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STUDIES ON LIVE ATTENUATED MUMPS VIRUS VACCINE

1. ATTENUATION OF MUMPS VIRUS BY SERIAL PASSAGE IN THE CHORIOALLANTOIC CAVITY OF DEVELOPING CHICK EMBRYOS AND FIELD TRIALS BY THE INHALATION METHOD.[1]

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SUMMARY The Towata strain of mumps virus, isolated in human embryonic kidney (HEK) cells, was successfully cultivated in the chorioallantoic cavity of developing chick embryos after several passages in the amniotic cavity. Virus adapted to the chorioallantoic cavity showed a narrower range of susceptibility for tissue culture cells and a markedly lower pathogenicity for monkey parotid gland than virus which had not undergone passages in developing eggs.

After various numbers of passages in the chorioallantoic cavity, the Towata strain was used in field trials on children by the inhalation method. No significant clinical manifestations were observed. The seroconversion rate was approximately 80 percent in the trial, the average neutralizing antibody titer being 1 : 8 to 1 : 16.

INTRODUCTION

Mumps is usually an infectious disease of children causing mild fever and parotitis. However, mumps virus is one of the most important cause of viral meningoencephalitis (Azimi et al., 1969). When adults contract this disease, severe complications, such as orchitis, ovaritis or pancreatitis are not uncommon.

Live mumps vaccines were developed in U.S.A. and U.S.S.R. (Smorodintsev et al., 1965, Buynak et al., 1966, Weibel et al., 1967). In these countries, chick embryo fibroblastic cell cultures were mainly used for virus attenuation and vaccine preparation. It was reported that these vaccines caused no clinical reactions and induced good antibody responses. But the serum antibody titers induced with these vaccines were lower than those with natural mumps.

1. This work was presented at the 15th Annual Meeting of Japanese Virologists at Chiba City in November 1967.
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For several years we have also been studying the possibility of attenuated live mumps vaccine using developing chick eggs. Attempts were made to attenuate the Towata strain, isolated in human embryonic kidney (HEK) cells, by serial passages in the chorioallantoic cavity of developing chick embryos. After adaptation to the chorioallantoic cavity, its biological characteristics were examined, including its susceptibility for various kinds of cultured cells and its pathogenicity for monkey parotid glands. The attenuated virus was used for small field trials on children by the inhalation method.

MATERIALS AND METHODS

1. *Cells*

Human embryonic kidney (HEK) cells, green monkey kidney (GMK) cells, and chick embryonic fibroblasts (CEF) were cultured with LE medium (Earles' medium containing 5% lactalbumin hydrolysate). Ten percent bovine serum was added for growth medium and 3 percent for maintenance medium.

2. *Virus strains and vaccine preparation*

The Towata strain, isolated in our laboratory, was passaged twice in HEK cells and once in green monkey kidney (GMK) cells. Then it was cultivated in the amniotic cavity and chorioallantoic cavity of developing chick embryos. For cultivation in the amniotic cavity, 8-day-old eggs were inoculated with 0.1 ml of virus fluid and incubated for five days at 35 C. For cultivation in the chorioallantoic cavity, 7-day-old eggs were inoculated with 0.1–0.2 ml of virus fluid and incubated for 6 to 7 days at 35 C. The fluid was harvested and centrifuged at 1,500 rev/min for 15 min, and the supernatant was used for experiments.

3. *Experiment with monkeys*

Approximately 2 ml of virus fluid were injected into the bilateral parotid glands of cynomolgus monkeys through Stensen's ducts, the orifices of the glands. Swelling of the glands was checked and the antibody response was observed for one month after inoculation.

4. *Neutralization (NT) test*

All sera employed were inactivated by heating at 56 C for 30 min. Sera were diluted serially two-fold with LE medium. A mixture of equal volumes of

TABLE 1. *Susceptibility of Towata strain virus at various passage levels to culture cells*

Titration system / No. of Passage	Virus Titer (log_{10}/0.1 ml)					
	CEF		GMK		HEK	
	CPE[d]	HAD[e]	CPE	HAD	CPE	HAD
HEK-2-GMK-1[a]	5.55	5.67	6.33	6.33	5.67	5.67
HEK-2-Am-1[b]	NE	NE	5.33	5.33	NE	NE
HEK-2-Am-3	4.0	5.33	5.50	6.0	NE	NE
HEK-2-Am-5	4.33	5.67	5.33	6.67	3.50	4.50
HEK-2-Am-5-A11-3[c]	6.50	7.0	6.50	6.50	<0	4.50
HEK-2-Am-5-A11-5	6.0	6.33	6.50	6.50	<0	3.50
HEK-2-Am-5-A11-9	5.67	6.50	3.75	3.75	<0	2.50
HEK-2-Am-5-A11-12	<0	6.25	3.50	3.50	NE	NE
HEK-2-Am-5-A11-18	<0	6.50	4.50	4.50	<0	<0

