

Induction of SMON-Type Virus from Avian Infectious Laryngotracheitis Virus (39216)

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The biological properties of SMON virus, a new human herpesvirus, isolated from human cases of subacute myelo-optico-neuropathy (SMON) were reported in previous papers (1, 2). Especially, the relationship between SMON virus and avian infectious laryngotracheitis (ILT) virus was demonstrated by both neutralization tests and isolation of SMON-type virus from ILT virus (3). These results suggested that SMON virus may be a variant of ILT virus. In the present report, we examined the factors affecting the induction of SMON-type virus from ILT virus by use of Y-1000 strain which did not produce SMON-type virus under ordinary culture-conditions for ILT virus.

Materials and methods. *ILT virus used.* Y-1000 strain propagated in chorioallantoic membrane (CAM) was described in a previous paper (3). For the experiment, Y-1000 strain was plaque-purified three times in primary chicken kidney (CHK) cell cultures, and Y-1000 strain purified and grown in CHK cells was used as the parental ILT virus.

Cell cultures. BAT-6 cells were derived as a clonal line from hamster tumor induced by type 3 bovine adenovirus (1). CHK cell cultures were prepared as described previously (3).

Virus titration. TCD₅₀ titers of SMON-type virus were determined in BAT-6 tube cultures. ILT virus was titrated by plaque formation on CHK bottle cultures (3).

Cultivation in CAM of embryonic hen's eggs. Virus in the amount of 0.1 ml was inoculated into CAM of 8- to 12-day-old eggs, and incubated at 36° for 3 to 7 days. Infected CAM was made into 20% emulsion with yolk saline, which consisted of Dulbecco's phosphate-buffered saline of pH 7.2 containing 0.1% egg yolk and antibiotics. Cellular debris was removed by centrifugation at 5000 rpm for 30 min.

Growth curve experiment in CHK cell cul-

tures. The experimental method was essentially similar to that described previously (4). Inoculated bottle cultures were held at 37° for 1 hr to allow adsorption. At the end of the adsorption period, excess inocula were removed, and bottles were washed three times with cold Eagle's minimum essential medium (MEM), then 4 ml of maintenance medium was added. Cultures were incubated at 32°, 37°, and 40° respectively. At each time interval, cultures were removed and total virus yield per milliliter was assayed.

Pathogenicity test in C57BL/6 mice. C57BL/6 strain newborn mice were used in the same manner as described previously (3, 5). Virus materials diluted to 10⁻¹ or 10⁻² were inoculated intracerebrally at birth.

Mutagenesis. Parental Y-1000 strain was mutagenized by either 5-bromodeoxyuridine (BrdU) or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG). Concentrations of 1 and 10 ng of BrdU or NG/ml were prepared in MEM containing 2% fetal calf serum and 0.1% yeast extract. CHK cells infected with ILT virus were treated by BrdU according to the method of Sambrook *et al.* (6), while treatment with NG was done according to Tegtmeyer *et al.* (7). Parental virus was inoculated onto CHK cells. After adsorption at 37° for 1 hr the cultures were washed three times, then 4 ml of medium containing BrdU or NG was added to cultures. The cultures were incubated at 37 or 40° for 3 days. Harvested cultures were frozen and thawed, and cell debris was removed by low-speed centrifugation. As mutagenized stocks, supernatants were titrated in BAT-6 tube cultures or assayed by plaque on CHK cells.

Results. *Effect of different-day-old eggs on the induction of SMON-type virus in CAM.* It was found that SMON-type virus, which produced weak CPE in BAT-6 cells and also induced pathogenicity in newborn C57BL/6 mice, appeared when different-

day-old eggs were used (Table I), viz., when CAM of younger than 10-day-old eggs were used, SMON-type virus could not be isolated in BAT-6 cells from the infected CAM suspensions. On the other hand, when Y-1000 strain was cultivated in CAM of 12-day-old eggs, SMON-type virus could be induced. The growth of the parental virus was depressed in 12-day-old eggs. The isolated SMON-type virus was neutralized by SMON virus immune serum and showed the pathogenicity in newborn C57BL/6 mice.

Effect of different incubation temperatures on the induction of SMON-type virus in CHK cells. Figure 1 shows the growth curves of Y-1000 strain at different temperatures in CHK cell cultures. The time required to reach the maximal production of virus was about 48, 72, and 144 hr at 40, 37, and 32°, respectively. Appearance of SMON-type virus was detected only from the culture cultivated at 32° for 7 days or more (Table II). Cultivation at 40 or 37° did not succeed to induce SMON-type virus constantly. At 32° the growth rate of the parental virus became significantly slow.

