Polymorphism of the Genomic RNAs Among the Avian Reoviruses

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SUMMARY

The genome of avian reoviruses is comprised of 10 segments of double-stranded (ds)RNA. Analysis by polyacrylamide gel electrophoresis of the genomic RNA from a small number of avian reoviruses has demonstrated a significant polymorphism in the migration pattern of the dsRNA segments among different isolates. Comparison of these patterns with that of the mammalian reovirus of serotype 1 has permitted calculation of the molecular weights of the avian dsRNA species.

Mammalian reoviruses have been extensively studied, not only in terms of biochemical characterization (Ramig & Fields, 1977; Joklik, 1974; Cross & Fields, 1977; Silverstein et al., 1976) but also as models for mechanisms of disease expression (Fields et al., 1978; Fields, 1972; Weiner et al., 1977; Rubin & Fields, 1980). These investigations have demonstrated that the reovirus genome is composed of 10 distinct segments of double-stranded RNA (dsRNA), each of which constitutes a single gene coding for the production of a unique protein. Examination of individual mammalian reovirus isolates by analysis of the pattern of migration of their genomic RNA upon polyacrylamide gel electrophoresis (PAGE) has demonstrated a striking polymorphism, not only among serotypes but also among different isolates within the same serotype (Shatkin et al., 1968; Ramig et al., 1977; Hrdy et al., 1979). The mechanism by which this polymorphism arises is not known, and its importance, if any, for the maintenance of the virus in an endemic state has not been determined.

Reoviruses have also been isolated from a variety of avian sources, particularly from individuals expressing clinical tenosynovitis (van der Heide, 1977; Olson, 1978). These isolates have been assigned to the reovirus family largely on the basis of physical properties shared with the mammalian reoviruses, namely a particle diam. of 70 to 80 nm, a double-capsid structure, and a genome consisting of dsRNA (van der Heide & Kalbac, 1975; Sekiguchi et al., 1968; Deshmukh & Pomeroy, 1969). Avian reoviruses may be distinguished, however, from their mammalian counterparts by their lack of haemagglutinating ability and their host range, which is restricted to the avian species (Glass et al., 1973; Petek et al., 1967).

Despite a large number of pathological studies with many different isolates (van der Heide, 1977; Olson, 1978; Cullen, 1977; Sahu & Olson, 1975; Jones & Onunkwo, 1978; Kircher, 1977), avian reoviruses have not been extensively investigated in biochemical terms. Only a single isolate has been partially characterized in terms of protein composition and genome structure (Spandidos & Graham, 1976). In an attempt to determine if individual avian reovirus isolates demonstrate the same type of polymorphism in regard to their genomic RNA as reported with their mammalian counterparts, several different avian reovirus isolates have been obtained and the pattern of migration of their genomic RNAs compared.

The S1133 (van der Heide et al., 1976) and P100 (van der Heide, 1980), avian reovirus isolates were kindly supplied by L. van der Heide, Storrs, Conn., U.S.A. The P100 virus represents the 100th tissue culture passage of S1133. The R19 and Lasswade isolates (MacDonald et al., 1978) were obtained from R. C. Jones, University of Liverpool, Wirral, U.K. Human reovirus type 1 (Lang) was obtained from B. N. Fields, Harvard Medical
School, Boston, Mass., U.S.A. All isolates were plaque-purified three times and then a final stock virus pool made and used in all assays. Avian reoviruses were plaque-purified and grown on primary or secondary cultures of chick embryo fibroblasts (CEF) prepared as described by Schnitzer \textit{et al.} (1977) and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% tryptose phosphate broth, 2% newborn calf serum (NCS), and 2% chick serum. Mammalian reoviruses were grown on mouse L cells maintained in DMEM with 10% NCS.

Virus RNA was prepared by a modification of the procedure described by Sharpe \textit{et al.} (1978). Monolayer cell cultures in 30 mm plastic Petri dishes (Nunc) were infected at a multiplicity of infection of 3 and virus allowed to adsorb at 37 °C for 1 h. After adsorption the inoculum was removed and 2 ml DMEM containing 2% dialysed, heat-inactivated NCS, 60 ng/ml actinomycin D and 100 μCi [5,6-3H]uridine (Amersham International) were added per plate. RNA from mammalian reovirus type 1 was prepared in the same manner as above except that the final actinomycin D concentration was 250 ng/ml. After 18 h (avian reoviruses) and 27 (mammalian reovirus) of incubation at 37 °C and in 5% CO₂, the infected cells were harvested and cytoplasmic RNA prepared. Briefly, the cells were scraped off the plates, washed with phosphate-buffered saline (PBS) and treated with 0.5% Nonidet P40 at 4 °C for 30 min. Nuclei were removed by low-speed centrifugation. The cytoplasmic extract was adjusted to 0.25 M-NaCl and the RNA precipitated with 2.5 vol. ethanol overnight at −20 °C. The samples were centrifuged, the pellet dried under vacuum and resuspended in 200 μl of gel sample buffer. Electrophoresis of reovirus dsRNA was carried out on SDS slab gels containing 10% acrylamide–0.267% bisacrylamide in a discontinuous tris–glycine buffer system as described by Cross & Fields (1976). Samples were boiled for 1 min just before loading and electrophoresis was carried out at 140 V for 17 h. The gels were then treated with En³Hance (New England Nuclear), dried on to filter paper and fluorography performed by exposing X-Omat film (Kodak) for 1 to 3 days.

