Strain-specific selection of genome segments in avian reovirus coinfections

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To determine whether selection of genome segments in coinfections is strain-specific, chicken embryo fibroblasts were coinfected with avian reovirus strain 883 and one of three other avian reovirus strains (176, S1133 and 81-5). Viral progeny from each coinfection (883×176, 883×S1133 or 883×81-5) was serially passaged at a low m.o.i. The electropherotypes of the coinfection progeny and those of the plaque-derived clones obtained from passages 1 and 20 were analysed. Two 883 segments (M2 and S2) were found to be selected in the 883×176 coinfection, three 883 segments (M2, M3 and S2) in the 883 x S1133 coinfection, and only one 883 segment (M3) in the 883×81-5 coinfection, i.e. different 883 genome segments were selected in the three coinfections. It was, therefore, concluded that selection of genome segments in a coinfection of a given cell line is virus strain-specific. The selection of genome segments in coinfections was shown to be due to enhanced infectivity of the reassortants that were formed in the coinfections. In addition, defective interfering particles that lack the S1 segment were identified in the 883×81-5 coinfection progeny following serial passage. Selection of genome segment(s) in coinfections as described herein may have potential importance on the effect and production of divalent or multivalent vaccines.

Introduction

Avian reoviruses are the major cause of viral arthritis syndrome or tenosynovitis in chickens (Robertson & Wilcox, 1986; Rosenberger & Olson, 1991). Being members of the reovirus genus of the Reoviridae family (Matthews, 1982), avian reoviruses have a genome consisting of 10 dsRNA segments. By using PAGE, the genome segments can be separated into three size classes: large (L), medium (M) and small (S) (Spandidos & Graham, 1976; Joklik, 1981).

Two avian reovirus strains, 883 and 176, that exhibited divergent electropherotypes and pathogenic properties (Clark et al., 1990) were used to coinfest chicken embryo fibroblasts (CEFs) and generate reassortants (Ni & Kemp, 1990). A comparison of the growth curves of 883 and 176 showed that 883 replicated more slowly than 176 and to an infectious titre at least 100-fold lower. Despite the poor growth of 883, the 883 M2 segment was shown to be preferentially selected over the 176 M2 segment following limited serial passage of coinfection progeny.

Coinfection or mixed infection is one way for virus evolution or new virus strains to emerge, i.e. recombinants or reassortants can be formed in the coinfected cells. Recombinants or reassortants that replicate more efficiently or are better adapted to the host system should be preferentially selected. The selection of reassortants in coinfections has been observed for rotavirus (Graham et al., 1987; Ward et al., 1988), bluetongue virus (Ramig et al., 1989) and avian reoviruses (Ni & Kemp, 1990).

It is conceivable that a genome segment of virus ‘A’, preferentially selected when ‘A’ is used to coinfest cells with virus ‘B’, may not be selected when virus ‘A’ is used to coinfect cells with virus ‘C’, i.e. selection of genome segments in coinfections is virus strain-specific. Our previous studies on the preferential selection of the 883 M2 segment in the 883 x 176 coinfection and availability of other avian reovirus strains with different electropherotypes (Clark et al., 1990) provided a system to test this hypothesis.

We report here the analysis of three coinfections made with avian reovirus strain 883 and one of three other avian reovirus strains. The results indicated that selection of genome segments in coinfections is virus strain-specific and that the genome segment selection was due to increased infectivity of the reassortants formed in the coinfection. We also found that defective interfering (DI) particles having an S1 segment deletion were generated in the 883×81-5 coinfection.

Methods

Cells. Primary CEFs were prepared from 10-day-old embryos obtained from eggs purchased from SPAFAS. CEFs were maintained in Eagle's basal medium (BME) supplemented with 5% foetal calf serum.
Viruses. Avian reoviruses 883, 176, S1133 and 81-5 used for this study have been previously described (Clark et al., 1990). Two reassortants, F42 (883 M2) and F59 (883 M2, S2) were isolated from a 883 × 176 coinfection as previously described (Ni & Kemp, 1990). Before coinfections were done, all viruses were plaque-purified twice, and stocks of known titre were stored at −70 °C.

