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STUDIES ON LIVE MUMPS VIRUS VACCINE

III. EVALUATION OF NEWLY DEVELOPED LIVE MUMPS VIRUS VACCINE

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SUMMARY The Urabe strain of mumps virus was serially passaged in the amniotic cavity of chick embryos. Virus samples at the 4th, 6th, 9th and 15th passage levels, respectively were used in inoculation tests in children. After vaccination no clinical manifestations were observed and all inoculated children showed an antibody response. The geometric mean neutralizing antibody titer to all the respective vaccine viruses except 4th passage virus was approximately 5.7, the latter being 22.6. The virus could not be reisolated and no contact infection from the vaccinees was noted.

INTRODUCTION

The preceding paper (Yamanishi et al., 1970), reported the biological characteristics of a newly isolated mumps virus strain (designated Urabe) and results of inoculation tests on cynomolgus monkeys with it. Monkeys injected with this mumps virus showed a good antibody response and no clinical reactions. This Urabe strain was serially passaged in the amniotic cavity of developing chick embryos and prepared as a live mumps virus vaccine. We used this strain at various passage levels in field trials by the injection method. This report describes the results of these field trials.

MATERIALS AND METHODS

1. *Preparation of vaccine*

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

2. *Vaccination*

Vaccination was carried out on children with a negative history of mumps during 1969–1970 in Nishinomiya City, Hyogo and Suita City, Osaka. Doses of 0.5 ml or 0.25 ml of the vaccine were

injected subcutaneously into healthy children living at home. Blood specimens were collected just before and four or six weeks after vaccination. Serum was inactivated by heating at 56C for 30 min before the serum test.

3. *Virus titration*

Infectivity of vaccine virus was titrated in HEK (human embryonic kidney) cells by the hemadsorption method. The titer was read after 7 days incubation at 37C.

4. *Organ culture of cynomolgus monkey testis*

The procedures used were as described previously (Yamanishi et al., 1970).

5. *Neutralization (NT) test*

Serum neutralization tests were performed in LLC-MK2 cell (established cell line of monkey kidney cell) cultures, using the Miyake strain, isolated in our laboratory in 1967 and serially passaged since then in LLC-MK2 cells. Serial 2 fold dilutions of sera were mixed with an equal volume of virus containing 30–100 $TCID_{50}$/0.1 ml. The mixture was incubated at 37C for 1 hr and then kept overnight at 4C. Volumes of 0.2 ml each mixture were inoculated into 3 LLC-MK2 cell tubes.

Neutralizing antibody titers were read after four or five days incubation at 37C by the hemadsorption method.

6. *Hemagglutination inhibition (HI) test*

To remove non-specific inhibitor, three volumes of 25% Kaolin (Difco)and one volume of phosphate buffered saline (PBS) were added to one volume of inactivated serum. After standing at room temperature for 20 min, mixtures were centrifuged at 3,000 rev/min for 20 min. The supernatant was mixed with one volume of 10% suspension of chicken red blood cells and kept at 0C for 1 hr and then clarified by centrifugation at 2,000 rev/min for 20 min. Tween-80 ether treated antigen was employed in the HI test. Sera was mixed with 4 units of this antigen and after standing at room temperature for 3 hr the HI titer was read. The micro-titer system was used in this HI test.

7. *Virus recovery test*

Throat swab specimens were collected from 5 vaccinees on the 4th, 6th, 9th and 14th day after vaccination and these materials were inoculated onto HEK cells. Cells were incubated with Eagle's minimal essential medium containing 5% calf serum. After 7 days, the fluid was transferred to other HEK cells and these were cultivated for 1 week. The result was judged by the hemadsorption method.

8. *Collection of blood samples from sibling of the vaccinees*

To test for contact infection, children were injected with mumps vaccine and blood samples from their siblings were collected 49 to 133 days after vaccination and the NT test was performed on these samples.

RESULTS

1. *Biological characteristics of the four vaccine viruses*

As shown in Table 1, the ratio of virus titers in HEK and CEF cells varied from 1 : 1,000 to 1 : 1 with serial passages of the virus in the amniotic cavity.

However, the virus yields from these four

TABLE 1. *Ratios of CEF/HEK titer of the four vaccine viruses*

Vaccine virus	Virus titer in HEK cells log_{10}/0.1 ml	Virus titer in CEF cells log_{10}/0.1 ml	CEF/HEK ratio
U-Am-4	7.0	4.0	1/1,000
U-Am-6	6.0	3.5	1/300
U-Am-9	6.5	4.5	1/100
U-Am-15	7.0	7.0	1/1

TABLE 2. *Growths of the four vaccine viruses in organ cultures of monkey testis*

Vaccine virus→	U-Am-4 (log_{10}/0.1 ml)	U-Am-6 (log_{10}/0.1 ml)	U-Am-9 (log_{10}/0.1 ml)	U-Am-15 (log_{10}/0.1 ml)
Input titer→ Day ↓	5.5	5.5	6.5	6.5
2	0.0	0.0	0.0	0.0
4	2.0	1.0	2.0	2.5
6	2.5	1.5	3.5	2.5
8	2.5	2.5	3.0	2.0

viruses in organ cultures of monkey testis were similar (Table 2).

2. *Serological response in children*

The four kinds of Urabe strain virus were tested in field trials. First, U-Am-4 (Urabe strain, 4 times passaged in amniotic cavity) was injected subcutaneously into children. To find the effective dose serial dilutions of the virus were tested. As shown in Table 3, NT and HI antibody developed in all children. Children who were injected with a dose of $2\times 10^{6.0}$ $TCID_{50}$ acquired higher antibody titers than children who were injected with a dose of $2\times 10^{5.0}$ $TCID_{50}$ or $2\times 10^{4.0}$ $TCID_{50}$.