The results in Fig. 1 clearly demonstrate that the genome of avian reoviruses can be separated into distinct segments of RNA and that significant polymorphism of the genomic RNA exists among the different isolates examined. Under ideal conditions of PAGE, 10 distinct bands can be seen with each avian reovirus isolate. With the exception of the S1133 and P100 isolates, which are related by \textit{in vitro} passage, most avian reovirus isolated thus far have given unique patterns of migration of their genomic RNA (data not shown). Attempts to distinguish the genomic RNAs of P100 from S1133 by PAGE using different conditions (different buffer systems, different percentage of acrylamide or bisacrylamide ratio) have not demonstrated any differences in migration.

The RNA bands from the avian isolates shown in Fig. 1 have been arbitrarily grouped into large, medium, and small size categories in a manner similar to that indicated for the mammalian reoviruses, and within each size class the bands have been classified by numerical sequence. By extrapolation from the migration of the mammalian reovirus T1 dsRNAs, whose mol. wt. are known (Ramig \textit{et al.}, 1977), the mol. wt. of the genomic RNA segments of the four avian reoviruses have been calculated and are included in Table 1. As the assignment of individual RNA segments to their respective protein products has not yet been made for the avian reoviruses, the correspondence between any individual RNA band in the avian species and that found in the mammalian reovirus genome is not known.

Recent examinations of the genome structures of viruses have demonstrated that variability is a common occurrence, not restricted to any single class of viruses but found with DNA viruses as well as single-stranded and double-stranded RNA viruses (Hrdy \textit{et al.}, 1979; Kalica \textit{et al.}, 1978; Palese & Schulman, 1976; Linnemann \textit{et al.}, 1978). Studies of the genomic variability among mammalian reoviruses have been extensive and indicate no specific relationship between migration pattern of the genomic RNA segments and serotype.
Fig. 1. [3H]uridine-labelled dsRNA from avian and mammalian reoviruses. Electrophoresis was from top to bottom in a single 10% acrylamide gel under conditions described in the text. (a) Avian R19; (b) avian P100; (c) avian S1133; (d) avian Lasswade; (e) human type 1 (Lang). RNA genome segments of R19 and type 1 (Lang) are identified (Ramig et al., 1977).

Table 1. Molecular weights of double-stranded RNAs from some avian reoviruses*

<table>
<thead>
<tr>
<th>dsRNA species</th>
<th>Mammalian reovirus</th>
<th>Avian reoviruses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M1 Lang</td>
<td>R19</td>
</tr>
<tr>
<td>L1</td>
<td>2.48</td>
<td>2.40</td>
</tr>
<tr>
<td>L2</td>
<td>2.37</td>
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</tr>
<tr>
<td>M1</td>
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</tr>
<tr>
<td>M2</td>
<td>1.54</td>
<td>1.42</td>
</tr>
<tr>
<td>M3</td>
<td>1.42</td>
<td>1.34</td>
</tr>
<tr>
<td>S1</td>
<td>0.82</td>
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</tr>
<tr>
<td>S2</td>
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<td>0.70</td>
</tr>
<tr>
<td>S3</td>
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<td>0.68</td>
</tr>
<tr>
<td>S4</td>
<td>0.63</td>
<td>0.64</td>
</tr>
</tbody>
</table>

* Mol. wt. of dsRNA were calculated from the mol. wt. of dsRNA of human reovirus type 3 taken from Ramig et al. (1977).

(Hrdy et al., 1979). Examination of isolates of reoviruses from different mammalian hosts has not revealed distinctions that were species-specific; however, such differences may exist between reoviruses believed to be more distantly related, such as the avian and mammalian...
reoviruses. In particular, it appears that the bands designated M3 and S1 in the avian species migrate in a manner quite distinct from any RNA band of the mammalian reoviruses examined but show little variability among the avian isolates.

The ability to distinguish individual avian reovirus isolates by the migration pattern of their genomic RNA allows a series of further studies aimed at examining the significance of this polymorphism. The fact that continued in vitro passage fails to result in alterations of genomic migration patterns, as demonstrated here with the P100 and S1133 isolates, suggests that the variability seen with other isolates may arise as a consequence of processes other than simple failure of integrity of copying of genomic RNA during virus replication. The importance in vivo of selective pressure arising from the host immune response and the significance of reassortment of genomic segments among different reovirus isolates in the field are not known but can be examined by characterization of appropriate isolates. Furthermore, valuable epidemiological data regarding the spread of virus in flocks and the importance of horizontal and vertical transmission for infection can be obtained, and the relationship, if any, between RNA genomic patterns and serotype, type of disease produced, geographic localization, and host of origin may be determined by these methods. Such studies should provide a better insight into the mechanism of virus variability and its consequences for both disease production as well as maintenance of the endemic state within the host species.

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REFERENCES


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