Large and Small Plaque Variants of Canine Adenovirus 2 Isolate

Yukinobu TOHYA, Masayuki AZETAKA, Eiji TAKAHASHI, and Shin-ichiro KONISHI

Department of Veterinary Microbiology, Faculty of Agriculture, the University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan

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Canine adenovirus 2 (CAV-2) is one of the important agents of canine respiratory diseases [1, 12]. The virus has been isolated in Canada [5], the United States [3], the United Kingdom [4] and Japan [9]. Several strains of CAV-2 have been examined for their properties in comparison with CAV-1 (infectious canine hepatitis virus) [2, 5, 6, 8, 9], but little is known about plaque morphology of CAV-2 [7].

We isolated a strain of CAV (A2 strain) from the lung of a dog which died of pneumonia. This isolate was serologically identified as CAV-2. This report describes the plaque formation by the A2 strain in the Madin and Darby canine kidney (MDCK) cells and the preliminary characterization of large and small plaque variants of the isolate.

The A2 strain was passaged in dog kidney cell (DKC) culture grown at 37°C in Eagle's minimum essential medium (MEM) (Nissui, Japan) supplemented with 10% calf serum, 10% tryptose phosphate broth (Difco, USA), and antibiotics. For virus propagation, 2% newborn calf serum was used for the maintenance medium (MM).

For the plaque assay, MDCK cell monolayers grown in 60 mm Petri dishes at 37°C in the same medium described above were infected with 1.0 ml volumes of virus diluted in MEM. Virus was adsorbed at 37°C for 2 hr, and then monolayers were overlaid with 5 ml of 1% agarose (Nakarai, Japan) in MEM containing 2% fetal calf serum and incubated at 37°C in humidified air containing 5% CO₂. Two and a half ml of overlay medium was added onto the cultures on the 5th and 10th days after infection. The cultures were stained with neutral red 14 days after infection and were photographed on the next day.

Plaques produced by the A2 strain were a mixture of large and small plaques, and the plaque boundaries were diffuse (Fig. 1b). The plaque diameters were approximately 4 mm for the large ones and less than 1 mm for the small

ones, and medium-sized plaques were also observed.

To examine the nature of large and small plaque variants, both were plaque-cloned 3 times in MDCK cells and designated as Lp and Sp, respectively (Figs. 1c and 1d). They were not differentiated from each other by the plaque reduction neutralization test or by the hemagglutination inhibition test (data not shown).

In order to determine the growth curves of the variants, DKC monolayers were infected with Lp or Sp strains at a multiplicity of 10 plaque-forming-units (PFU)/cell. After adsorption of virus at 37°C for 60 min, the monolayers were washed with Dulbecco's phosphate-buffered saline and MM was added. The virus-infected cells and culture fluids were collected up to 60 hr at varying intervals. The virus-infected cells were frozen and thawed three times to release intracellular virus. The intracellular and extracellular fractions (culture fluids) were tested for infectivity by the plaque assay.

The growth curves of Lp and Sp viruses are shown in Fig. 2. The growth characteristics of

Fig. 1. Plaques produced by CAV-2 isolate and its large and small plaque variants. (a) MDCK cell control, (b) CAV-2 isolate, A2 strain, (c) large plaque variant, Lp, (d) small plaque variant, Sp.

Fig. 2. Growth of Lp and Sp in dog kidney cell cultures. ●—●, intracellular virus; ○—○, extracellular virus.

intracellular virus of Lp and Sp were similar. The infectivity began to rise 12 hr after infection and reached a plateau 20 hr after infection. On the other hand, the infectivity of the extracellular viruses of Lp and Sp was detected at 20 hr. The infectivity of Lp increased to $10^{7.0}$ PFU/ml 36 hr after infection, while that of Sp was $10^{3.3}$ PFU/ml. The infectivities of Lp were at least 1000 times higher than those of Sp after 40 hr. These results indicate that the amount of Lp and Sp in the infected cells are similar, but the release rates of these viruses from the cells into the culture medium are different from each other.

It was reported that the spread of human adenovirus type 5 (Ad 5) in HeLa cells from cell to cell was minimal and that the spread by released virus via the medium was much more important [10]. Williams [11] showed that $MgCl_2$ enhances the number and size of plaques formed by Ad 5 in HeLa cells and that the effect was due to an increase in the rate of virus release from infected cells. Although we did not examine the

effect of the divalent cations on enhancement of CAV-2 plaque formation in MDCK cells, the differences in plaque size of naturally occurring plaque variants might be due to a more extensive spread of the Lp virus from cell to cell in agar-overlaid cultures as a result of a more rapid release of the virus.

The large and small plaque variants were found in CAV-2 isolate during the early passages in DKC cultures. Plaque size variations such as the A2 strain were also observed in both the CAV-2 type strain of Toronto A26/61 and the vaccine strain of Manhattan (data not shown). These observations suggest that CAV-2 found in nature may be a mixture of large and small plaque type variants or that these variants may have arisen during early passages of the virus. In spite of these possibilities, it will be of great significance to further examine some other biological characteristics, especially the virulence of these two variants.

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要 約

イヌアデノウイルス2型分離株の大・小ブラック変異株（短報）：遠矢幸伸・畔高政行・高橋英司・小西信一郎（東京大学農学部獣医微生物学教室）——イヌアデノウイルス2型分離株には，MDCK細胞において大・小のブラックを形成する変異株が混在していた．両変異株は血清学的に区別されなかったが，犬腎培養細胞における増殖曲線を比較したところ，感染後40時間以上経過しても大ブラック変異株の細胞外ウイルス量は，小ブラック変異株のその1000倍以上であり，ブラックサイズと細胞外ウイルス量の関係が示唆された．