Virus purification. Infected cells were harvested by two freeze–thaw cycles. Cell debris was removed by centrifugation at 8000 r.p.m. for 30 min. Virions were pelleted at 27000 r.p.m. for 2 h in a Beckman SW28 rotor, then suspended in TN buffer (10 mM-Tris–HCl, 140 mM-NaCl, pH 7.5). They were then layered onto a 30% (v/v) glycerol–50% (w/w) potassium tartrate gradient. Following centrifugation at 40000 r.p.m. for 2 h at 4 °C in a Beckman SW41 rotor, the virus band was collected by aspiration after piercing the side of the centrifuge tube. The viral band was then diluted in TN buffer and virions were pelleted by centrifugation at 40000 r.p.m. for 1 h in a Beckman SW41 rotor. The viral pellet was suspended in TN buffer and stored at −70 °C before use.

Coinfections. Coinfection was initiated by first infecting CEF monolayers cultured in T-25 flasks with the 883 parental strain at an m.o.i. of 0.1. Prior infection of CEFs with 883 was necessary because of the poor growth properties of this virus (Ni & Kemp, 1990). 883-infected cells were then superinfected with either 176, S1133 or 81-5 at an m.o.i. of 0.1, 24 h later. Coinfected cells were incubated for an additional 72 h, and then frozen and stored at −70 °C. Viral progeny from each coinfection was subsequently serially passaged at m.o.i.s of 0.1 to 0.5 in CEFs.

Plaque assay. CEFs cultured in 60 × 15 mm Petri dishes were inoculated with serial virus dilutions. After a 1 h adsorption period infected monolayers were washed twice with BME, and overlaid with 1% agar in BME containing 0.0075% neutral red. Infected monolayers were incubated at 37 °C in 5% CO2 until plaques were detected (usually 3 to 5 days). Plaques were picked and passaged in CEFs cultured in T-25 flasks.

Determination of viral growth curves. Viral growth curves were determined by inoculating CEFs cultured in T-25 flasks at an m.o.i. of 0.1. After a 1 h adsorption period infected monolayers were washed twice with BME and, following the addition of fresh BME, they were incubated at 37 °C. Infected monolayers were harvested at the indicated time intervals, and stored at −70 °C.

Serial dilutions of each infected cell lysate were then used to infect CEFs cultured in 24-well plates (four wells per dilution). After a 1 h adsorption period infected cells were washed twice with BME and, after addition of fresh BME, incubated at 37 °C in 5% CO2 for 5 days. The TCID50/ml for each time point was determined using the procedures of Reed & Muench (1938). Titres were calculated in terms of TCID50/ml.

Extraction and electrophoretic analysis of viral RNAs. Infected cell lysates or purified virus preparations (0.5 ml) were mixed with 50 ml of 10% SDS and 5 ml of proteinase K (20 mg/ml) and incubated at 37 °C for 30 min. The mixture was then extracted with phenol, and viral RNAs were precipitated from the aqueous phase by the addition of 50 ml of 3 M-sodium acetate (pH 5.2) and two volumes of absolute ethanol.

Precipitated viral RNAs were analysed on 10% SDS-polyacrylamide gels (Laemmli, 1970). After electrophoresis, gels were stained with silver nitrate using a procedure modified from that of Herring et al. (1982). After electrophoresis, the gel was fixed with 10% alcohol and 1% acetic acid for 1 h, and then stained with 0.011 M-silver nitrate for 30 min. The stained gel was developed in 250 ml of developer containing 7.5 g NaOH, 22 mg NaBH4 and 1.5 ml 37% formaldehyde.

Fig. 1. Serial passage of 883 × 176 coinfection progeny. Viral RNAs extracted from infected cell lysates at each passage were separated by PAGE and stained with silver. Numbers above the lanes indicate the passage number. 883 and 176 indicate the lanes in which viral RNAs of parental viruses 883 and 176 were electrophoresed.

