Inhibition of Influenza C Virus Replication by Actinomycin D, α-Amanitin, and UV Irradiation

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Actinomycin D and α-amanitin caused similar reductions in the yields of influenza A/WSN and influenza C/JHB/1/66 viruses in a chicken kidney cell culture system. Irradiation of host cells with UV light before virus infection also produced a similar reduction in yields of the two viruses. The results indicate a close similarity between the replication processes of influenza C and other orthomyxoviruses.

Influenza C virus is immunologically unrelated to influenza A or B virus. However, because of morphological and biochemical similarities, influenza C has been considered as a member of the orthomyxovirus group (12). The similarities include the segmented genome structure (7, 8, 20, 22) and the types of major structural polypeptides (7, 11, 16). On the other hand, influenza C viruses exhibit several characteristic features which distinguish them from other orthomyxoviruses, including a different erythrocyte receptor (9) and a receptor-destroying enzyme which does not liberate sialic acid from well-known substrates (11). The carbohydrate composition of the virion glycoproteins is also distinct for influenza C virions, and sialic acid has been identified in influenza C but not in influenza A virions (16-18). In addition, influenza C differs in biological parameters such as host range and optimal growth temperatures (12), as well as in some aspects of viral envelope morphology (1, 7, 15). Virtually no information has been obtained concerning the molecular events in influenza C virus replication, which has been due in large part to the lack of suitable cell culture systems. Therefore, some uncertainty remains concerning the classification of the virus, and it has been provisionally classified as a separate genus.

In contrast to paramyxoviruses, influenza A and B viruses require nuclear functions of the host cell for their replication (4, 6, 21, 23, 24). Inhibitors such as α-amanitin (α-A) or actinomycin D (AMD) block replication if added at an early stage of the growth cycle, and it has been shown that DNA-dependent RNA polymerase II is required for virus replication (14, 25). Using a chicken kidney (CK) cell culture system that we have recently described (19), we have determined that influenza C virus replication is also dependent on such a host cell function.

To examine the effect of AMD and α-A on virus yield, CK cells infected with influenza C/JHB/1/66 virus (C/1/66) were incubated at 33°C and maintained with different concentrations of drugs in the culture medium from 1 to 24 h postinfection (p.i.). The medium was harvested 24 h p.i., and assayed for infectious virus and hemagglutinin. Table 1 shows that 0.3 μg of AMD per ml resulted in a >100-fold reduction in the yield of infectious virus compared with controls. To suppress the virus yield 100-fold using α-A in the same system, a concentration of 50 μg of the drug per ml was needed. When lower amounts of the drugs were tested, the suppression of virus growth was correspondingly smaller. Concentrations of 0.01 μg of AMD or 1 μg of α-A per ml caused no reduction in virus yields. The background of 2 × 10^4 to 5 × 10^4 PFU/ml at even the highest concentrations of drugs could be due to residual inoculum virus, which may not be completely removed by washes after the adsorption period. The effect of α-A and AMD on the growth of influenza A/WSN in this cell system is included for comparison (Table 1). It can be seen that the sensitivity of the A/WSN virus to the two inhibitors was approximately parallel to that shown by influenza C/1/66 virus.

The effect of AMD on the yield of influenza C/1/66 in CK cells was also tested when the drug was added to the culture medium at different times p.i. Table 2 shows that the addition of AMD (5 μg/ml) at 4 h p.i. completely suppressed the replication of influenza C virus. However, the addition of AMD in the same concentration at 8 or 10 h p.i. had little effect. A similar experiment with influenza A/WSN virus is in-
Table 1. Inhibition of influenza A and C viruses by AMD and α-A

<table>
<thead>
<tr>
<th>Conc of drug (µg/ml)</th>
<th>Virus yield*</th>
<th>C/1/66</th>
<th>WSN</th>
<th>Control</th>
<th>AMD</th>
<th>α-A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HAU/m</td>
<td>PFU/ml</td>
<td>(HAU/m</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>256</td>
<td>8 × 10⁷</td>
<td>1,024</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>256</td>
<td>6.6 × 10⁷</td>
<td>1,024</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>16</td>
<td>4 × 10⁶</td>
<td>128</td>
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<td></td>
</tr>
<tr>
<td>0.3</td>
<td>4</td>
<td>2.3 × 10⁵</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1.0</td>
<td>4</td>
<td>1.8 × 10⁴</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>4</td>
<td>1.6 × 10⁴</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* CK cells were infected with influenza C/1/66 or influenza A/WSN virus at a multiplicity of 20 PFU/cell. After a 1-h adsorption period at 33°C, drug-containing medium was added, and cells were further incubated at 33°C. Virus yields in cell culture medium were assayed at 24 h p.i.

