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STUDIES ON LIVE ATTENUATED MUMPS VIRUS VACCINE

II. BIOLOGICAL CHARACTERISTICS OF THE STRAINS ADAPTED TO THE AMNIOTIC AND CHORIOALLANTOIC CAVITY OF DEVELOPING CHICK EMBRYOS

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SUMMARY A newly isolated strain of mumps virus (named Urabe) was serially cultivated in the amniotic cavity of developing chick embryos. After only a few passages in the amniotic cavity, the virus showed a higher titer in HEK (primary human embryonic kidney) cells than in CEF (chick embryonic fibroblast) cells and growth of virus was demonstrated in organ cultures of cynomolgus monkey testis and parotid gland.

On the contrary, after serial passages of another strain (Towata) in the chorioallantoic cavity of embryonated hen's eggs, it showed a higher titer in CEF cells than in HEK cells and scarcely grew in the organ culture system.

Both T-All-5 (Towata strain, passaged 5 times in the chorioallantoic cavity) and U-Am-5 (Urabe strain, passaged 5 times in the amniotic cavity) were immunogenic for monkeys. High antibody titers were obtained in monkeys by two injections at a 77 day interval.

INTRODUCTION

The preceding paper (Hosai et al., 1970) reported that the Towata strain of mumps virus is easily attenuated by serial passages in the chorioallantoic cavity of developing chick embryos after cultivation in the amniotic cavity. However, from field trials it seemed that virus adapted to the chorioallantoic cavity was over-attenuated, as the seroconversion rate in vaccinees was approximately 80% even with a large dose of virus. With this virus, the seroconversion rate was also unsatisfactory using the injection method (unpublished data).

To obtain an attenuated virus with an appropriate immunogenicity, we cultivated a newly isolated strain of mumps virus serially in the amniotic cavity of developing chick embryos. This report describes the biological characteristics of this virus and results of inoculation tests in monkeys.

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MATERIALS AND METHODS

1. *Virus strains*

In the winter of 1967, a strain of mumps virus was isolated from the saliva of a child (named Urabe) showing the typical clinical symptoms of mumps. The virus was isolated using primary human embryonic kidney (HEK) cells and passaged once in primary green monkey kidney (GMK) cells. The strain was named the Urabe strain and was passaged further in the amniotic cavity (Am).

The Towata strain isolated by Dr. Hosai using HEK cells was passaged 5 times in the Am and then in the chorioallantoic cavity (All) (cf. preceding report).

These two virus strains were tested after various passage histories. The passage number is expressed as a suffix after an abbreviation of the type of cells used. For example, Urabe strain virus passaged 5 times in the amniotic cavity is named U-Am-5, while the Towata strain, passaged 30 times in the chorioallantoic cavity is named T-All-30.

2. *Virus titration*

Virus was titrated by the hemadsorption method. The titer was read after 7 days incubation at 32 C (CEF cells) or 37 C (HEK cells). The virus titer is expressed as the 50% tissue culture infectious dosis ($TCID_{50}$/0.1 ml).

3. *Virus preparation*

Urabe strain virus was inoculated into the amniotic cavity of 7- or 8-day-old chick embryos. After 5–6 days incubation at 35 C, the amniotic fluid was harvested and centrifuged at 3,000 rev/min for 15 min. at 4 C. Then the supernatant was centrifuged again at 20,000 rev/min for 1 hr at 4 C. The pellet was resuspended in phosphate buffered saline (PBS) containing 0.5% gelatin.

4. *Neutralization (NT) test*

The NT test was done as described previously (Hosai et al., 1970).

5. *Organ cultures of cynomolgus monkey testis and parotid gland*

The testis or parotid gland of cynomolgus monkeys was washed with PBS and cut into small pieces. These were incubated in an atmosphere of 5% CO_2 in air at 37 C. Cultures were maintained with Eagle's minimal essential medium containing 10% calf serum and penicillin and streptomycin. The medium was changed every two days.

6. *Inoculation of cynomolgus monkeys*

Two monkeys were injected subcutaneously with 0.5 ml of U-Am-5 with a titer of $10^{6.0}$ $TCID_{50}$/0.1 ml. Another two monkeys were injected subcutaneously with 0.5 ml of T-All-5 with a titer of $10^{6.0}TCID_{50}$/0.1 ml in HEK cells. Blood samples were taken from the femoral vein at intervals after inoculation and their neutralizing antibody titers were measured.