Results

Effect of viral strains on selection of 883 genome segments

To determine whether selection of genome segments in coinfections is strain-specific, CEFs were coinfected with avian reovirus strain 883 and one of three other avian reovirus strains (176, S1133 and 81-5). It was observed that 883 replicated poorly compared to the three other virus strains, i.e. to an infectious titre usually at least 100-fold lower than that of any of the others. Coinfections were therefore done asynchronously by infecting cells with 883 first and superinfecting the 883-infected cells with either 176, S1133 or 81-5 at the same m.o.i. (0.1) 24 h later. Comparative electrophoretic analysis of the genome segments of the four parental virus strains showed that the 883 S3 segment exhibited an electrophoretic mobility indistinguishable from that of the S3 of 176 and S1133, and the S2 of 81-5 (Fig. 1, 2 and 3). Genome segments, M3 of the 883 and 176 strains could not be differentiated (Fig. 1). The electrophoretic mobilities of the 81-5 S2 and S3 segments were shown to be inverted relative to those of the 883 S2 and S3 segments by analysis of electropherotypes of the plaque-derived reassortants. Thus, the parental origin of nine of the genome segments in the 883 × S1133 and 883 × 81-5 coinfections and eight of the genome segments in the
Fig. 2. Serial passage of 883 × S1133 coinfection progeny. Viral RNAs extracted from infected cell lysates at each passage were separated by PAGE and stained with silver. Numbers above the lanes indicate the passage number. 883 and S1133 indicate the lanes in which viral RNAs of parental viruses 883 and S1133 were electrophoresed.

883 × 176 coinfection could be determined by electrophoretic analysis.

Analysis of genome RNAs extracted from coinfected cells (passage 1) showed that the amount of 883 genome segments detected in the coinfected cells was much lower than those of the superinfecting viruses (Fig. 1, 2 and 3). Moreover, all plaque-derived clones obtained from passage 1 coinfection progeny exhibited the electropherotype of the superinfecting virus, except for one reassortant (Table 1). This reassortant was isolated from the 883 × S1133 coinfection and it possessed the M2 segment of strain 883 (Table 1). Therefore the 883 genome segments appeared to be present at a ratio unfavourable for them to be selected in the coinfections.

Previously we showed that the 883 M2 segment was preferentially selected over the 176 M2 segment when the 883 × 176 coinfection progeny (passage 1) was serially passaged (Ni & Kemp, 1990). Enrichment of the amount of detectable 883 M2 occurred when the 883 × S1133 coinfection and it possessed the M2 segment of strain 883 (Table 1). Therefore the 883 genome segments appeared to be present at a ratio unfavourable for them to be selected in the coinfections.

At passage 20 the electropherotypes of the plaque-derived clones obtained from the coinfections were determined. Incidence frequencies of the 883 M2 segment in the clones from 883 × 176 and 883 × S1133 coinfections were 93% and 92% (Table 1), respectively. This result is consistent with the selection of the 883 M2 segment in both these coinfections. In the 883 × S1133 coinfection, no 883 M2 segment was detected in the clones obtained from passage 20 progeny, indicating an effect of virus strain on selection of genome segments.

Besides selection of the 883 M2 segment, serial passage of coinfection progeny also resulted in preferential selection of 883 S2 in the 883 × 176 coinfection, 883 M3 and S2 in the 883 × S1133 coinfection, and 883 M3 in the 883 × 81-5 coinfection (Fig. 1, 2 and 3). The amount of 883 M3 segment exceeded that of its cognate segment at passage 11 in the 883 × S1133 coinfection and at passage 22 in the 883 × 81-5 coinfection. The 883 S2
segment became dominant at passages 9 and 15 in the $883 \times 176$ and $883 \times S1133$ coinfections respectively.

The selection of the $883$ M3 and S2 segments in the coinfections was also shown by their high incidence frequencies (over 50\%) in the plaque-derived clones obtained from passage 20 coinfection progeny (Table 1). Almost all the plaque-derived clones isolated at passage 20 were reassortants, and most of the reassortants had the selected $883$ genome segments (M2, M3 and S2) (Table 2); other $883$ genome segments were either present at frequencies of less than 50\% in the plaque-derived clones, or became undetectable (Fig. 1, 2 and 3; Table 1).

In general, the $883$ M2 and S2 segments were selected in the $883 \times 176$ coinfection, $883$ M2, M3 and S2 were selected in the $883 \times S1133$ coinfection, and only the $883$ M3 segment was selected in the $883 \times 81-5$ coinfection. Since different $883$ genome segments were selected in the three coinfections, it is concluded that selection of genome segment(s) in coinfections is virus strain-specific.

