Interaction Between Cytomegalovirus and Newcastle Disease Virus as Mediated by Intrinsic Interference

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Cytomegalovirus (CMV) was demonstrated to induce intrinsic interference to Newcastle disease virus (NDV) in human fibroblast cells under noncytopathic conditions. This interference is unique in that (i) cytomegalovirus is the first DNA virus demonstrated to have this property and (ii) the state of interference was transient and progressively lost as the condition of the cells changed with the development of cytopathic effect. These observations are consistent with the view that the newly formed protein responsible for interference with NDV has a limited half-life and is no longer made when cytopathic conditions are produced by CMV.

MATERIALS AND METHODS

Cell lines. Human fibroblast cells (WI38; BBL) were maintained as confluent monolayers in screw-cap tubes on Eagle minimal essential medium (MEM) supplemented with 5% fetal calf serum, streptomycin (100 μg/ml), and penicillin (100 units/ml).

Viruses. Human cytomegalovirus AD169 was obtained from Wallace P. Rowe, National Institutes of Health, and titered 10^3.4 tissue culture infective doses (TCID_{so}) per ml in WI38 cells. Newcastle disease virus California strain was prepared in chick allantoic fluid and had a titer of 10^8 plaque-forming particles (PFP) per ml. Sindbis virus was propagated in primary chick embryo monolayers and had a titer of 10^8 PFP per ml.

Hemadsorption-negative plaque test. Confluent monolayers of WI38 cells in screw-cap tubes were inoculated with 0.3 ml of serial 10-fold dilutions of a stock of CMV which had a titer of 10^3.4 TCID_{so} per ml. After 1 hr of viral adsorption at 37 C, the monolayers were covered with 2 ml of MEM with 5% fetal calf serum. The tubes were kept at 37 C for 21 days, and the maintenance medium was changed every 3 days. During this period, the cells were observed daily for the development of CPE, and at various intervals of 1 to 6 days, replicate monolayers were challenged with NDV at a multiplicity of about 10 PFP per cell. The hemadsorption-negative plaque test was performed as described by Marcus and Carver (1).

RESULTS

Table 1 compares the sequential development of intrinsic interference and CPE in WI38 cells infected with 10 to 1,000 TCID_{so} of CMV. The cells infected with CMV resisted superinfection...
TABLE 1. Comparison of the sequential development of intrinsic interference and CPE in CMV-infected cells

<table>
<thead>
<tr>
<th>Development of</th>
<th>Viral inoculum</th>
<th>Time after inoculation (days)</th>
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<tr>
<td></td>
<td></td>
<td>Onset</td>
<td>Maximum</td>
<td>Cessation</td>
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<tr>
<td>HAD—a</td>
<td>1,000 TCID&lt;sub&gt;50&lt;/sub&gt;</td>
<td>1</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>CPE</td>
<td>100 TCID&lt;sub&gt;50&lt;/sub&gt;</td>
<td>5</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>CPE</td>
<td>10 TCID&lt;sub&gt;50&lt;/sub&gt;</td>
<td>7</td>
<td>10</td>
<td>21</td>
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* Hemadsorption negative.

with NDV and therefore did not demonstrate hemadsorption with bovine erythrocytes. The extent of intrinsic interference was measured by the percentage of the monolayer demonstrating hemadsorption-negative cells. This was graded increasingly from 0 (0%) to 4+ (100%). Appropriate cell controls that had not been infected with CMV were tested simultaneously and were continually found to be 100% hemadsorption positive after NDV infection.

The times at which intrinsic interference developed was dependent on initial viral inocula, developing earlier with higher input. The initial development of this state did not appear to have the focal nature observed with CPE, but was more diffuse, demonstrating streaks of hemadsorption-negative cells interspersed with hemadsorption-positive cells. The percentage of cells refractory to NDV progressively increased until the entire monolayer soon became hemadsorption negative. This progression was more evident as the amount of virus used in the initial cell infection was decreased, thus delaying the onset of intrinsic interference and the subsequent appearance of CPE. This finding is consistent with the view that the gradual development of interference represented spread of infectious virus. Intrinsic interference preceded the development of CPE in all instances. However, despite this difference in the sequence of development, these two conditions eventually became approximately equal in extent.

The state of intrinsic interference was transient. As CPE occurred, cells became hemadsorption positive after NDV challenge. Thus, the cells reverted to a state of susceptibility to NDV infections that contrasted to the refractoriness to NDV infection exhibited earlier by replicate cultures. This susceptibility was confirmed by demonstrating that cells in which CMV-induced CPE had occurred and which were now hemadsorption positive produced the same quantity of infectious NDV as the cell controls. This hemadsorption was not attributable to CMV, since CMV-infected cells demonstrating CPE did not show any hemadsorption. As CPE progressed to involve the entire cell sheet, intrinsic interference was entirely lost irrespective of the initial vira

![Fig. 1. Development and loss of intrinsic interference in CMV-infected cells. WI38 cells were infected with 100 TCID<sub>50</sub> of CMV, and at varying intervals challenged with NDV (100 PFP/cell). (A) At 10 days, interference of NDV was demonstrated by the total absence of hemadsorption. (B) At 21 days, when CPE was 4+, the interference was lost and the monolayer demonstrated hemadsorption similar to the cell control. × 200.](http://jvi.asm.org/)

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inoculum. The development of resistance to NDV replication and the subsequent development of susceptibility to NDV infection are illustrated in Fig. 1.

The interference induced by CMV was specific for NDV, since cells refractory to NDV demonstrated CPE when challenged with a high multiplicity of Sindbis virus. The unimpeaded adsorption of NDV to these cells suggests that CMV acted at a stage after adsorption.

The capacities of 100 TCID<sub>50</sub> of CMV to produce CPE and to induce interference to NDV were neutralized by a 1:32 dilution of serum containing neutralizing antibodies against CMV (AD169). These capacities were also inactivated by exposure to ultraviolet (UV) light.

DISCUSSION

The data presented indicate that CMV can induce intrinsic interference to NDV. The characteristics demonstrated that are consistent with this type of viral interference were (i) a rate of induction that was a function of the multiplicity of the inducing virus, (ii) a loss of capacity to induce this interference upon exposure of the virus to UV irradiation, which suggests the requirement of a functional viral genome (2), and (iii) a cellular state of resistance to NDV existing simultaneously with a state of susceptibility to a virus relatively sensitive to the action of interferon.

The intrinsic interference induced by CMV is unique in that (i) CMV is the first DNA virus demonstrated to have this property and (ii) the state of interference is transient and is progressively lost as the condition of the cells changes with the development of CPE. These observations suggest that the newly formed protein responsible for interference with NDV (2) has a limited half life and is no longer made when cytopathic conditions are produced by CMV. Under cytopathic conditions, cellular synthesis of proteins coded for by viral genomes seems to be selective, since the infected cell can still produce NDV hemagglutinin as demonstrated by the hemadsorption of bovine erythrocytes.

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LITERATURE CITED