Variation in Homotypic and Heterotypic Interference by Defective Interfering Viruses Derived from Different Strains of Semliki Forest Virus and from Sindbis Virus

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SUMMARY

There was strong interference between various virulent and avirulent strains of Semliki Forest virus (SFV) and their respective defective interfering (DI) viruses but in other combinations interference was variable: it could be equally strong, weak or could not be demonstrated. On passage, this spectrum of interfering activity changed, some combinations showing greater interference than before and others less. Heterotypic interference between DI SFV, DI Sindbis virus and standard viruses was clearly demonstrated although this was strongest between DI SFV preparations and Sindbis standard virus than in the reciprocal combinations. Variation in interference between DI SFVs and different SFV strains was similar in magnitude to that between DI SFVs and Sindbis virus, suggesting that a similar DI RNA sequence is recognized by both viruses.

Defective interfering (DI) viruses are generated by serial undiluted passage of most, if not all, animal viruses (Huang & Baltimore, 1977) and have a genome which is a deleted form of the infectious (standard) virus genome. Since these viruses are defective and are unable to replicate unaided they are propagated by co-infecting cells with DI and standard virus. The progeny from these co-infected cells contain reduced quantities of standard virus, hence the term interference (Holland et al., 1980; Perrault, 1981). Although DI virus-mediated interference is generally thought to occur only with the homologous strain of standard virus, work with the negative-stranded RNA virus vesicular stomatitis virus has shown heterotypic interference where certain types of DI particles of the Indiana serotype (HR DI 0-46) can interfere with standard virus of the New Jersey serotype (Prevec & Kang, 1970; Schnitzlein & Reichmann, 1976). Interference between different strains of type A influenza virus, another negative-stranded RNA virus, has also been observed (Nayak et al., 1981; L. McLain & N. J. Dimmock, unpublished observations). In this report we show that DI Semliki Forest virus (SFV) interferes with different strains of SFV and with Sindbis virus, demonstrating that heterotypic interference can also take place with positive-stranded RNA viruses.

DI virus was obtained from all standard virus stocks by inoculating BHK cells with virus at a multiplicity of infection (m.o.i.) of 50 p.f.u./cell and harvesting after 48 h at 37 °C. This preparation, passage one (p1), contained biologically active DI virus as previously demonstrated for the ts + strain of SFV (Barrett et al., 1981). DI virus p2 was obtained by inoculating DI virus p1 together with a m.o.i. of 50 of standard virus for 24 h at 37 °C in BHK cells. In this way DI virus stocks were derived from the avirulent A774 (Bradish et al., 1971) and virulent L10 (Bradish et al., 1971) strains of SFV (hereafter termed AV and L10 respectively) and Sindbis virus. DI virus of the virulent ts + strain of SFV (Tan et al., 1969) was propagated as described by Barrett et al. (1981) to p19 and p20 and was a descendant of DISFV p8 described by Dimmock & Kennedy (1978). The viruses were all high passage laboratory stocks received from various sources. In our laboratory they received further passages as follows: SFV A774, CEF 6, mouse brain 1; SFV L10, BHK 1; SFV ts +, mouse brain 1, CEF 1; Sindbis virus, BHK 1. Standard virus stocks for each of the four viruses were prepared by inoculating virus at a m.o.i. of 0.1 in
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Table 1. Interference between DI and standard viruses* of three strains of SFV

<table>
<thead>
<tr>
<th>DI virus</th>
<th>AV</th>
<th>L10</th>
<th>ts⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>AV p1</td>
<td>50</td>
<td>≤4</td>
<td>≤4</td>
</tr>
<tr>
<td>L10 p1</td>
<td>13</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>ts⁺ p19</td>
<td>12</td>
<td>50</td>
<td>79</td>
</tr>
</tbody>
</table>

* See text for details of the preparation and origins of DI and standard viruses.

Table 2. Interference by SFV DI strains after further passage* and interference by Sindbis DI virus

<table>
<thead>
<tr>
<th>DI virus</th>
<th>AV</th>
<th>L10</th>
<th>ts⁺</th>
<th>Sindbis</th>
</tr>
</thead>
<tbody>
<tr>
<td>AV p2</td>
<td>16</td>
<td>16</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>L10 p2</td>
<td>6</td>
<td>14</td>
<td>≤4</td>
<td>8</td>
</tr>
<tr>
<td>ts⁺ p20</td>
<td>63</td>
<td>501</td>
<td>501</td>
<td>562</td>
</tr>
<tr>
<td>Sindbis p2</td>
<td>≤4</td>
<td>18</td>
<td>10</td>
<td>100</td>
</tr>
</tbody>
</table>

* DI viruses from Table 1 were passaged once more as described in the text.

