Isolation of a Recombinant between Simian and Bovine Rotaviruses

(Accepted 19 December 1979)

SUMMARY

A recombinant between simian rotavirus, simian agent 11 (SA-11) and bovine rotavirus, neonatal calf diarrhoea virus (NCDV), was obtained by mixed infection of MA-104 cells with NCDV and u.v.-irradiated SA-11 virus, and isolation of a plaque formed in the presence of anti-NCDV serum. The genome of the recombinant contained dsRNA segments 4, 5 and 10 derived from SA-11 virus and segments 1, 2, 3, 6 and 11 derived from NCDV, and segments 7, 8 and 9 of undetermined origin. Polypeptides VP4, VP5, VP7a, VP7b, NCVP1 and NCVP3 were derived from SA-11 virus and polypeptides VP1, VP2, VP3, VP6, VP8, NCVP2a and NCVP2b from NCDV. Haemagglutination of the recombinant was inhibited and its infectivity neutralized by the antiserum against SA-11 virus but not by anti-NCDV serum.

Rotaviruses have been reported to be associated with diarrhoeal disease in humans and animals (Wyatt et al. 1978). Rota-, reo- and orbiviruses have all been shown to possess genomes consisting of segments of dsRNA (Shatkin et al. 1968; Verwoerd et al. 1970; Newman et al. 1975; Rodger et al. 1975). The genome of rotavirus consists of 11 segments as studied by polyacrylamide gel electrophoresis (Newman et al. 1975; Rodger et al. 1975; Kalica et al. 1976; Schnagl & Holmes, 1976). Those segments were classified into four size classes: class I contains four genome segments (1 to 4), having mol. wt. of $1.61 \times 10^6$ to $1.89 \times 10^6$; class II two segments (5 and 6) with mol. wt. of $1.10 \times 10^6$ and $1.18 \times 10^6$; class III three segments (7 to 9) with mol. wt. of $0.84 \times 10^6$ to $0.88 \times 10^6$ and, finally, class IV has two segments (10 and 11), with mol. wt. of $0.52 \times 10^6$ and $0.63 \times 10^6$ (Kalica et al. 1976). Different rotaviruses have been distinguished by the difference in electrophoretic migration of RNA segments in polyacrylamide gels (Kalica et al. 1976; 1978b). Simian agent 11 (SA-11) virus and bovine rotavirus (neonatal calf diarrhoea virus, NCDV) cross-react in complement fixation tests but are distinguishable by serum neutralization and haemagglutination-inhibition (HI) tests (see review by McNulty, 1978). The production of recombinants between other Reoviridae groups was reported by several workers (Fields, 1971; Gorman et al. 1978). We present here the isolation and characterization of a recombinant clone derived from mixed infection of MA-104 cells with SA-11 virus and NCDV.

Growth of SA-11 virus and NCDV (Lincoln strain), plaque assay and plaque reduction neutralization tests were performed in MA-104 (Macacus Rhesus monkey kidney) cells by methods described previously (Matsuno et al. 1977). SA-11 virus and NCDV were plaque-purified three times. Haemagglutination (HA) and HI tests were carried out by the methods of Fauvel et al. (1978) and Kalica et al. (1978a). Production of recombinants was performed by the following method. SA-11 virus diluted in phosphate-buffered saline (PBS) was irradiated as a thin film of solution on a Petri dish (diam. 10 cm) with a 15 W germicidal lamp at a distance of 20 cm for 30 to 40 s periods during which the dish was constantly rocked. The infectivity was reduced about 1000-fold. MA-104 cells were mixedly infected with 1 p.f.u./cell of NCDV and u.v.-irradiated SA-11 virus (0.01 and 0.1 p.f.u./cell based on pre-irradiation titre). The viruses were adsorbed to the cells for 60 min at 37 °C. The
inoculum was then removed and 3 ml of minimal essential medium (MEM) was added. After 24 h incubation at 37 °C, the cells were freeze-thawed three times. The harvested material was then inoculated into MA-104 cells. Plaques were made under anti-NCDV serum. After incubation for 4 days a block of agar overlay from each plaque was picked and suspended in MEM. Virus contained in a plaque was grown under the fluid medium in the absence of antiserum. Plaques in the presence of antiserum followed by the growth under the fluid medium without antiserum, was repeated twice more. Clones were finally plaque purified in the absence of antiserum and were grown to virus stocks. Out of eight clones thus obtained, seven appeared to be SA-11 virus by electrophoresis of RNA as described below. Isotopic labelling and purification of virus were as previously described (Matsuno & Mukoyama, 1979).
Table 1. Cross neutralization and haemagglutination-inhibition tests between SA-I1, clone 8 and NCDV

