The Matrix Protein Gene Determines Amantadine-Sensitivity of Influenza Viruses

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SUMMARY

The genetic composition of several recombinant strains, produced by mixed infection with an amantadine-sensitive and an amantadine-resistant influenza virus, have been compared with their response to amantadine. It is concluded that transfer of resistance or sensitivity to amantadine is determined by a single gene, that coding for the matrix protein.

Influenza viruses differ in the sensitivity of their replication to amantadine hydrochloride (Davies et al. 1964; Neumayer et al. 1965) and drug-resistant variants can readily be isolated from normally sensitive strains (Cochran et al. 1965; Oxford et al. 1970). It has also been shown that amantadine-resistance can be transferred genetically (Tuckova et al. 1973) and it has been suggested that this trait, which is independent of either the haemagglutinin or neuraminidase, is controlled by a single virus gene (Appleyard, 1977). To obtain further information on this point, the genome compositions of recombinants formed during mixed infections with an amantadine-resistant and an amantadine-sensitive influenza virus have been determined and compared with their drug-resistance and the results are reported in this communication.

Recombinant viruses of antigenic composition H0N2 were produced in 11-day-old embryonated eggs by mixed infection with A/Singapore/1/57 (H2N2) (amantadine-sensitive) and an amantadine-resistant variant of A/BEL/42 (H0N2) [BEL(AR)] and were tested for their sensitivity to amantadine, as described by Appleyard (1977). The genetic composition of each recombinant was determined by RNA–RNA hybridization analyses (Hay et al. 1977a) in which transcripts produced in recombinant virus-infected chick cells were hybridized with the virion RNAs of the parent viruses, as shown in Fig. 1.

The data in Table 1 show a correlation between the response of the recombinant to amantadine and the parental origin of genome RNA7. No correlation with any other RNA segment was detected. Furthermore, the results obtained for the recombinants J10 and J11 indicate that transfer of RNA 7 alone is sufficient to determine the amantadine-sensitivity of the recombinant.

From comparisons of the genetic compositions of these recombinants and the virus-specified polypeptides synthesized during infection (our unpublished results) we have shown that as for other influenza viruses (Ritchey et al. 1976) genome RNA 7 codes for the matrix protein. Investigations on the mechanism of inhibition of influenza virus replication by amantadine have indicated that it acts on some unknown event following virus infection before transcription of the genome (Hoffman et al. 1965; Kato & Eggers, 1969; Dourmashkin & Tyrrell, 1974; Skehel et al. 1978). The present results suggest that the matrix protein may be involved in this process; however, whether or not amantadine acts by direct interaction with the matrix protein remains to be determined.
**Fig. 1.** Genome analysis of recombinants of A/Singapore/1/57 and BEL(AR). Chick cell monolayers (5 x 10^2 cells/culture) were pre-treated for 30 min with cycloheximide (100 μg/ml), infected with the appropriate virus (10 to 100 p.f.u./cell) in the presence of cycloheximide, washed and then incubated in tris-Gey's medium containing cycloheximide (100 μg/ml). ^3H-uridine (200 μCi culture) was added at 2 h after infection and at 4 h the RNA was extracted from cytoplasmic extracts of these cells and washed, as described previously (Hay et al. 1977b). Each RNA preparation was divided into 2 portions and virion RNA (10 μg) of either (a) A/Singapore/1/57 or (b) BEL(AR) was added. The RNA samples (0.05 ml) were denatured by incubating for 30 min at 45 °C following the addition of 9 vol. of dimethyl sulphoxide. NaCl, tris-HCl, pH 7.5, and EDTA were added to give final concentrations of 3 x 10^{-2}M, 10^{-2}M and 1.5 x 10^{-3}M, respectively, the concentration of dimethyl sulphoxide was reduced to 63 % and the RNA was allowed to anneal at 37 °C for 14 h. The RNA was precipitated with ethanol, washed and redissolved in sodium acetate, pH 4.5 (10^{-2}M, 0.5 ml) containing ZnSO₄ (10^{-3}M) and digested with nuclease S₁ (Sigma Chemical Co.; 2500 units/ml) at 37 °C for 3 h. The RNA was reprecipitated with ethanol, washed and dissolved in 7 M-urea, EDTA (5 x 10^{-3}M), tris-acetate (2 x 10^{-3}M), pH 7.8 and the double-stranded RNAs separated by electrophoresis on 4.5 % polyacrylamide slab gels and detected by fluorography as described by Hay et al. (1977b). The analyses shown are of the two parent viruses A/Singapore/1/57 (A) and BEL(AR) (B) and three recombinant viruses J₅, J₁₀ and J₁₂. Identification of the homologous hybrids is indicated by the numbering in the appropriate columns. In this particular experiment some of the heterologous hybrids, e.g. those of RNAs 5, 7 and 8, were fairly resistant to the nuclease digestion; however, these could be distinguished by their intensities and altered electrophoretic mobilities relative to the homologous hybrids. RNAs 1, 2 and 3 are numbered according to their separation for BEL(AR) since RNAs 1 and 2 of A/Singapore were not resolved.
**Table 1. Genetic composition of amantadine sensitive and resistant recombinants**

<table>
<thead>
<tr>
<th>Recombinant virus</th>
<th>Response to amantadine</th>
<th>RNA segment*</th>
</tr>
</thead>
<tbody>
<tr>
<td>J23</td>
<td>Sensitive</td>
<td>A A A B A A A A</td>
</tr>
<tr>
<td>J2</td>
<td>Sensitive</td>
<td>A A A B A A B</td>
</tr>
<tr>
<td>J20</td>
<td>Sensitive</td>
<td>A A B B A A A A</td>
</tr>
<tr>
<td>J18</td>
<td>Sensitive</td>
<td>A A B B B A B B</td>
</tr>
<tr>
<td>J10</td>
<td>Sensitive</td>
<td>B B B B A A B</td>
</tr>
<tr>
<td>J14</td>
<td>Resistant</td>
<td>B B B B A B B</td>
</tr>
<tr>
<td>J5</td>
<td>Resistant</td>
<td>B B A B B A B</td>
</tr>
<tr>
<td>J6</td>
<td>Resistant</td>
<td>B B A B A B B</td>
</tr>
<tr>
<td>J15</td>
<td>Resistant</td>
<td>A B B B A B B</td>
</tr>
<tr>
<td>J12</td>
<td>Resistant</td>
<td>B A A B A A B</td>
</tr>
<tr>
<td>J11</td>
<td>Resistant</td>
<td>A A A B A A B</td>
</tr>
</tbody>
</table>

* A: derived from A/Singapore/1/57; B: derived from BEL(AR). The results were obtained from two independent analyses carried out as described in Fig. 1.

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**Division of Virology**

National Institute for Medical Research

Mill Hill, London NW7 1AA and

Wellcome Research Laboratories

Langley Court

Beckenham, Kent, U.K.

**REFERENCES**


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