BHK cells and harvesting after 18 h incubation at 37 °C. Interference by DI virus was measured using the RNA synthesis inhibition assay (RSIA; Barrett et al., 1981). Briefly, this measures the ability of DI virus to inhibit virus RNA synthesis in cells co-infected with standard virus. The quantity of DI virus present in a DI virus preparation (defective interfering virus units, DIU/ml) is defined as the reciprocal of the dilution of DI virus required to inhibit standard virus RNA synthesis by 50%. Actinomycin D is used throughout the assay to avoid possible effects of interferon.

DI viruses derived from SFV strains AV, L10 and ts⁺ were assayed for interfering activity using all combinations of DI and standard virus. The results (Table 1) show that AV DI virus p1 interfered with the replication of AV standard virus and gave no detectable interference with either of the virulent strains. L10 DI virus p1 interfered with all three standard viruses equally, and ts⁺ DI virus p19 interfered strongly with L10 and ts⁺ standard viruses but weakly with AV standard virus. These results show that DI viruses derived from different strains of SFV do not show the same pattern of interference with the various standard viruses and we conclude that some mechanism is operating which allows discrimination in the interference event.

These observations were extended by investigating the stability of the observed spectrum of SFV interference activity after one further passage and by measuring interference with Sindbis virus, another alphavirus which is serologically distinct but has a similar molecular biology (Table 2). Interference by ts⁺ DI virus was unchanged upon passage and in addition this DI virus demonstrated strong heterotypic interference with Sindbis virus. After the additional passage L10 DI virus no longer interfered equally with the other SFV strains, showing reduced interference against AV standard virus and none with ts⁺ standard virus. DI virus L10 p2 interfered heterotypically with Sindbis standard virus. The second passage of AV DI virus interfered with all four standard viruses equally, in contrast to p1 virus which only interfered in the homologous reaction. Sindbis DI virus p2 gave strong homologous interference, reduced interference with L10 and ts⁺ and none with AV standard virus. Control experiments showed no detectable interference between the various combinations of different standard viruses, indicating that the results obtained above were due to DI virus-mediated interference. From Table 2 we conclude that the specificity of interference between DI viruses of different strains of SFV can vary on passage; for instance, a single passage is sufficient to increase interference by ninefold (ts⁺ DI virus and L10 standard virus). However, in other combinations, passage caused no change in interference titres. Table 2 also clearly demonstrates heterotypic interference between SFV and Sindbis viruses. All SFV DI viruses showed interference with Sindbis virus
but the reciprocal reaction was < 20% of the homologous titre. The similarity in patterns of interference between SFV strains and Sindbis virus suggests that, although these viruses are serologically distinct, the replication mechanism of Sindbis virus differs no more from SFV than the various SFV strains differ from each other. This may be explained by sequence studies which have shown that certain non-coding regions (possibly polymerase recognition sites) of alphaviruses are highly conserved (Ou et al., 1981, 1982a, b, 1983).

Why does interference differ between DI viruses of closely related SFV strains and why does interference vary upon passage? Examination of the sequences of DI RNA of SFV and Sindbis virus (Kääriäinen et al., 1981; Pettersson, 1981; Söderlund et al., 1981; Lehtovaara et al., 1981, 1982; Monroe et al., 1982; Monroe & Schlesinger, 1983) has shown that they contain extensive deletions of the standard virus genome with subsequent rearrangements and duplications. Different clones of SFV DI genomes from the same DI virus preparations have different sequences (Lehtovaara et al., 1982). Therefore, we suggest that the relative abilities of DI viruses to interfere with various standard viruses is dependent upon the sequence of a particular DI RNA, and it may be variation in polymerase recognition site sequences or variation in secondary structure affecting this region which is important. The variation of interfering abilities which we have observed when DI viruses are passaged is consistent with the variations in sequence of DI virus genomes observed under similar conditions by Kääriäinen et al. (1981). As these authors found that the population of DI SFV genomes is heterogeneous, we cannot distinguish between the possibilities that the sequence varies de novo from passage to passage or that the proportions of the various pre-existing genome sequences change on passage. Finally, our results recall those of Weiss & Schlesinger (1981) who found that DI Sindbis virus failed to interfere with the homologous virus (Sindbis virus isolated from persistently infected BHK cells) but did interfere with SFV.

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REFERENCES


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