Reassortment of Human Rotaviruses Carrying Rearranged Genomes with Bovine Rotavirus

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SUMMARY

Rotaviruses isolated from chronically infected immunodeficient children were previously shown to contain RNA yielding abnormal migration profiles on gels: normal RNA segments were lost or decreased in concentration, and additional bands of dsRNA were found which were derived (rearranged) from genome segments of lower molecular weight by concatemer formation. These viruses grew very slowly during passage in secondary rhesus monkey kidney cells. Upon superinfection with the tissue culture-adapted UK Compton strain of bovine rotavirus (BRV) extensive genome reassortment occurred. Clones with the following reassorted genome patterns were isolated: (i) RNA segments 5 or 6 of BRV were replaced by the corresponding RNA segments of human rotavirus; (ii) RNA segments 9 or 11 of BRV were replaced by different rearranged bands of RNA of human rotavirus; (iii) reassortants were observed containing more than one segment/rearranged band of human rotavirus RNA in different combinations. The reassortant viruses possessed functional proteins coded for by the genome segments and/or by rearranged bands of RNA of the human rotaviruses. Rearrangement of parts of the rotavirus genome may be a mechanism of evolution of these viruses.

INTRODUCTION

Rotaviruses, comprising one genus of the family Reoviridae, are the main cause of acute gastroenteritis in infants and in young animals of a variety of species (Flewett & Woode, 1978; McNulty, 1978; Estes et al., 1983; Holmes, 1983). They possess a genome of 11 segments of dsRNA and can be distinguished by serological procedures (Greenberg et al., 1983; Wyatt et al., 1983; Coulson et al., 1985), analysis of RNA profiles (‘electropherotypes’; Rodger et al., 1981; Schnagl et al., 1981; Follett et al., 1984) and by more detailed genome analysis such as hybridization (Flores et al., 1982), one-dimensional terminal fingerprinting (Clarke & McCrae, 1981, 1982) and two-dimensional oligonucleotide mapping (Follett & Desselberger, 1983b). Rotaviruses of human and animal origin were found to reassort easily in vitro (Greenberg et al., 1981; Kalica et al., 1981; Garbarg-Chenon et al., 1984), and there is evidence which suggests that this may also happen in nature (Street et al., 1982; Pedley & McCrae, 1984).

Recently, we described the genomes of rotaviruses isolated from chronically infected children who suffered from severe combined immunodeficiency (SCID) (Pedley et al., 1984). The genomes of these isolates possessed additional bands of dsRNA which consisted of concatemers containing segment-specific sequences, and which migrated more slowly on gels; that is, they were larger than the segments from which their sequences were derived. In one case the normal RNA segment 11 had completely disappeared from the RNA profile and other RNA segments were decreased in concentration. However, these viruses seemed to be viable and replicated over a period of several months in the intestinal tract of their hosts. Viruses with similar genome rearrangements have also been isolated from immunodeficient hosts by others (Dolan et al., 1985).

At the time of isolation we were unable to grow these viruses in tissue culture. However, by passaging the tissue culture-adapted UK Compton strain of bovine rotavirus (BRV) serially at
high m.o.i. we produced and cloned viruses with genome rearrangements similar to those observed in the human isolates (Hundley et al., 1985).

Here we report attempts to grow human rotaviruses with genome rearrangements in tissue culture and describe experiments involving co-infection with BRV. It was discovered that the human rotaviruses, which grew very slowly when propagated on their own in tissue culture, readily reassorted with BRV: not only did normal RNA segments of the human rotaviruses replace corresponding segments of BRV but also several rearranged pieces of dsRNA of the human rotavirus genomes were able to replace normal RNA segments of the BRV genome, taking over their function by coding for the corresponding proteins.

METHODS

Viruses. The following rotavirus isolates and strains were used in this work: two isolates of rotaviruses with genome rearrangements obtained from the chronically infected, immunodeficient child U.H. on 17.6.80 and 14.8.80 (Dr I. Chrystie, Department of Virology, St Thomas's Hospital, London, U.K.; Pedley et al., 1984) and the tissue culture-adapted UK Compton strain of BRV (Follett & Desselberger, 1983a).

Rotaviruses were purified from clinical faecal specimens as described previously (Follett & Desselberger, 1983a). Suspensions of purified human rotavirus were filtered once through 0.45 μm Millex GS filters (Millipore) before infection. Bovine rotavirus (six times plaque-purified) was passaged at low m.o.i. in MA104 cells (Follett & Desselberger, 1983a).