RESULTS

1. *Growth of the two strains after various numbers of passages in HEK and CEF cells*

The infectivity titers of the Urabe and Towata strains in HEK and CEF cells are shown in Table 1. After only a few passages in Am the Urabe strain showed higher titers in HEK cells than in CEF cells, but its virus titer in CEF cells increased with the number of serial passages in Am. After further passages in All the Towata strain gave low titers in HEK cells.

Fig. 1 and 2 show the growth curves of the Urabe and Towata strains in HEK cells. U-Am-5 and U-Am-11 strains grew well in HEK

TABLE 1. *CEF/HEK titer ratio of the virus after various passages*

Virus strain and passage history	Virus titer in HEK cells	Virus titer in CEF cells	CEF/HEK ratio
	log/0.1 ml	log/0.1 ml	
U-HEK-1 GMK-1	4.0	2.5	3/100
U-Am-4	7.0	4.0	1/1000
U-Am-8	8.5	6.0	1/300
U-Am-10	6.5	4.5	1/100
U-Am-14	6.0	6.0	1
U-Am-15	7.0	8.0	10
T-A11-6	6.0	6.0	1
T-A11-16	3.5	6.0	300
T-A11-29	1.0	6.0	100,000

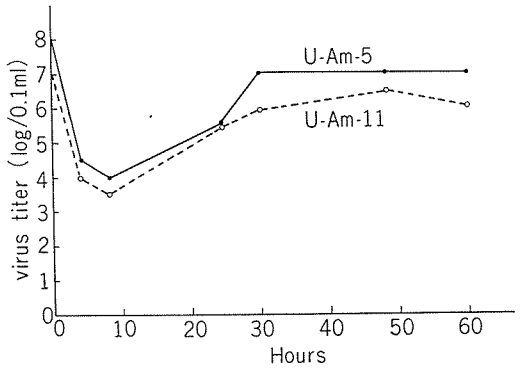

FIGURE 1. *Growth of U-Am-5 and U-Am-11 strains in HEK cells.*

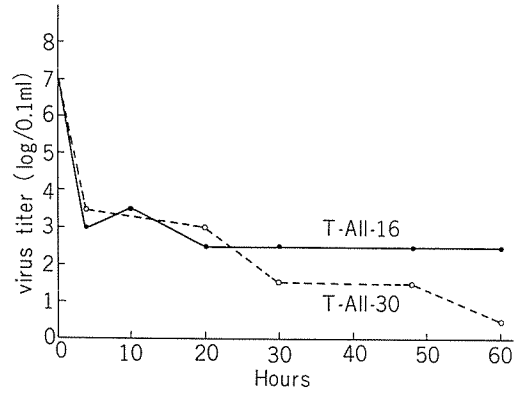

FIGURE 2. *Growth of T-All-16 and T-All-30 strains in HEK cells.*

cells, but the T-All-16 and T-All-30 strains grew very badly.

2. *Growth of the two strains in organ cultures of cynomolgus monkey testis and parotid gland*

The growth of U-HEK-2, U-Am-5, U-Am-14 and T-All-32 were compared in organ cultures of the testis and parotid gland. The titers of these strains inoculated were $10^{5.0}$, $10^{5.0}$, $10^{6.0}$ and $10^{5.0}$ $TCID_{50}$/0.1 ml, respectively. Volumes of 0.2 ml of these virus strains were dropped onto small pieces of tissue and samples were collected every two days. Then the fluid and tissue specimens were titrated in HEK cells (U-HEK-2 and U-Am-5 strains) or in CEF cells (U-Am-14 and T-All-32 strains). Replication of U-HEK-2 strain occurred in organ cultures of the testis and parotid gland. As shown Table 3, no growth of the T-All-32 strain was detected in the testis and replication of U-Am-5 or U-Am-14

TABLE 3. *Growth of U-HEK-2 and T-A11-32 strains in organ cultures of monkey testis*

Day	U-HEK-2		T-A11-32	
	Tissue (log/0.1 ml)	Fluid (log/0.1 ml)	Tissue (log/0.1 ml)	Fluid (log/0.1 ml)
2	0.5	<0	<0	<0
4	1.5	1.5	<0	<0
6	2.5	3.5	<0	<0
8	3.0	3.5	<0	<0

TABLE 2. *Growth of the two strains after different passage histories in organ cultures of monkey parotid gland*