Induction of SMON-type virus by either BrdU or NG. Y-1000 strain was mutagenized by BrdU or NG for induction of SMON-type virus. The multiplication of virus in the presence of mutagen, BrdU, or NG, resulted in decreased infectivity and proportional increase of mutants. Y-1000 strain mutagenized-stocks prepared at temperatures of 37 and 40° showed CPE in BAT-6 cells and SMON-type virus was induced at 37 and 40° by the action of mutagen (Table III). On the other hand, non-

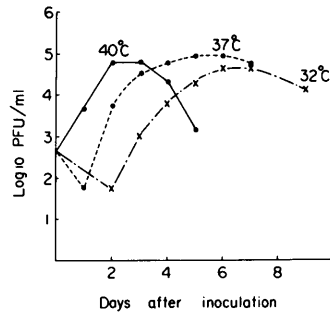


Fig. 1. Growth curves of ILT virus in CHK cell cultures at different temperatures.

TABLE II. EFFECT OF INCUBATION TEMPERATURE ON INDUCTION OF SMON-TYPE VIRUS FROM ILT VIRUS Y-1000 STRAIN IN CHK CELLS.

Temp. of incubation	Days	Log ₁₀ titer per ml		Pathogenicity to C57BL/6 mice
		PFU CHK cells	TCD ₅₀ BAT-6 cells	
32°C	3	3.0	<1.5	ND
	5	4.3	<1.5	ND
	6	4.5	<1.5	-
	7	4.5	3.3	ND
	9	4.1	3.8	+
37°C	1	1.7	<1.5	ND
	2	3.7	<1.5	ND
	3	4.4	<1.5	ND
	4	4.7	<1.5	ND
	5	5.0	<1.5	-
40°C	2	4.7	<1.5	ND
	3	4.7	<1.5	-
	4	4.3	<1.5	ND
	5	3.1	<1.5	ND

ND: Not determined

TABLE I. EFFECT OF DAY-OLD EGGS ON INDUCTION OF SMON-TYPE VIRUS FROM ILT VIRUS Y-1000 STRAIN IN CAM.

Day-old	Days of cultivat.	Log ₁₀ titer per ml		Pathogenicity to C57BL/6 mice
		PFU CHK cells	TCD ₅₀ BAT-6 cells	
8	4	5.0	<1.5	ND
	7	5.3	<1.5	-
10	4	4.7	<1.5	ND
	5	4.9	<1.5	-
12	3	3.3	3.5	+
	4	3.4	3.5	ND
Parental virus		4.5	<1.5	-

ND: Not determined

mutagenized control culture did not induce SMON-type virus at both 37 and 40°.

Discussion. It was found previously that some strains of ILT virus are pathogenic for C57BL/6 newborn mice and frequently produce SMON-type virus, which produces weak and incomplete CPE in BAT-6 cells (3). SMON-type virus was already confirmed to be not a contaminant virus by the isolation from a cloned ILT virus which was preliminarily exposed to anti-SMON virus serum. However, Y-1000 strain could not induce SMON-type virus under ordinary culture conditions for ILT virus. Therefore, using Y-1000 strain, we demonstrated in this work that SMON-type virus was successfully induced by the cultivation under unsuitable conditions for the growth of the

TABLE III. MUTAGENESIS OF ILT VIRUS Y-1000 STRAIN EITHER BY BrdU OR NG.

Temp. of incubation	Mutagen concentration ng/ml	Log ₁₀ titer per ml	
		PFU CHK cells	TCD ₅₀ BAT-6 cells
37°C	Control	50	< 1.5
	BrdU	1	3.3
		10	< 1.0
	NG	1	5.0
10		4.7	
40°C	Control	50	< 1.5
	BrdU	1	1.0
		10	< 1.0
	NG	1	4.4
10		4.0	

parental virus or by the mutagenesis in the presence of either BrdU or NG. Also, it was previously observed that cloned NS-175 strain of ILT virus could induce SMON-type virus only after successive propagation in CHK cells at ordinary temperature at 37° (3). The inducibility of SMON-type virus seemed to be significantly different depending upon the strains of ILT virus. However, induction of SMON-type virus, which is pathogenic to mammals, from ILT virus is more likely to be not a rare phenomenon, and this is, of course, a very important fact in respect to zoonosis.

Furthermore, a number of more or less fixed differences among strains of ILT virus have been reported (8, 9). These include differences in pathogenicity for CAM and

for birds, antigenicity, tissue tropism, survival rate during storage, and various epidemiological characters. Therefore, comparative studies of ILT virus at international level will be required for providing more detail information about the relationship between ILT virus and SMON virus.

Summary. SMON-type virus which shows different host range was induced from ILT virus under unsuitable culture conditions for the growth of the parental ILT virus, such as cultivation in CAM of 12-day-old eggs or cultivation in CHK cells at 32°. Furthermore, it was induced from ILT virus by mutagenesis by either BrdU or NG in CHK cell cultures.

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Received July 25, 1975. P.S.E.B.M. 1976, Vol. 151.