Cells. Secondary rhesus monkey kidney (RMK) cells (obtained from Flow Laboratories) and the continuous MA104 cell line (obtained from Microbiological Associates, through Laboratory Dupex Ltd, Twickenham, U.K.) were grown as roller cultures to confluency in Eagle's MEM supplemented with 10% foetal calf serum. They were washed twice with sterile phosphate-buffered saline (PBS) before infection. Infection of RMK cells with human rotaviruses was exactly as described by Ward et al. (1984).

Reassortment experiments and analysis of RNA profiles. RMK cells were infected with human rotaviruses possessing genome rearrangements and grown under liquid overlay (Eagle's MEM) containing 2 μg/ml trypsin (Difco 1:250; cat. no. 0152-15). Replication was monitored by testing the supernatant in an ELISA test for the presence of group-specific antigen (Rotazine, Abbott Laboratories, North Chicago, Ill., U.S.A.; Follett & Desselberger, 1983a).

Cells infected with human rotavirus containing a rearranged genome were then superinfected with BRV at low m.o.i. (<0.005) and passaged twice under liquid overlay until complete c.p.e. was reached. No deliberate selective pressures were applied during these steps (Garbarg-Chenon et al., 1984). Virus from these passages was purified and the RNA extracted and analysed on polyacrylamide gels as described by Follett & Desselberger (1983a). Virus suspensions of the third passage after superinfection were serially diluted in tenfold steps and grown under a semi-solid agar overlay containing 0.5% foetal calf serum. They were washed twice with sterile phosphate-buffered saline (PBS) before infection. Infection of RMK cells with human rotaviruses was exactly as described by Ward et al. (1984) and grown under liquid overlay containing no serum and 2 μg/ml trypsin. When full c.p.e. was reached, the virus was purified and the RNA extracted and separated on polyacrylamide gels as described by Follett & Desselberger (1983a). Viruses with reassorted genomes were plaque-to-plaque purified four times.

The sizes of rearranged bands of dsRNA were estimated from their electrophoretic mobility in relation to that of genomic BRV RNA. The sizes of individual BRV RNA segments had been determined independently (Rixon et al., 1984), and plots of their size (log scale) against distance of electrophoretic migration on gels served as calibration lines (Shatkin et al., 1968).

Production of radio-labelled segment-specific cDNA probes. Viral RNA was electrophoretically separated (Follett & Desselberger, 1983a) and the gel stained with ethidium bromide (4 μg/ml). Gel pieces containing RNA segments were cut out, and the RNA electroeluted and re-precipitated as described by Taylor et al. (1985). Segmental RNA was used as template in a reverse transcriptase reaction with calf thymus DNA fragments as primers. These had been obtained by digestion with DNase I (Sigma D-0751) and purification over a DEAE-cellulose (Whatman DE-52) column (Maniatis et al., 1982). The cDNA was produced as described by Maniatis et al. (1982) except that unlabelled dNTPs were at 5 μM final concentration, and 30 μCi of each [α-32P]dNTP (sp. act. >3000 Ci:mmol) was added; template (1 to 2 μg) and primers (300 μg) were denatured together before the other components were added. By this method between 15 and 40% of the [α-32P]dNTPs were incorporated into the cDNA.

Northern blotting and hybridization. These experiments were carried out as described by Pedley et al. (1984) with minor modifications. In brief, RNA segments separated on a 7.5% polyacrylamide gel using the discontinuous buffer system of Laemmli (1970) were electroblotted onto Biodyne membrane (Pall Process Filtration Ltd, Portsmouth, U.K.) using a Bio-Rad Trans-Blot Cell and TBE buffer (50 mm-Tris, 50 mm-boric acid, 1 mm-EDTA, pH 8.3) for 1 h at 200 V and approx. 150 to 180 mA. The RNA was fixed to the membrane by u.v. irradiation.
Reassortment of rearranged rotavirus genomes

Growth in tissue culture of human rotaviruses with normal and with rearranged genomes

The procedure for growing human rotaviruses in secondary RMK cells as described by Ward et al. (1984) has been successfully used by us routinely to grow human rotavirus isolates possessing a normal genome of 11 RNA segments (results not shown). We have tried to grow in tissue culture several rotavirus isolates obtained from a chronically infected, immunodeficient child which had previously been shown to possess rearranged genomes (Pedley et al., 1984). In contrast to rotaviruses obtained from acutely infected children, the viruses with genome rearrangements were found to be fastidious during passaging on RMK cells. Virus was passaged after an incomplete c.p.e. had slowly (7 to 10 days) developed. Genomic RNA, including rearranged bands of dsRNA, was replicated at low levels in several passages as identified by analysis of RNA extracts on polyacrylamide gels, and there was also moderate production of group-specific antigen (results not shown).