Day	U-HEK-2		U-Am-5	U-Am-14	T-A11-32	
	Tissue (log/0.1 ml)	Fluid (log/0.1 ml)	Fluid (log/0.1 ml)	Fluid (log/0.1 ml)	Tissue (log/0.1 ml)	Fluid (log/0.1 ml)
2	0	0	<0	<0	<0	<0
4	4.0	3.5	0	1.5	<0	<0
6	>5.5	>5.5	4.0	3.0	0.5	0.5
8	4.5	3.5	2.5	3.5	<0	<0

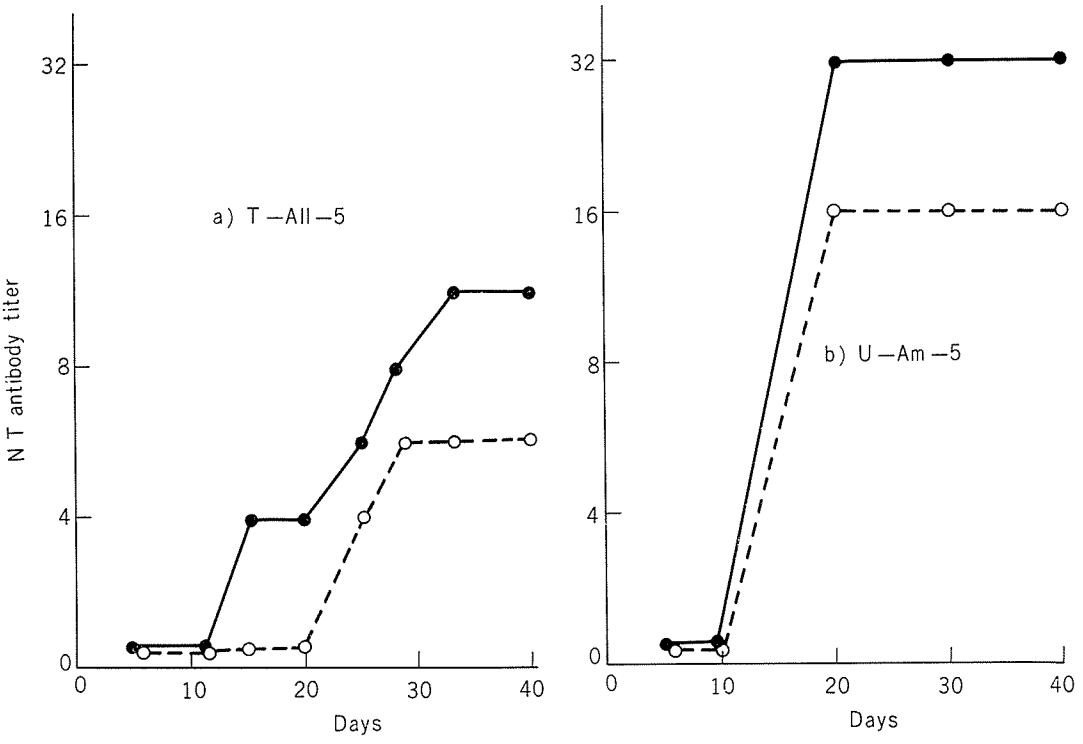

FIGURE 3. *Neutralizing antibody responses in monkeys inoculated with the T-All-5 and U-All-5 strains.*
a) ●——● *monkey No. 1* ○·····○ *monkey No. 2*
b) ●——● *monkey No. 3* ○·····○ *monkey No. 4*

was far slower than that of U-HEK-2 in cultures of parotid gland.

3. *Inoculation into cynomolgus monkeys*

Fig. 3 shows the neutralizing antibody responses of monkeys. The neutralizing antibody titers induced with the U-Am-5 strain were higher than those with the T-All-5 strain and reached a peak on about the 20th day. Those of the T-All-5 strain reached a peak about 10 days later than those of the U-Am-5 strain. No clinical reactions (swelling of parotid gland, etc.) were detected. Fig. 4 shows the persistence of neutralizing antibody induced with the T-All-5 strain. The effect of a second injection of the T-All-5 strain after an interval of 77 days was examined. Higher antibody titers were found on the 9th days after the second injection.

DISCUSSION

It has been reported that mumps virus adapted to embryonated hen's eggs does not cause clinical reactions in monkeys or in children (Henle et al., 1951, Enders et al., 1946). Moreover, over-attenuation readily occurs on serial passage in CEF cells (Buynak et al., 1966).