[a] Towata strain, passaged twice in HEK cells and once in GMK cells
[b] Towata strain, passaged twice in HEK cells, once in GMK cells and once in chick amniotic cavity
[c] Towata strain, passaged twice in HEK cells, once in GMK cells, 5 times in chick amniotic cavity and 3 times in chick cherioallantoic cavity
[d] CPE: Cytopathogenic effect
[e] HAD: Hemadsorption
NE: Not examined

diluted serum and virus fluid, containing 30–100 $TCID_{50}$/0.1 ml, was incubated at 37 C for one hour and then kept at 4 C overnight. The NT antibody titer was measured by the hemadsorption (HAD) method following incubation at 37 C for 6 days.

5. *Hemagglutination inhibition (HI) test*

To eliminate non-specific HI inhibitor, three volumes of receptor destroying enzyme (RDE) solution were added to one volume of serum, and the mixtures were incubated at 37 C overnight and then heated at 56 C for 30 min. The HI titer was read after incubation for one hour at room temperature.

RESULTS

1. *Comparison of the biological characteristics of the Towata strain virus at various passage levels*

Samples of Towata strain virus at various passage levels were compared. Values for their infectivities in CEF, GMK, and HEK cells measured by their cytopathic effects (CPE) and hemadsorption (HAD) are shown in Table 1. After passage in the amniotic cavity, virus retained its cytopathogenicity for all these cells, but after passage in the chorioallantoic cavity, it lost its cytopathogenicity for HEK cells.

Furthermore, after more than 12 passages in the chorioallantoic cavity, the virus lost its cytopathogenicity for CEF cells.

TABLE 2. *Pathogenicity and Immunogenicity of Towata strain to Monkeys*

Strains and Virus Titers Inoculated (in $TCID_{50}$/0.1 ml)	Monkey No.	Swelling of the Parotid Glands		Excretion of Virus		Antibody Response HI Titer (Log_2)	
		Beginning (Days)	Duration (Days)	Beginning (Days)	Duration (Days)	(Pre)	(Post)
Towata HEK-2 ($10^{5.5}$ in HEK cells)	19	7	1	NE		2	7
	20	—	—	NE		2	7
	21	7	3	NE		2	NE
	22	6	3	NE		2	7
	23	6	2	NE		2	7
	24	7	3	NE		2	7
Towata HEK-2GMK-1Am-5A11-4 ($10^{6.5}$ in CEF cells)	35	7	2	7	2	2	8
	36	—	—	—	—	2	8
	37	7	2	7	1	2	7
	38	—	—	—	—	2	7
	39	7	1	—	—	2	6
Towata HEK-2GMK-1Am-5A11-12 ($10^{5.5}$ in CEF cells)	15	—	—	NE		2	7
	16	—	—	NE		2	6
	17	—	—	NE		2	7
	18	—	—	NE		2	6
Towata HEK-2GMK-1Am-5A11-15 ($10^{6.5}$ in CEF cells)	31	—	—	—	—	2	5
	32	—	—	—	—	2	6
	33	—	—	—	—	2	8
	34	—	—	—	—	2	6

NE : not examined

The virulence of the virus at various passage levels for monkey parotid glands was examined. After two passages in HEK cells (HEK-2) and two in HEK cells, one in GMK cells, five in the amniotic cavity and then five in the chorioallantoic cavity (HEK-2, GMK-1, Am-5, ALL-5) virus induced swelling of the parotid glands and excretion of virus was detected.

On the contrary, after more than 12 passages in the chorioallantoic cavity, virus was apparently nonpathogenic for monkey parotid and no excretion of virus was observed. No difference was found between the antibody responses of samples of various histories of passage.

2. *Field trials by the inhalation method*

Towata strain virus after cultivation in the chorioallantoic cavity was employed for field trials, as shown in Table 3. The virus titer of the inoculum was $10^{6.0}$ to $10^{7.5}$ $TCID_{50}$/0.1 ml in CEF cells. Blood specimens were collected just before and four weeks after vaccination, and NT and HI tests were performed. The possible development of clinical manifestations, including swelling or tension of the parotid glands, and fever were checked for one month after vaccination. However, in no case were clinical manifestations recognized and no significant fever developed after vaccination.