Upon superinfection of cultures of human rotaviruses possessing a rearranged genome with BRV (UK Compton strain) a rapid c.p.e. developed. The RNA profiles of viruses obtained from such cultures showed increasing amounts of RNA segments of the BRV genome (Fig. 1 a, b, lanes M); however, some RNA segments (labelled h5 and h6) and bands (labelled B, D, F, G) of the human rotavirus genome seemed to be disproportionally amplified.

Genome analysis of rotaviruses plaque-purified from mixed infections

Serial dilutions of suspensions of viruses showing mixed RNA profiles were grown on MA104 cells under semi-solid agarose overlay. Plaques were picked, and the viruses replicated under liquid overlay on MA104 cells. Virus was then purified, the RNA extracted and analysed on silver-stained polyacrylamide gels as described previously (Follett & Desselberger, 1983a). Some of the results are shown in Fig. 1 (a, b). It can be seen that extensive reassortment had taken place. (i) Genome segments 5 or 6 of BRV were replaced by genome segments 5 or 6, respectively, of the human rotaviruses (Fig. 1 a, lanes 1, 6, 10, 11; Fig. 1 b, lane 2); (ii) genome segment 11 of BRV was consistently replaced by rearranged RNA bands F or G (Fig. 1 a, lanes 1, 6 to 12; Fig. 1 b, lane 7); (iii) additional RNA bands B and D were reassorted into the BRV genome without (Fig. 1 a, lanes 4, 5; Fig. 1 b, lanes 3, 5) or with further reassortment events (additional reassortment of segment 11 by replacement with rearranged bands F or G: Fig. 1 a, lane 8 and Fig. 1 b, lane 7; additional reassortment of segment 11 by replacement with rearranged band G and of segment 5: Fig. 1 a, lanes 1, 6 and 10). When the genomes of reassorted viruses carrying additional RNA bands B and D were separated on a 7.5% polyacrylamide gels using Laemmli's buffer system it was found that these RNA bands had replaced segment 9 (Fig. 2 a).

Representatives of all the different kinds of reassortant viruses described (Fig. 1 a, b) have so far been plaque-to-plaque purified four times without reappearance of a standard BRV genome. Thus, it was concluded that reassortant viruses possessing rearranged bands of dsRNA in their genome were genetically stable in their rearrangements and fully replication-competent on their own (Hundley et al., 1985).

We have at present analysed approximately 25 plaques from each of the two human rotavirus–BRV co-cultivations. The different genotypes observed in this collection (Table 1) show a distribution in which reassortment of rearranged bands of dsRNA of the human rotaviruses was a frequent event. Further investigation of the randomness of reassortment is in progress.

It should be noted that bands G and F have an apparent size of approximately 1.92 to 1.97 times the size of RNA segment 11 and bands D and B of 1.73 to 1.87 times the size of RNA segment 9.
Fig. 1. RNA profiles of rotaviruses grown from single plaques (denoted 1 to 12 on top of (a) and 1 to 7 on top of (b)) which were derived from mixed infections of BRV and human rotaviruses with rearranged genomes (a, isolate U.H. of 14/8/80; b, isolate U.H. of 17/6/80; Pedley et al., 1984). The RNA profiles of the virus populations from the mixed infections are designated M. RNA segments of BRV are denoted 1 to 11, RNA segments 5 and 6 of the human rotavirus are called h5 and h6, respectively, and rearranged bands of dsRNA derived from the human rotavirus are designated with capital letters (a: D

*Genome relationship of reassortants as determined by Northern blotting and hybridization*