We titrated two mumps virus strains in HEK and CEF cells. Viral growth in HEK cells decreased with the number of serial passages in the chorioallantoic cavity (Hosai et al., 1970) and after numerous passages in the chorioallantoic cavity the strain grew very poorly in HEK cells. The virus titer in HEK cells

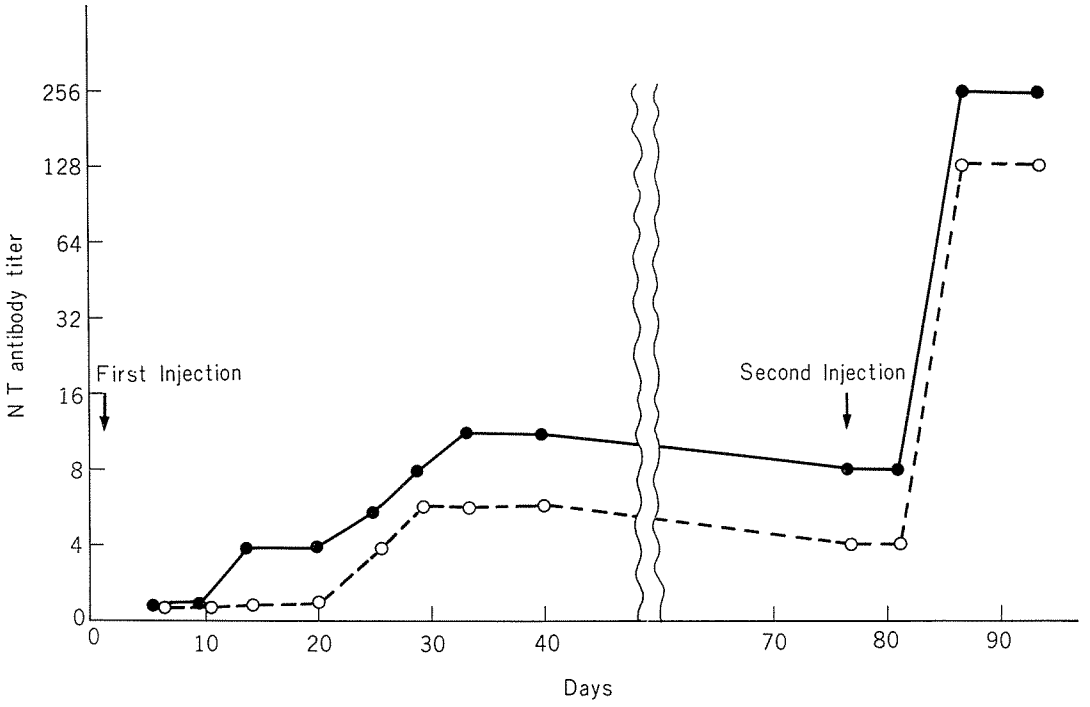

FIGURE 4. *Neutralizing antibody responses in monkeys inoculated with the T-All-5 strain twice at an interval of 77 days.*

●——● *monkey No. 1* ○·····○ *monkey No. 2*

decreased and that in CEF cells increased with the number of serial passages in the amniotic cavity. This suggests that passage of mumps virus in developing embryos is generally accompanied by loss of susceptibility of the virus for human cells and acquisition of susceptibility for chick cells.

Bigazzi et al. (1968) showed that mumps virus could grow in organ cultures of rhesus monkey testis. As shown in this report (Table 2, 3), the U-HEK-2 strain replicated well in organ cultures of monkey testis and parotid gland, whereas after numerous passages in the chorioallantoic cavity it did not replicate. These results seem relevant for attenation of virus.

The antibody titers induced with live mumps vaccines were lower than those observed in natural mumps infection (Weibel, 1967, Ennis, 1967). Ennis (1969) reported that some children with low levels of mumps antibody were really infected and showed a clinical reaction. This is one of the most important points ever noted about mumps virus vaccines. In our experiments higher antibody titers were obtained in monkeys by two doses of injections at a 77 day interval than by a single injection. Shramek (1969) also reported high antibody titers in guinea pigs after two injections. This method of inoculation is now under detailed investigation in our laboratory.

Field trials with attenuated viruses obtained by passage in the amniotic cavity are being carried out and the result will be reported in the next paper.

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