Plaque assays were carried out on CK cell monolayers (19), and hemagglutinin titrations were performed in microtiter plates in a final volume of 0.05 ml, using a 1% suspension of chicken erythrocytes as indicator. The values are means of at least two experiments. HAU, Hemagglutination units.

Table 2. Effect of time of addition of AMD on the yield of influenza virus

<table>
<thead>
<tr>
<th>Time of addition of AMD (h p.i.)</th>
<th>Virus yield*</th>
<th>C/1/66</th>
<th>A/WSN</th>
<th>Control</th>
<th>AMD</th>
<th>α-A</th>
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<tbody>
<tr>
<td></td>
<td>PFU/ml</td>
<td>HAU/m</td>
<td>(HAU/m</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.3 × 10⁴</td>
<td>&lt;4</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.1 × 10⁴</td>
<td>&lt;4</td>
<td>128</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2 × 10⁴</td>
<td>&lt;4</td>
<td>512</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1.6 × 10⁴</td>
<td>8</td>
<td>1,024</td>
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</tr>
<tr>
<td>6</td>
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<td>16</td>
<td>1,024</td>
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</tr>
<tr>
<td>8</td>
<td>5 × 10⁵</td>
<td>128</td>
<td>NT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>7 × 10⁵</td>
<td>256</td>
<td>NT</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>1.2 × 10⁴</td>
<td>256</td>
<td>1,024</td>
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</tr>
</tbody>
</table>

* CK cells were infected with influenza C/1/66 virus or influenza A/WSN at a multiplicity of 20 PFU/cell. Drugs were present in the culture medium from the time indicated to 24 h, and virus yields were assayed at 24 h p.i.

PFU and hemagglutinin titers are mean values of at least two experiments. HAU, Hemagglutination units; NT, not tested.

C. Figure 1 shows the effect of AMD on viral polypeptide synthesis. Compared with infected cells without inhibitors (lane 2), 5 µg of AMD per ml produced only a slight decrease in viral protein synthesis when added at 5.5 h p.i. (lane 3). Three major structural polypeptides, gp88, NP, and M, were resolved, as described previously (19). Addition of AMD at earlier times completely suppressed the synthesis of these viral polypeptides (lanes 4 and 5). A nonstruc-
tural viral polypeptide (NS) is tentatively identified in front of the M protein in lanes 2 and 3; its synthesis was also inhibited when AMD was added at early times p.i. (lanes 4 and 5). Thus, the drug-sensitive phase in the replication cycle is paralleled by the time during which viral protein synthesis is sensitive to inhibitors.

UV light pretreatment of the host cell is also very effective in suppressing influenza A virus replication (4). Confluent monolayers of CK cells were irradiated for different times and subsequently infected with 20 PFU of influenza C/1/66 or influenza A/WSN per cell. After a 1-h adsorption period, unadsorbed virus was removed and medium was added. The virus yield was tested by hemagglutinin titration of the culture medium at 24 h p.i. Figure 2 shows that influenza C and influenza A virus replication were similar in sensitivity to UV light pretreatment of the host cell. In contrast, when we compared the sensitivity of Newcastle disease virus replication to UV light pretreatment, using the same batch of CK cells, we observed a >10-fold-lower sensitivity than that found with influenza C (data not shown).

The present results indicate that influenza C viruses are as sensitive to AMD and α-A, as are influenza A and B. Since the replication cycle of influenza C viruses is slower than that of influenza A/WSN (19), it is not surprising that the inhibitor-sensitive phase of its replication cycle is also of longer duration. Previously it has been observed that influenza C antigens occur in nuclei of infected cells (2), a finding which also resembles results with other orthomyxoviruses.

Recent evidence indicates that RNA molecules synthesized by the host cell nuclear RNA polymerase II are required for influenza A virus transcription in vivo, and their synthesis is probably the function which is inhibited by α-A (6, 21). Further, the 5' cap of the primer RNA is transferred to the viral mRNA during the transcription process. Since RNA splicing also occurs with an in vitro transcription system, using the virion RNA polymerase and globin mRNA as the donor of the cap structure (21), it is likely that influenza A virion proteins possess the enzymatic activities involved in this splicing event. If the host cell function is of a similar nature in influenza C and influenza A virus-infected cells, it would follow that influenza C virions also possess enzymes which splice host cell RNA primers to viral transcripts.

The present results support the conclusion that influenza C virus resembles other orthomyxoviruses in the molecular events involved in virus replication. However, much more information is needed before the molecular biology of influenza C virus is thoroughly understood.

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LITERATURE CITED