In order to elucidate further the genome composition of the reassortants, Northern blots were made from the RNAs of some of them after separation on a polyacrylamide gel (Fig. 2a). Segment-specific, $^{32}$P-labelled cDNA probes, produced by using either RNA segment 11, mixtures of RNA segments 7 and 8 or mixtures of RNA segments 7, 8 and 9 of BRV as templates in a randomly-primed reverse transcriptase reaction (Maniatis et al., 1982), were hybridized to the blots, and the blots autoradiographed. It was found that the segment 11-specific cDNA probe hybridized to the homologous segment of BRV RNA (Fig. 2b, lanes 1, 2 and 5) and to
and G; h: B and F). Rotaviruses derived from plaques 2 and 3 of (a) and from plaques 1, 4 and 6 of (b) contained the standard BRV genome. Lanes 1 to 4 and M in (b) are from different parts of the same gel; lanes 5 to 7 are part of a different gel. RNAs were separated on 2·8% polyacrylamide–6 M-urea slab gels using Loening's buffer; electrophoresis was at 150 V for 16 h at room temperature, and the gels were then silver-stained (Follett & Desselberger, 1983a).

band G (Fig. 2b, lanes 3 and 4). Segment 7/8-specific cDNA probe (obtained by reverse transcription from RNA segments 7 and 8 of the reassortant genome shown in Fig. 2a, lane 1) hybridized only to segments 7 and 8 of the blots (Fig. 2c, all lanes). In contrast, segment 7/8/9-specific cDNA probe (obtained by reverse transcription from RNA segments 7, 8 and 9 of the standard BRV genome; see Fig. 2a, lanes 2 and 5) hybridized to the 7/8/9 RNA complex of the blots and to bands B and D (Fig. 2d, lanes 1 and 4, respectively). This was further evidence in support of the conclusion drawn from Fig. 2(a) that reassorted RNA bands B and D had replaced segment 9 of the BRV genome.
Fig. 2. RNA profiles of BRV and rotavirus reassortants (a) and Northern blot analysis (b to d). (a) RNA extracted from purified rotavirus was separated on a 7.5% polyacrylamide gel using Laemmli’s discontinuous buffer system. The gel was stained with 4 μg/ml ethidium bromide. Shown are the RNA profiles of standard BRV (lanes 2 and 5) and of several reassortant viruses showing: lane 1, exchange of segment 9 for band B (Fig. 1b, lane 5); lane 3, exchange of segment 11 for band G (Fig. 1a, lane 7); lane 4, exchange of segment 5, of segment 9 for band D and of segment 11 for band G (Fig. 1a, lane 1). The RNAs of reassortant viruses shown in lanes 1, 3 and 4 are identical with those of Fig. 1 as indicated. (b to d) A Northern blot of the RNAs shown in (a) onto Biodyne membrane was prepared (Pedley et al., 1984) and hybridization was carried out as described by Pedley et al. (1984) using 32P-labelled, segment-specific cDNAs (Maniatis et al., 1982) as probes. Between hybridizations with different probes, radioactive cDNA was removed from the blot by incubation in prehybridization buffer (Pedley et al., 1984) at 68 °C for 2 h. (b) Autoradiogram of Northern blot after hybridization to segment 11-specific cDNA. (c) Autoradiogram of Northern blot after hybridization to segment 7/8-specific cDNA probe (RNA template obtained from a preparative separation of the RNA shown in a, lane 1). (d) Autoradiogram of Northern blot after hybridization to segment 7/8/9-specific cDNA probe (RNA template obtained from a preparative separation of standard BRV RNA).
Table 1. Genotypes of 47 plaque-purified rotavirus reassortants obtained from co-cultivation of human rotaviruses carrying rearranged genomes with bovine rotavirus

<table>
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<tr>
<th>Genome composition*</th>
<th>s5--sh5</th>
<th>s9--D</th>
<th>s9--g</th>
<th>s11--G</th>
<th>s11--F</th>
<th>s11--G</th>
<th>s11--G</th>
<th>s6--*sh6</th>
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<td>Standard BRV</td>
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<td></td>
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<tr>
<td>Co-cultivation</td>
<td>s9--B</td>
<td>s9--D</td>
<td>s11--G</td>
<td>s11--F</td>
<td>s11--G</td>
<td>s11--G</td>
<td>s11--G</td>
<td>s6--*sh6</td>
</tr>
<tr>
<td>Human rotavirus U.H. of 17/6/80 with BRV</td>
<td>14‡</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Human rotavirus U.H. of 14/8/80 with BRV</td>
<td>4</td>
<td>-</td>
<td>2</td>
<td>6</td>
<td>-</td>
<td>4</td>
<td>2</td>
<td>6</td>
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<tr>
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<td>7</td>
<td>2</td>
<td>6</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>6</td>
</tr>
</tbody>
</table>

* Headings denote only deviation from standard BRV genome composition in reassortants. s, Segment.
† Denotes figure/lane in which RNA profile of reassortant is shown (only one lane is indicated).
‡ Indicates number of plaques showing genome composition as delineated in heading.