Selection of genome segments in coinfections is due to the increased infectivity of reassortants

The coinfection and subsequent serial passage of coinfection progeny in the present experiments were carried out at m.o.i.s of 0.1 to 0.5. Therefore it seems likely that the selection of genome segments is due to the increased infectivity of the reassortants generated in the coinfections. Growth curves of two reassortants, F42 ($883$ M2) and F59 ($883$ M2 and S2), isolated from passage 3 progeny of an $883 \times 176$ coinfection (Ni & Kemp, 1990), were compared with those of parental $883$ and 176. Both reassortants have the M2 segment from virus $883$. The $883$ M2 segment was the first $883$ segment selected during serial passage of $883 \times 176$ coinfection progeny (Fig. 1), and therefore the $883$ M2 segment may contribute the most among the $883$ genome segments in increasing the infectivity of the coinfection progeny. Along with $883$ M2, F59 contains $883$ S2. The $883$ S2 segment was also selected during serial passage of the $883 \times 176$ coinfection progeny, but it took four more

### Table 1. Incidence frequencies (%) of $883$ genome segments in the plaque-derived clones obtained from passages 1 (P1) and 20 (P20) progeny of $883 \times 176$, $883 \times S1133$ and $883 \times 81-5$ coinfections

<table>
<thead>
<tr>
<th></th>
<th>883 $\times$ 176</th>
<th>883 $\times$ 1133</th>
<th>883 $\times$ 81-5</th>
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</thead>
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<tr>
<td>Segment</td>
<td>P1</td>
<td>P20</td>
<td>P1</td>
</tr>
<tr>
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<td>0</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>L2</td>
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</tr>
<tr>
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<td>44</td>
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</tr>
<tr>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M2</td>
<td>0</td>
<td>93*</td>
<td>5</td>
</tr>
<tr>
<td>M3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No. of clones</td>
<td>14</td>
<td>32</td>
<td>18</td>
</tr>
</tbody>
</table>

* The incidence frequencies of $883$ genome segments above 50\% are underlined.
† The parental origin of the M3 segment in the clones from the $883 \times 176$ coinfection and the S3 segment in the clones from all three coinfections could not be determined under the electrophoretic conditions used.

† The electrophoretic migration order of the 81-5 S2 and S3 segments is inverted in relation to that of the $883$ S2 and S3 segments.

### Table 2. Electropherotypes of the plaque-derived clones obtained from passage 20 progeny of $883 \times 176$, $883 \times S1133$ and $883 \times 81-5$ coinfections

<table>
<thead>
<tr>
<th></th>
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<th>883 $\times$ 1133</th>
<th>883 $\times$ 81-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clone*</td>
<td>Number</td>
<td>Clone</td>
<td>Number</td>
</tr>
<tr>
<td>L2, S2</td>
<td>1</td>
<td>M2</td>
<td>1</td>
</tr>
<tr>
<td>L3, M2</td>
<td>1</td>
<td>M3</td>
<td>2</td>
</tr>
<tr>
<td>M2, S2</td>
<td>14</td>
<td>M2, M3</td>
<td>14</td>
</tr>
<tr>
<td>L1, M2, S2</td>
<td>3</td>
<td>M3, S2</td>
<td>1</td>
</tr>
<tr>
<td>L2, L3, S2</td>
<td>1</td>
<td>M2, M3, S2</td>
<td>21</td>
</tr>
<tr>
<td>L3, M2, S2</td>
<td>10</td>
<td>L2, M3</td>
<td>2</td>
</tr>
<tr>
<td>L1, L3, M2, S2</td>
<td>2</td>
<td>L3, M3</td>
<td>3</td>
</tr>
<tr>
<td>Total no. of clones</td>
<td>32</td>
<td>39</td>
<td>40</td>
</tr>
</tbody>
</table>

* The reassortants were identified by the segments of $883$ origin; the parental origin of the M3 segment in the clones from the $883 \times 176$ coinfection and the S3 segment in the clones from all three coinfections could not be determined under the electrophoretic conditions used.
passages for the 883 S2 to be enriched over its cognate segment (176 S2) than for the 883 M2 segment (Fig. 1).

