RNA patterns of the eight isolates were reproduced after more than 20 passages of the virus in cell culture, indicating the stability of the RNA.

A study on the identity of three strains of culture-adapted HRVs had been previously performed by serological examinations (Urasawa et al., 1981). In this study, we analysed the
RNA segments of eight strains of the HRVs cultivated in cell culture by PAGE. This technique has proved a useful tool for examining the genetic diversity of the rotavirus genome (Kalica et al., 1978; Rodger & Holmes, 1979). The RNA gel patterns of the tissue culture-adapted HRVs were the same as those of HRVs from the original stools. In contrast, the migrations of the RNA segments of the culture-adapted HRVs were markedly different from those of calf rotavirus (Lincoln strain) and the Wa strain of HRV. These findings eliminate the possibility of contamination of the culture with some animal rotavirus or rescue of the fastidious HRVs by gene reassortment. Also, calf rotavirus was not handled during the period of HRV cultivation. Several clones, which were picked randomly, produced the same RNA electrophoretic migrations, showing the homogeneity of each strain of the HRVs propagated in cell culture. Thus, we reconfirmed the successful and reliable in vitro propagation of HRVs from stool specimens.

The adequate culture conditions, such as pretreatment of the HRVs in stool suspensions with trypsin, the use of MA-104 cells, and rotary culture with maintenance medium containing trypsin, appeared to enable the propagation of the fastidious HRVs. In our laboratory, out of 24 strains that we tried to cultivate, 20 strains of HRVs have been successfully grown in cell culture.

Considerable heterogeneity in electrophoretic migrations of the RNA segments of the HRVs grown in cell culture was observed, as has been found in many animal species including humans (Kalica et al., 1978; Rodger & Holmes, 1979). The gel patterns of the eight strains of culture-adapted HRVs were grouped into five different electropherotypes. More electropherotypes, however, may be obtained by examination of more samples. The correlation between the serological properties and the RNA electrophoretic patterns of the HRVs has not yet been elucidated. Recently, Rodger et al. (1981) suggested that serotypes determined by enzyme-linked immunosorbent assay are dependent on the products of segment 10 or 11. On the other hand, Kalica et al. (1981) indicated that segments 6 and 9 code for subgroup and neutralization antigen respectively. Based on these observations, investigations of the relationship between the antigenicity and electropherotypes of HRVs are in progress, using culture-adapted HRVs.

We wish to thank Dr S. Chiba, Department of Pediatrics, Sapporo Medical College, for providing the Wa strain of HRV and the MA-104 cells.

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REFERENCES


(Received 28 September 1981)