Suppression of Canine Parvovirus Growth in CRFK Cells by Canine Distemper Virus

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ABSTRACT. The growth of canine parvovirus (CPV) in Crandell feline kidney cells was suppressed when canine distemper virus (CDV) was simultaneously inoculated. The both virus antigens were found in the same cells. The synthesis of capsid protein antigen of CPV decreased in the co-inoculated cells. The growth of CDV was not affected by co-inoculation with CPV. These results suggest that the CDV showed suppressive effects on the CPV growth in cells when they are inoculated simultaneously.—KEY WORDS: canine distemper virus, canine parvovirus, suppression.

Since the titers of CPV in cell cultures corresponded to those obtained by hemagglutination (HA) test [4], the infectivity of CPV was titrated by HA test at pH 6.0 as described previously [8]. Briefly the virus was diluted in borate-buffered saline with 0.2% bovine serum albumin at pH 9.0, and porcine erythrocytes were diluted in sodium phosphate-NaCl buffer. The test was performed in plastic microplates with 96-wells and V-shaped bottoms. Serial two-fold dilution of test samples were prepared in 50 μl, mixed with 50 μl of 0.5% porcine erythrocyte suspension and then incubated at 4°C overnight. The HA titer was expressed as the reciprocal of the highest antigen dilution showing complete HA.

The titration of CDV was performed using microplates with 96-wells and flat-bottoms. Fifty μl of a Vero cell suspension (2 × 10³ cells/ml) was seeded to each well and cultured for 24 hr. After the supernatant in the wells was discarded, 50 μl of 10-fold dilution of CDV was added to each well. After incubation for 7 days at 37°C, the cultures were examined for CPE under the microscope, and the titer was expressed as TCID₅₀/50 μl.

CRFK cells incubated at 37°C for 24 hr were washed with serum-free media and used for virus inoculation. The cells inoculated with CPV were incubated for 1 hr at 37°C. After being washed twice with serum-free media, the cells were incubated with serum-free MEM. At 0, 12 and 24 hr post-inoculation (hpi) of CPV, the cells were incubated with CDV and incubated for 1 hr at 37°C in the same way as described above. Aliquots of culture supernatants were collected for HA test at the indicated times, and the remaining supernatants and cells were frozen-thawed and sonicated. After centrifugation, the supernatants were titrated for CDV.

For indirect immunofluorescence studies, the cells inoculated with viruses were fixed in acetone at −20°C for 30 min, and reacted with the anti-CPV rabbit serum or a monoclonal antibody (Yal) against capsid protein of CPV, both of which were kindly provided by National Veterinary Assay Laboratory, Japan.

For immunochemistry, the avidin-biotin-alkaline phosphatase complex (ABC) method was employed on CDV-and/or CPV-inoculated CRFK cells. Briefly, CRFK cells on coverslips were fixed in methanol and incubated with 1:100 diluted anti-measles virus rabbit serum which cross-reacts to CDV, and with anti-CPV monoclonal antibody (Yal) at 37°C for 1 hr. This anti-CPV antibody
recognizes the epitope on capsid protein of CPV. Subsequently the cells were incubated with biotinylated anti-rabbit serum and peroxidase conjugated anti-mouse serum at 37°C for 30 min, and subjected for Vectastain ABC-AP method (Vector Laboratories Inc., CA, U.S.A.) for detection of the CDV antigen. After the ABC staining, the cells were reacted with substrate solution (0.5 mg/ml 3, 3′ diaminobenzidine-tetrahydrochloride and 0.02% H₂O₂ in Tris buffer) for detection of the CPV antigen.

The individual growth curves of CPV and CDV are shown in Fig. 1. Both CPV and CDV replicated well in CRFK cells. Infectivities of the two viruses increased gradually until 72 hpi, and the titers sustained the same levels.

Effects of co-inoculation with CDV on the growth of CPV in CRFK cells were examined at 48 and 72 hpi. As shown in Fig. 2, the growth of CPV apparently decreased in the cells co-infected with CDV, especially one at 72 hpi was 1/8 as that of CPV alone. This suppressive effect on CPV growth was more significantly observed when CDV was co-inoculated with high multiplicity of infection (m.o.i.) than with lower m.o.i.

Productivity of CPV-antigens was compared using monoclonal antibody against CPV-capsid protein in CRFK cells infected CPV alone and those infected with both CPV and CDV. Both intensity and distribution of the virus-specific fluorescence were seemed to be decreased in the cells co-infected with CPV and CDV compared to those infected with CPV alone (data not shown).

Subsequently, the effect of co-infection with CDV on the growth of CPV was examined by CDV inoculation at the same time or at 6 h before or after CPV inoculation. The growth of CPV was most markedly suppressed when CDV was inoculated simultaneously (Fig. 3b). The growth of CDV co-inoculated with CPV was not suppressed (Fig. 3a). In the cells previously inoculated with CPV, the growth of CDV was found to be not affected by CPV (Table 1). The results suggest that co-inoculation with CPV did not affect on CDV growth.

In the co-infected cells, CPV antigens as well as CDV antigens were detected in the syncytiot. Both antigens were

![Fig. 1. Growth curves of CPV and CDV in CRFK cells. The titers of CDV (open rectangle) were expressed as log₁₀ TCID₅₀/50 μL and the titers of CPV (solid triangle) as log₂ HAU/50 μL.](image)

![Fig. 2. Growth of CPV in CRFK cells co-inoculated with CDV at 48 and 72 hpi. CRFK cells were inoculated with CPV alone (solid column) and CRFK cells were co-inoculated with CPV and CDV (shaded column).](image)

![Fig. 3. Growth of CDV in CRFK cells co-inoculated with CPV.](image)

**Table 1. CDV growth in CRFK cells after CPV**

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Virus</th>
<th>CDV alone</th>
<th>CDV after</th>
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<tbody>
<tr>
<td>1</td>
<td>CPV alone</td>
<td>2.50</td>
<td>2.5</td>
</tr>
<tr>
<td>2</td>
<td>CPV alone</td>
<td>3.0</td>
<td>2.7</td>
</tr>
</tbody>
</table>

*The titers were expressed as log₁₀ TCID₅₀/50 μL.*
SUPPRESSION OF CPV GROWTH BY CDV

detected by immunochemistry with anti-CPV monoclonal antibody and anti-measles rabbit serum in the same cells. It was shown that CDV antigens were detected in the cytoplasms and nuclei and CPV antigens were detected in the nuclei (data not shown). The localization of virus antigens of these viruses were in agreement with previous studies [1, 4].

Immunosuppression by CDV is well known [5]. The interaction between CDV and other virus including CPV, however, has not been intensively examined to our knowledge.

In the present study, m.o.i.-dependent suppressive effect of CDV on CPV growth suggests that CDV growth can associate with suppressive effect. In the present study, the growth of CPV was suggested to be suppressed by CDV in CRFK cells. On the other hand, CPV did not suppress CDV growth. Since both CPV and CDV were shown to infect the same cells, CDV may affect the absorption of CPV to the cells or its replication in the cells. A study for the receptor competition between CPV and CDV is now underway.

The results of immunofluorescence studies may account for suppression of CPV protein synthesis in the co-inoculated cells, indicating that the suppressive effect of CDV on the growth of CPV could occur in the cells. Further studies on the mechanism(s) of suppressive effect on the growth of CPV by CDV in cells are required.

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REFERENCES