A comparison of the growth curves of the two reassortants with those of the parental viruses (883 and 176) demonstrated that both reassortants and parental 176 replicated to a titre far higher than parental 883 throughout the growth cycle and the two reassortants replicated to a titre at least 10-fold higher than that of parental 176 at early time intervals, i.e. 12 and 24 h post-infection (p.i.) (Fig. 4a). The titres of both reassortants were still considerably higher than that of 176 at 36 h p.i. No noticeable difference between reassortants and parental 176 was observed at 48 and 60 h p.i. This result indicates that the reassortants having the 883 M2 segment replicate better than both parental viruses at early times during the growth cycle, i.e. the 883 M2 segment confers a replicative advantage on the reassortants. In a coinfection, higher infectious yields of certain reassortants generated at early times during the growth cycle should lend them an advantage for infecting any other cells that are available. This suggests that the selection of 883 M2 segment in the 883 × 176 coinfection is due to the increased growth ability or infectivity of the reassortant having this segment. No obvious difference was seen between the two reassortants. F59 did not exhibit better growth properties than clone F42 although it has another selected 883 segment (S2). Since 883 S2 became dominant after several more passages than did the 883 M2 (Fig. 1), it is possible that 883 S2 segment contributes much less to enhance viral growth ability compared to the 883 M2.

To support the hypothesis that the 883 M2 segment was indeed able to confer the replicative advantage to reassortants, additional coinfection studies with F42 and parental 176 were carried out. Cells were coinfected at a 1 to 10 ratio, i.e. an m.o.i. of 0-1 for F42 and 1 for 176. The coinfection progeny were then serially passaged at m.o.i.s of 0-1 to 0-5 as described above. Only parental 176 was used in this experiment because it replicates much more efficiently than parental 883 and would therefore be expected to be the major competitor with reassortants formed in the coinfection. The results showed that the 883 M2 segment present at a lower amount following coinfection (passage 1) was rapidly enriched and became dominant over the 176 M2 segment by passage 3 (Fig. 4b). Since F42 has only the M2 segment from 883, this result further shows that the 883 M2 segment confers a replicative advantage or increased infectivity on the reassortants.

Along with a replicative advantage, it seemed possible that preferential packaging could be involved in the selection of 883 M2. To determine whether this is the case, 883 × 176 coinfection progeny at passages 2, 3 and 4 were used to infect cells at an m.o.i. of 0-1. Coinfection progeny at these passages were selected because the amount of detectable 883 M2 was enriched to a point equal to or exceeding that of the cognate 176 M2 segment during these three passages (Fig. 1). At 72 h p.i., infected cells were harvested and virions were purified as described in Methods. A comparison of viral RNAs extracted from purified virions to those extracted from infected cell lysates showed that the relative amounts of 883 and 176 M2 were essentially invariant at all three passages (data not shown). Thus it is not likely that preferential packaging is involved in the selection of the 883 M2 segment.

**Generation of DI particles lacking the S1 segment in 883 × 81-5 coinfection**

Besides the preferential selection of the 883 M3 segment, serial passage resulted in deletion of the S1 segment in the 883 × 81-5 coinfection. Deletion of the S1 segment,
shown by the reduced amount of detectable S1 segment as compared to other genome segments, was first observed around passage 6, and was exaggerated by further passage (Fig. 3). After passage 10, only a small amount of 81-5 S1 segment could be detected, and the 883 S1 segment became undetectable. Deletion of the S1 segment was also shown when viral RNAs extracted from purified passage 20 progeny were analysed (data not shown). These observations suggest that a majority of the virus population at late passages of the coinfection progeny was composed of defective particles that lack the S1 segment.

These defective particles were also shown to be interfering by infectivity studies. The average infectious titre (2 × 10^5 p.f.u./ml) of the coinfection progeny at late passages (20 and 22) was found to be 50-fold lower than that of the coinfection progeny (1 × 10^7 p.f.u./ml) at the early passages (1 and 3). Therefore, these defective particles were in fact DI particles.

**Discussion**

The data presented herein show that selection of genome segments in coinfections is virus strain-specific. This was demonstrated by coinfecting cells with avian reovirus strain 883 and one of three other avian reovirus strains (176, S1133 and 81-5). Selection of genome segments in coinfections was shown to be due to the increased infectivity of reassortants formed in the coinfections by studying the selection of 883 M2 in the 883 × 176 coinfection. Together these results show that different genetic component(s) of one virus strain can be combined with the genetic background of different virus strains to generate viruses with better growth characteristics. The results also show that a genetic element selected onto the genetic background of another virus strain thereby resulting in the generation of a virus having higher infectivity need not come from a more infectious virus strain.

