Rotavirus 993/83, isolated from calf faeces, closely resembles an avian rotavirus

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Polypeptides from purified virions of the calf rotavirus (RV) isolate 993/83 and those from the pigeon RV isolate PO-13 comigrated on SDS-polyacrylamide gels. Two polypeptides of 45K and 47K were detected at the position of VP6. Both proteins behaved like authentic VP6 protein with EDTA and heat treatment.

RV 993/83 and PO-13 showed identical one-dimensional peptide maps for VP2, and the 45K and 47K proteins. More than 70% of sera from German cattle older than 1 year showed neutralizing serum antibodies to RV 993/83 and RV PO-13.

Rotaviruses (RVs) are a major infectious cause of diarrhoea in calves (McNulty, 1978). In a survey of 1430 faecal samples from calves with diarrhoea conducted in Germany (Eichhorn et al., 1985) we identified a group A RV that differed from conventional bovine RV in its subgroup, serotype and genogroup (Brüssow et al., 1992). This isolate, RV 993/83, was a serotype 7 RV and it showed RNA sequence homology with a pigeon RV (Minamoto et al., 1988; Brüssow et al., 1992). In the present report we extend the biochemical and serological comparison of RV 993/83 with pigeon RV PO-13.

MA104 cells were labelled throughout the entire infection cycle with [35S]methionine (15 μCi/ml; DuPont, NEN) in a medium containing one-tenth the normal methionine content. RV particles were isolated from the cell-free culture supernatant by high-speed centrifugation (90000 g, 2 h, 4 °C) followed by equilibrium centrifugation on preformed CsCl gradients (1-25 to 1-42 g/ml). Protein electrophoresis was carried out in slab gels using the method of Laemmli (1970) with 13% running and 3% stacking gels. Gels were fluorographed with Enlightening (DuPont, NEN), dried and exposed on X-ray films (YAR-5; Eastman Kodak).

Putative VP1, VP2, VP3, VP5* (the major tryptic cleavage product of VP4), VP6 and VP7 (37K and 35K bands) were detected in double-shelled RV 993/83 purified by CsCl density gradient centrifugation (Fig. 1a). Pigeon RV PO-13 showed a very similar polypeptide migration pattern. In addition, RV 993/83 and RV PO-13 contained two polypeptides that migrated to near the position of authentic VP6: a major 47K band which migrated slower than bovine RV VP6 and a minor 45K band which migrated at approximately the same position as bovine RV VP6. The 45K and 35K bands varied in intensity between different preparations from identical virus stocks (data not shown).

Rotavirus particles banding at 1.36 g/ml (double-shelled particles) were recovered from the CsCl gradients using a Pasteur pipette, diluted with TNC (50 mM-Tris HCl pH 7.5 containing 150 mM-NaCl and 10 mM-CaCl2) or alternatively with TNE (containing 10 mM-EDTA in place of 10 mM-CaCl2) and pelleted by high-speed centrifugation (130000 g, 1 h, 4 °C). EDTA treatment resulted in loss of putative VP5* and VP7, whereas both polypeptide bands at the approximate VP6 position (the 47K and 45K bands) remained associated with single-shelled particles (Fig. 1b).

When RV 993/83 was loaded onto SDS-polyacrylamide gels without prior heating (2 min at 100 °C) in sample buffer (containing 2-mercaptoethanol), it was found that the autoradiographic intensity of the 47K and 45K polypeptide bands decreased and a polypeptide band with an Mr of approximately 140K appeared (Fig. 1c). Thus, with EDTA treatment and omission of sample boiling, the 47K and 45K polypeptides behave like authentic VP6 (Cohen et al., 1979; Gorziglia et al., 1985; McCrae & Faulkner-Valle, 1981; Sabara et al., 1987). Limited proteolysis analysis was performed using the procedure described by Cleveland et al. (1977). Staphylo-
Table 1. Prevalence and geometric mean titre of neutralizing antibodies to rotavirus 993/83, pigeon RV PO-13 and chicken RV Ch-2

<table>
<thead>
<tr>
<th>Age group</th>
<th>n*</th>
<th>993/83 (%)</th>
<th>PO-13 (%)</th>
<th>Ch-2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1 month</td>
<td>22</td>
<td>41 (44)‡</td>
<td>27 (24)</td>
<td>0 (10)</td>
</tr>
<tr>
<td>1-2 months</td>
<td>19</td>
<td>37 (47)</td>
<td>32 (17)</td>
<td>5 (15)</td>
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<tr>
<td>2-4 months</td>
<td>18</td>
<td>22 (31)</td>
<td>28 (14)</td>
<td>0 (10)</td>
</tr>
<tr>
<td>4-12 months</td>
<td>16</td>
<td>31 (21)</td>
<td>25 (17)</td>
<td>6 (13)</td>
</tr>
<tr>
<td>1-3 years</td>
<td>27</td>
<td>67 (100)</td>
<td>81 (83)</td>
<td>11 (16)</td>
</tr>
<tr>
<td>3-5 years</td>
<td>26</td>
<td>88 (165)</td>
<td>73 (100)</td>
<td>0 (11)</td>
</tr>
<tr>
<td>&gt;5 years</td>
<td>13</td>
<td>100 (197)</td>
<td>92 (176)</td>
<td>8 (12)</td>
</tr>
</tbody>
</table>

* n, Number of serum samples tested.
† A serum was counted as neutralizing if a 1:90 dilution neutralized 100 TCID₅₀ of the rotavirus indicated in the peroxidase focus reduction test (Brüssow et al., 1991b).
‡ Figures in parentheses indicate the geometric mean titre.

In conclusion, RV 993/83 isolated from the faeces of a German calf with diarrhoea closely resembles a pigeon RV. We do not believe that RV 993/83 is a laboratory contaminant (Brüssow et al., 1992). However, contamination of the original bovine faeces with avian faeces in the field remains a possibility that we cannot exclude. Only infecting a calf with RV 993/83 to determine whether it replicates in the seemingly heterologous host can provide experimental evidence that RV 993/83 has the capacity to cross the species barrier.
We thank David Baker for reading the manuscript and Marie-Claude Gysi for typing the manuscript.

References


(Received 30 December 1991; Accepted 27 March 1992)