Protein profiles of reassortants

Virus-coded proteins produced in MA104 cells after infection with BRV and different reassortants were labelled with [35S]methionine and separated on polyacrylamide gels as described previously (Hundley et al., 1985). The autoradiograph of one of these gels is shown in Fig. 3(a). For the sake of clarity the corresponding RNA profiles are presented in Fig. 3(b). The following observations were made.

(i) VP6, the gene product of RNA segments 6 (McCrae & McCorquodale, 1982a), differed in migration from the VP6 of BRV in the reassortant possessing the human VP6 gene only (Fig. 3a, b, lanes 2); (ii) VP5, the gene product of RNA segment 5 (McCrae & McCorquodale, 1982a), migrated differently from the VP5 of BRV in the case of replacement of the BRV segment 5 by the corresponding segment of the human rotavirus (Fig. 3a, b, lanes 4 and 6); (iii) in all cases of replacement of BRV RNA segment 9 by rearranged RNA bands B or D, a VP9 differing in mobility from the BRV VP9 was found (Fig. 3a, b, lanes 5 to 9); (iv) in cases of replacement of BRV RNA segment 11 by rearranged bands F or G no apparent change in the relative migration of VP11, the gene product of RNA segment 11 (McCrae & McCorquodale, 1982a), was observed (Fig. 3a, b, lanes 3 to 7). Thus, RNAs in bands F and G apparently code for a protein which is closely related to the BRV VP11.

DISCUSSION

In this communication, we have reported that rearranged pieces of genomic RNA of human rotaviruses can be rescued by reassortment into BRV and functionally replace normal BRV RNA segments. In following the procedure described by Ward et al. (1984) we were able to replicate human rotaviruses with genome rearrangements in secondary RMK cells although they were fastidious in this tissue culture system when propagated on their own. Dolan et al. (1985) have also reported an unusual RNA profile of rotaviruses isolated from a child with SCID. Their diagram (Fig. 4, Dolan et al., 1985) shows eight additional RNA bands and only two genome segments in the region of the triplet of segments 7, 8 and 9 which normally migrate closely together. All additional bands except one disappeared from the RNA profile when the virus was cultured (Dolan et al., 1985). The human rotaviruses with genome rearrangements which we were able to propagate showed moderate replication of the genome including at least four different rearranged bands of dsRNA.

When passages of human rotaviruses with genome rearrangement were superinfected with the tissue culture-adapted BRV (UK Compton strain), the BRV overgrew the human rotavirus to a large extent but extensive reassortment also took place. These events occurred without any deliberate external selective pressure (such as u.v. irradiation of superinfecting virus or addition
Fig. 3. (a) Protein profiles of cell extracts infected with standard BRV (BRV; lanes 1 and 10, derived from plaques of reassortment mixture and shown to contain the standard BRV genome) and with different reassortants (lanes 2 to 9). MI, Mock-infected. Labelling was at 16 h post-infection with [35S]methionine for 2 h as described by Hundley et al. (1985). Separation was on a 15% polyacrylamide gel using Laemmli's buffer at twofold higher concentration. Protein designations (to right of panel) are according to the nomenclature of McCrae & Faulkner-Valle (1981). Changes in the migration of proteins relative to the migration of corresponding proteins of BRV are indicated by open arrows. (b)

of specific antibody to cultures. In vitro reassortment of human rotavirus strains differing in their RNA profiles was recently also observed by Garbarg-Chenon et al. (1984) following co-infection of tissue cultures in the absence of any external selection.

RNA analysis of plaque-purified viruses obtained from doubly infected cultures revealed several kinds of genetic interaction.

RNA segments 5 or 6 of BRV were replaced by the corresponding segments of the human rotavirus. This is a well established finding (Greenberg et al., 1981; Kalica et al., 1981).

Segment 11 of the BRV was replaced by the rearranged dsRNA bands F or G of the human rotavirus as indicated by the analyses of RNA profiles and by Northern blot hybridization data.