Ward et al. (1988) observed in rotaviruses that genome segments selected in one coinfection were not the same as those selected in another coinfection. However, the virus strains used for each coinfection were totally different or unrelated in this study. Thus the present experiments, by using a single avian reovirus strain (883) to coinfect cells separately with one of three other avian reovirus strains, directly demonstrated that the selection of genome segments in coinfections of a given cell line is virus strain-specific.

In addition to those 883 segments which were selected (present in an amount greater than that of the cognate segment as demonstrated by viral RNA analysis and having an incidence frequency above 50% in the plaque-derived clones), several other 883 segments, L1, L2 and L3 in the 883 × 176 coinfection and L2, L3 and S2 in the 883 × 81-5 coinfection, were also enriched and exhibited higher incidence frequencies following serial passage (Fig. 1 and 3; Table 1). No such 883 segments were detected in the 883 × S1133 coinfection.

The 883 S1 segment could be detected at later passages of the 883 × 176 and 883 × S1133 coinfections by analysing coinfection progeny viral RNAs (deletion of the S1 segment was evident in the 883 × 81-5 coinfection at late passages and no 883 S1 segment could be detected). However, the 883 S1 segment was never detected in the plaque-derived clones from these two coinfections. This is consistent with our previously reported observation that in more than 30 clones derived from a 883 × 176 coinfection no 883 S1 segment was detected, whereas all other 883 segments were present in these clones (Ni & Kemp, 1990). It has been suggested that the S1 segment codes for a major determinant of virus growth (Ni & Kemp, 1990) and plaque formation (unpublished data), i.e. the poor growth and inefficient plaque formation of 883 virus is determined by the 883 S1 segment. Thus the zero incidence of 883 S1 is probably due to the poor growth and inefficient plaque formation of the viruses with the 883 S1 segment because all clones were derived from well isolated clear plaques at a dilution greater than 10^4 of the coinfection progeny.

It was noted that the selection of different 883 segments (M2, M3 and S2) progressed at different rates in 883 × 176 and 883 × S1133 coinfections, i.e. certain selected 883 segments became dominant (enriched beyond that of the cognate segment) at early passages, whereas others became dominant at later passages. Such differences in selection rate may reflect the interdependence of the selected genome segments, i.e. selection of one genome segment may be dependent upon the selection of another one. Graham et al. (1987) observed that certain genome segments were selected non-randomly in plaque-derived clones isolated from a rotavirus coinfection.

DI particles lacking the S1 segment were generated in the 883 × 81-5 coinfection progeny during serial passage. Mammalian reovirus DI particles lacking mainly large-size genome segments (L1, L2, L3 and/or M1) have been reported (Nonyoyama et al., 1970; Ahmed & Graham, 1977; Brown et al., 1983). The generation of DI particles appears to be incidental because repeated coinfection with 883 and 81-5 and serial passage of the coinfection progeny did not yield any DI particles up to passage 15. DI particles are generally generated and maintained after passage at a high m.o.i. Under low m.o.i. conditions, DI particles can be generated, but they may not be maintained or amplified under such conditions.
because DI particles and helper viruses have less chance to enter the same cell (Holland, 1985). In the present experiments, DI particles were generated and maintained at a low m.o.i. The coinfection progeny were passaged without sonication. Thus, it is possible that virus aggregates were present in the virus stocks. DI particles and helper viruses, under such conditions, may be able to enter the same cell at a frequency high enough to maintain the DI particles even though cells were infected at a low m.o.i.

Live divalent or multivalent vaccines are often used to achieve effective immunization against more than one viral serotype. An uneven immune response may occur following immunization with these types of vaccines, as for example in one case with rotaviruses (Perez-Schael et al., 1990). Preferential selection of genome segments has been observed under in vivo conditions (Gombold & Ramig, 1986). Thus, studies on genome segment selection may be of interest in the study of divalent or multivalent live attenuated vaccines of segmented RNA viruses.

References


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