Growth of Rat Sialodacryoadenitis Viruses in LBC Cell Culture

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(Received 19 September 1985/Accepted 13 November 1985)

ABSTRACT. Four strains of rat sialodacryoadenitis virus isolated in Japan were shown to readily propagate in the LBC cell culture showing marked cytopathic effect with syncytia formation. Viral antigen was demonstrated in the syncytia produced by 4 isolates by indirect immunofluorescence. The LBC cell culture might provide a useful assay system for sialodacryoadenitis virus being difficult to propagate using previously described cell system.—**KEY WORDS:** coronavirus, LBC cell culture, sialodacryoadenitis.

Jpn. J. Vet. Sci. 48(1): 193–195, 1986

Rat sialodacryoadenitis virus (SDAV), a coronavirus, was first isolated from the affected salivary glands [1, 9]. SDAV is known to share common antigen with mouse hepatitis virus (MHV) and Parker's rat coronavirus (RCV) [8]. Routine serosurvey demonstrated rat antibody to MHV at high incidence [2, 6]. The outbreaks of sialodacryoadenitis have been reported in many rat breeding colonies and laboratories [5, 7, 10].

Kojima *et al.* [5] isolated SDAV in Japan using a primary rat kidney (PRK) cell culture and newborn mice, and Yamaguchi *et al.* [11] also isolated the virus from diseased rats using suckling mice. However, the growth of SDAV and RCV *in vitro* as well as *in vivo* was of no satisfactory level [1, 5, 8, 11].

Recently, we reported a successful propagation of RCV and SDAV strain 681 in rat cell line LBC culture, established from mammary tumor of Lewis rat, with marked cytopathic effect (CPE) and the yield of high-titered virus [3, 4]. The present note is to see growth of 4 SDAV isolates, strains 930-10, M [5], TG and KA [11] on the LBC cell culture.

SDAV strains 930-10 and M were kindly supplied by Dr. A. Kojima, Tanabe Seiyaku, Toda, and TG and KA were by Dr. R.

Yamaguchi, Institute of Medical Science, University of Tokyo. The LBC cells were grown at 37°C in Eagle's minimum essential medium (MEM) containing 10% fetal calf serum (FCS) and kanamycin (0.06 mg/ml). The FCS concentration was reduced to 5% for harvesting the virus and maintaining the cells. The cell monolayers prepared in 50-ml culture bottles were washed once with MEM and inoculated with 0.2 ml of virus material. After virus adsorption at 37°C for 60 min, the inoculated cultures were given 5 ml of maintenance medium and incubated at 37°C.

In LBC cell monolayers inoculated with 4 strains, cell rounding or syncytium formation was first detected within 48 hr postinoculation (p.i.), and marked CPE with syncytia was produced at 72 hr p.i. as seen previously on PRK cell cultures infected with 930-10 and M strains [4] as well as LBC cells infected with RCV and SDAV strain 681 [3, 4]. Passages of 4 isolates using LBC cell monolayers were carried out at 3-day-intervals with undiluted infected culture fluid. More remarkable CPE developed within 48 hr p.i. after 6 to 8 passages, as shown in Fig. 1. The infected culture fluid was assayed for infectivity titers using LBC cells grown in 13×100 mm test tubes, showing a 50% tissue

Fig. 1. Cytopathic effect of sialodacryoadenitis virus of rat in LBC cell culture (hematoxylin and eosin stained). Cells uninfected (A), and 48 hr after infection with the virus; 930-10 (B), M (C), TG(D) and KA (E) strains.

Table 1. Infectivity of culture fluid

Strain ^{a)}	Infectivity ^{b)}
930-10	7.0
M	6.8
TG	7.0
KA	6.5

a) At the 10th passage level in LBC cells.

b) log₁₀ TCID₅₀/0.2 ml, assayed at 48 hr after inoculation.

culture infective dose (TCID₅₀) of 10⁶ or more/0.2 ml at the 10th passage level. The maximum infectivity titers in PRK cell cultures were 10^{3.8} TCID₅₀/0.1 ml of 681 strain by Bhatt *et al.* [1], and 10^{5.7} and 10^{4.7} TCID₅₀/0.1 ml of 930-10 and 681 strains, respectively, by Kojima *et al.* [5]. Infectivity titers obtained in LBC cells were higher than those in PRK cell cultures [1, 4, 5].

The LBC cells infected with the SDAV strains were found to contain viral specific antigen by indirect immunofluorescence [3, 4] using rat antiserum against SDAV strain 681, kindly supplied by Prof. A. M. Jonas, The Jackson Laboratories, and Yale University, U.S.A., and fluorescein isothiocyanate conjugated anti-rat IgG rabbit serum (Miles Biochemicals U.S.A.). The cytoplasm of syncytia showed strong immunofluorescence, as shown in Fig. 2.

The LBC cells might provide a very useful tool for assay of rat coronaviruses and a satisfactory source of the viruses for experimental works.

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Fig. 2. Immunofluorescence of LBC cells 24 hr after inoculation with TG strain.

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要 約

LBC 細胞でのラット唾液腺涙腺炎ウイルスの増殖 (短報): 平野紀夫・鈴木義久・小野勝彦・村上敏明・藤原公策¹⁾ (岩手大学農学部家畜微生物学教室, ¹⁾ 東京大学農学部家畜病理学教室) ——ラット唾液腺涙腺炎ウイルスの分離株 4 株は著明な細胞変性効果を示し LBC 細胞で増殖した。感染細胞細胞質内にはウイルス抗原が検出され、従来の細胞系にくらべ、LBC 細胞では高いウイルス産生が得られた。