Throat swabs from 34 vaccinees were collected 2, 3, 7 and 14 days after vaccination in exp. 6. All attempts to reisolate mumps virus from these swabs failed.

The seroconversion rate and mean antibody titer by HI and NT tests are given in Table 4. In general the NT test was more sensitive than the HI test, and a seroconversion rate of approximately 80 percent was observed in most trials irrespective of the passage number in the chorioallantoic cavity of the virus sample employed. The mean HI antibody titer was 1 : 4 to 1 : 8 and the NT antibody titer was 1 : 8 to 1 : 16 in every trial.

DISCUSSION

It has been observed that mumps virus which has been passaged in developing chick embryos fails to multiply in human or simian cells, while freshly isolated virus or virus that has undergone only a few passages in cell cultures, propagates well with CPE in various cell cultures (Henle and Deinhardt, 1955, Utz et al., 1957, Henle et al., 1954). The Towata strain of

TABLE 3. *Vaccination Schedule by the Inhalation Method*

Exp.	Vaccination		No. of Vaccinees	Vaccine Strain	Inhalation Time (sec.)
	Date	Place			
1	April 1967	Muyuen (a nursery school) in Osaka City	32	Towata HEK-1 GMK-1Am-5A11-5	60
2	June 1967	Osaka University	18	A11-13	60
3	June 1967	Hamakoshien in Nishinomiya City	213	A11-15	60
4	July 1967	Public Health Institute in Sakai City	70	A11-18	60
5	Nov. 1967	Muyuen	115	A11-15	30
6	Dec. 1967	Muyuen	101	[a]A11-15	30
7	June 1968	Minamisenri in Suita City	97	A11-15	30
8	July 1968	Minamisenri	97	[a]A11-12	30

A11-5 : Figures indicates the passage numbers in the chorioallantoic cavity
a : vaccine purified by ultracentrifugation twice at 20,000 rpm. for 60 min.

mumps virus showed similar characteristics. With increase in the number of passages in the chorioallantoic cavity, the virus lost its susceptibility to GMK and HEK cells.

It has been reported that mumps virus is readily attenuated and loses its immunogenicity during passages in developing chick embryos or chick cell cultures (Enders et al., 1946, Buynak et al., 1966). Buynak et al., reported that their level A vaccine of the Jeryl Lynn strain (12nd passage in CEF) was more immunogenic than level B vaccine (17th passage in CEF), but the former induced swelling of the parotid glands in some vaccinees. When the latter was further passaged 10 times in CEF, the immunogenicity decreased considerably. The antibody levels obtained with the Towata strain, after more than 5 passages in the chorioallantoic cavity were similar to those given in previous reports (Luizyanina et al., 1965, Buynak et al., 1966, Somorodintsev et al., 1967, Weibel et al., 1967). However, the the seroconversion rate of our vaccine was approximately 80 percent. This might be due to over-attenuation of the virus, or to the inhalation method.

Improvements of these points are presented in the following paper.

TABLE 4. *Summary of Results on Antibody Responses in Field Trials*

Exp.	Vaccine Strains	HI Antibody Response		NT Antibody Response	
		Antibody development (%)	G. M. antibody titer (log_2)	Antibody development (%)	G. M. antibody titer (log_2)
1	Towata HEK-2 GMK-1 Am-5-A11-5	6/8[b] (75.0)	2.6		
2	Towata HEK-2 GMK-1 Am-5-A11-13	6/7 (85.7)	3.0	6/7 (85.7)	3.5
3	Towata HEK-2 GMK-1 Am-5-A11-15	21/32 (65.6)	2.0	26/33 (78.8)	3.1
4	Towata HEK-2 GMK-1 Am-5-A11-18	13/22 (59.1)	2.2	21/25 (84.0)	4.0
5	Towata HEK-2 GMK-1 Am-5-A11-15	6/7 (85.7)	2.3		
6	Towata HEK-2 GMK-1 Am-5-A11[a]-15	14/20 (70.0)	2.2		
7	Towata HEK-2 GMK-1 Am-5-A11-15	18/27 (66.7)	2.6	9/11 (81.8)	3.8
8	Towata HEK-2 GMK-1 Am-5-A11[a]-12	5/8 (62.5)	2.6		

Exp. No. 1-4: Inhalated for 60 sec.
5-8: Inhalated for 60 sec.
[a] Chorioallantoic fluid was purified by ultracentrifugation.
[b] Number of vaccinees showing antibody response / Total number of vaccinees
G. M.: geometric mean

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