<table>
<thead>
<tr>
<th>Virus</th>
<th>Neutralization test† against SA-I1</th>
<th>Neutralization test‡ against NCDV</th>
<th>Haemagglutination-inhibition test* against SA-I1</th>
<th>Haemagglutination-inhibition test* against NCDV</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA-I1</td>
<td>340,000‡</td>
<td>700</td>
<td>51,000§</td>
<td>1,600</td>
</tr>
<tr>
<td>Clone 8</td>
<td>20,000</td>
<td>3,400</td>
<td>12,800</td>
<td>400</td>
</tr>
<tr>
<td>NCDV</td>
<td>2,600</td>
<td>40,000</td>
<td>400</td>
<td>6,400</td>
</tr>
</tbody>
</table>

* Haemagglutination-inhibition test was performed in U-bottom microplates using phosphate-buffered saline, pH 7.2, containing 1% bovine serum albumin and 0.0001% gelatin and 0.5% human 'O' erythrocytes.
† Antisera against SA-I1 virus and NCDV were prepared in guinea-pigs as described previously (Matsuno et al. 1977).
‡ The figures represent the reciprocal of the serum dilution causing 50% plaque reduction.
§ The figures represent the reciprocal of the serum dilution inhibiting three out of four haemagglutinating doses of virus.

In Fig. 1(A) the migration patterns of dsRNA in polyacrylamide slab gel from SA-I1 virus, NCDV and the remaining clone (clone 8) are shown. Electrophoresis of dsRNA from SA-I1 virus and NCDV revealed differences in segments 1, 2, 3, 4, 5, 6, 10 and 11. However, the differences in other segments (7, 8, 9) were too small to be distinguished. For clone 8 it appears that segments 4, 5 and 10 are derived from SA-I1 virus and segments 1, 2, 3, 6 and 11 from NCDV. The origin of segments 7, 8 and 9 could not be determined by this method.

A comparative study of virus-specific polypeptides synthesized in MA-104 cells by NCDV, SA-I1 virus and clone 8, was carried out by SDS-polyacrylamide gel electrophoresis (Fig. 1 B, C). Thirteen virus-specific polypeptide bands were resolved in the electropherograms of infected cells. Previously we reported the appearance of 11 polypeptide species in NCDV-infected cells (Matsuno & Mukoyama, 1979). An increased number of polypeptides resulted from the separation of VP7 into VP7a and VP7b and NCVP2 into NCVP2a and NCVP2b, probably due to increased resolution of a small polypeptide species by the higher voltage used in this study. Some polypeptides were considered to be formed as a result of post-translational modification, but the exact derivation of the polypeptides is not known. Polypeptides VP1, VP2, VP3, VP6, VP8, NCVP2a and NCVP2b of clone 8 had electrophoretic mobilities similar to those of the corresponding polypeptides of NCDV and different from those of SA-I1 virus. Polypeptides VP4, VP5, VP7a, VP7b, NCVPV1 and NCVP3 seem to have derived from SA-I1 virus (Fig. 1 B, C).

SA-I1, NCDV and clone 8 were then compared by neutralization and HI tests using anti-SA-I1 and anti-NCDV sera. The results presented in Table 1 show that the recombinant virus was neutralized by the antiserum against SA-I1 virus but not by anti-NCDV serum. Apparent neutralization of clone 8 at low dilution of anti-NCDV serum may be due to the derivation of unidentified minor antigen(s) from NCDV. The haemagglutinating activity of clone 8 was also inhibited by anti-SA-I1 serum but not by anti-NCDV serum. The outer shell of rotavirus was shown to consist of VP5, VP7 and VP8 (Matsuno & Mukoyama, 1979). Therefore, haemagglutinin and major neutralization antigens should reside in either VP5 or VP7 polypeptide. The following experiment suggested that VP5 was the haemagglutinin. The outer shells were removed from purified virions of NCDV with 2 mM-EDTA. The absorption of released outer shell proteins with human erythrocytes markedly diminished the intensity of VP5 band in the electropherogram in comparison with the unabsorbed sample (data not shown). However, the complete assignment of polypeptide species to RNA segments of rotaviruses has not been made.
Short communications

The production of recombinants which contained the outer shell proteins from human rotaviruses and were capable of growing in cell cultures would be of practical importance.

The authors are grateful to Dr Akira Sugiura, Department of Microbiology, the Institute of Public Health, for his excellent suggestions.

Central Virus Diagnostic Laboratory
National Institute of Health
4-7-1, Gakuen, Musashimurayama-shi
Tokyo 190-12, Japan and
* Laboratory of Infectious Diseases
National Institute of Allergy and Infectious Diseases
N.I.H., Bethesda, Md. 20014, U.S.A.

REFERENCES


(Received 11 September 1979)