The latter finding is of interest in several respects. Firstly, it indicates that the human rotaviruses of patient U.H. remained viable after the loss of RNA segment 11 from the profile
RNA profiles of standard BRV (BRV; lanes 1 and 10 derived from plaques of reassortment mixture and shown to contain the standard BRV genome) and of different reassortants (lanes 2 to 9) applied in the same order as protein extracts of cells infected with these viruses in (a). The RNAs of reassortants shown in lanes 2 to 9 are representative samples of the RNAs shown in Fig. 1(a) and (b). RNA segments of BRV (1 to 11) and of human rotavirus (h5, h6), and rearranged RNA bands of human rotavirus (B, D, F, G) are indicated to the right. Gel and electrophoresis conditions were as described in the legend to Fig. 1(a).

(Pedley et al., 1984) because the rearranged RNA bands F and/or G had retained the segment 11 coding functions. As shown here, these bands can also replace segment 11 of other rotaviruses such as BRV. In the abnormal RNA profile of the tissue culture-adapted rotavirus which was obtained by Dolan et al. (1985) from isolates of a chronically infected, immunodeficient child it seems possible that the single rearranged band maintained after tissue culture adaptation (migrating between normal RNA segments 6 and 7) is derived from and has taken over the functions of RNA segment 8. Secondly, this finding could shed some light on the evolution of
human rotaviruses of the short electropherotype. It has been shown previously that segment 10 of the short electropherotype is equivalent to segment 11 of the long electropherotype and segment 11 of the short electropherotype to segment 10 of the long electropherotype (Dyall-Smith & Holmes, 1981). It could be argued that viruses of the short electropherotype evolved from those of the long electropherotype by a rearrangement event of segment 11 with maintenance of the segment 11 coding function and gene product expression, and that this rearrangement evolved to a genetically stable new lineage of rotaviruses. (Other electropherotype variations within rotavirus groups may turn out to be due to genome rearrangement events as well.)

Additional dsRNA bands B and D replaced segment 9 in the BRV genome as shown by the analysis of RNA profiles, of Northern blot hybridization data and of the profiles of virus-coded proteins. In both cases of segment 11 and segment 9 replacement in the BRV genome, the reassorting bands of dsRNA (F and G, B and D, respectively) coded for protein products very similar in size to those of the normal BRV genome segments. Thus, in these cases rearrangement of RNA segments of the human genome probably has left their open reading frames unchanged. Rearrangement of RNA segment 5 in clones of BRV after serial passage at high m.o.i. had led to either loss of the coding capacity (no VP5 product is made) or, in one case, to the appearance of a novel abnormal protein product (Hundley et al., 1985).

It is noteworthy that, although packaging of rotaviruses (Whitton et al., 1983) and reoviruses (Shatkin et al., 1968) is strictly equimolar for all RNA segments of the genome, there is still room for packaging of more genomic material: for example, the reassorted genomes shown in Fig. 1(a), lane 8 and Fig. 1(b), lane 7, contain approximately 1400 to 1550 base pairs (=7.5 to 8.3%) more than the genome of a normal bovine rotavirus, but the viruses are genetically stable and replication-competent. Further implications of this interesting phenomenon are under investigation at present.

It was observed in a limited number of plaque-purified reassortants that genome reassortment was a very frequent event in the absence of deliberate external selective pressure (Garbarg-Chenon et al., 1984), and that the rearranged bands of dsRNA were involved in the large majority of reassortment events recorded. Clearly, more data are needed to answer the question of whether or not this is a non-random event (Lubeck et al., 1979).

The reassortment of rearranged bands of the RNA genome of human rotaviruses into a virus possessing a mainly BRV genome opens new possibilities to investigate further the event(s) and mechanism(s) of genome rearrangements. Whereas the human rotaviruses carrying rearranged genomes grow on their own in tissue culture in a very fastidious way, some of the BRV reassortants possessing rearranged pieces from the human rotavirus genome grow to high titres similar to that of BRV, and therefore they are, with regard to the reassorted part(s) of the human genome, 'high yielding recombinants' (Kilbourne & Murphy, 1960; Kilbourne, 1969). The amplification of rearranged pieces of RNA in BRV reassortants will allow us to obtain enough material for molecular cloning and sequencing studies (McCrae & McCorquodale, 1982b; Both et al., 1982) in a way similar to that envisaged for the study of rearranged bands of RNA of the BRV genome (Hundley et al., 1985).

On the other hand, it was noticed that some of the BRV reassortants carrying rearranged pieces and/or segments of the human rotavirus genome differed in their plaque-forming and growth characteristics from those of BRV; a detailed comparative study of the replication of these viruses is under way.

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REFERENCES


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