Then, U-Am-6 (Urabe strain, 6 times passaged in chick amniotic cavity) virus was tested at doses of $2\times 10^{6.0}$ $TCID_{50}$ and $2\times 10^{5.0}$ $TCID_{50}$. All children showed an NT antibody response and the geometric mean titer was 5.3. There was no difference in the antibody titers after the two different doses (Table 4).

U-Am-9 (Urabe strain, 9 times passaged in chick amniotic cavity) and U-Am-15 (Urabe strain, 15 times passaged in chick amniotic cavity) also induced NT antibody in all vaccinated children. The geometric mean titers of NT antibody were 9.2 and 7.7 respectively and those of HI antibody were both 10.0 (Tables 5 and 6). As shown in Tables 5 and 6, there was no difference in antibody titers induced by the various virus dose of the vaccine. No clinical reaction due to vaccination were detected in any trial.

TABLE 3. *Antibody response to U-Am-4 strain*

Virus titer ($TCID_{50}$/dose)	NT antibody response		HI antibody response	
	Seroconversion rate	G.M. titer (range)	Seroconversion rate	G.M. titer (range)
$2\times 10^{6.0}$	7/7	22.6 (4–128)	6/6	23.0 (5–80)
$2\times 10^{5.0}$	24/24	5.7 (2–16)	23/23	10.0 (5–20)
$2\times 10^{4.0}$	6/6	5.7 (2–8)	5/5	10.0 (5–20)

TABLE 4. *Antibody response to U-Am-6 strain*

Virus titer ($TCID_{50}$/dose)	NT antibody response	
	Seroconversion rate	G.M. titer (range)
$2\times 10^{6.0}$	9/9	5.3 (2–16)
$2\times 10^{5.0}$	7/7	5.3 (2–8)

G.M.: geometric mean

3. *Communicability of U-Am-4 and U-Am-6 strains*

Throat swabs and saliva from 5 children vaccinated with U-Am-4 strain were collected,

TABLE 5. *Antibody response to U-Am-9 strain*

Virus titer ($TCID_{50}$/dose)	Initials	NT antibody response		HI antibody response	
		before	after	before	after
$10^{5.0}$	T.S.	<2	8	<5	5
	E.N.	<2	8	<5	5
	H.S.	<2	8	<5	10
	K.S.	<2	16	<5	20
$10^{4.0}$	H.A.	<2	4	<5	5
	M.M.	<2	16	<5	20

TABLE 6. *Antibody response to U-Am-15 strain*

Virus titer ($TCID_{50}$/dose)	NT antibody response		HI antibody response	
	Seroconversion rate	G.M. titer (range)	Seroconversion rate	G.M. titer (range)
$10^{6.0}$	6/6	7.5 (2–16)	5/6	12.3 (5–20)
$10^{5.0}$	4/4	8.0 (8–8)	4/4	7.1 (5–20)

but mumps virus could not be isolated from these materials. Moreover 5 families of children vaccinated with the U-Am-6 strain were tested for contact infection and none of the other children who were not vaccinated in these families showed an NT antibody response.

TABLE 7. *Virus recovery from vaccinees inoculated with U-Am-4 strain*

Initials	Age (Yr.)	Day after inoculation			
		4	6	9	14
M.T.	2	—	—	—	—
N.K.	5	—	—	—	—
A.K.	9	—	—	—	—
M.K.	7	—	—	—	—
Y.T.	5	—	—	—	—

TABLE 8. *Communicability of U-Am-6 strain*

Family No.	Initials		Age (Yr.)	NT antibody titer		Interval between blood samples(day)
				before	after	
1	T.T.	a	2	<2	2	28
	T.T.	b	6	NE	<2	90
2	T.O.	a	1	<2	16	28
	T.O.	b	4	NE	<2	90
	K.O.	b	7	NE	<2	90
3	K.T.	a	6	<2	8	73
	H.T.	a	5	<2	8	73
	N.T.	b	2	NE	<2	133
4	Y.Y.	a	2	<2	2	49
	T.Y.	b	1	<2	<2	49
5	T.H.	a	5	<2	2	30
	M.H.	b	2	NE	<2	71

a : vaccinated child b : unvaccinated child NE : not examined

DISCUSSION

For some years we have been studying the attenuation of mumps virus by serial passage in chick amniotic cavity. In the present study, virus at the 4 th to 15th passage level was tested in field trials. No clinical manifestations were detected following vaccination in these trials. Enders et al. (1946) reported that after a single egg-passage virus caused no clinical reactions but evoked a satisfactory antibody response when given by the oral spraying method. It

has been reported that mumps virus attenuation occurred after the 17th passage in chick fibroblast cells (Buynak et al., 1966). In our experiments, virus attenuation occurred after only 4 passages in chick amniotic cavity. These results suggest that mumps virus can easily be attenuated by passage in the chick amniotic cavity as in chick fibroblast cells.

It has been reported that immunogenicity is considerably lowered after 10 additional passages in chick fibroblast cells and so only a narrow range of passage levels can be used for production of effective vaccine (Buynak et al., 1966 and 1968). However, in our experiments, mumps virus was not over-attenuated until at least the 15th passage in chick amniotic cavity and there was no difference in the antibody responses to the four kinds of vaccine viruses.

The preceding report (Yamanishi et al., 1970), described markers for attenuation of mumps virus. U-Am-4, U-Am-6, U-Am-9 and U-Am-15 viruses like the wild strain grew in organ cultures of cynomolgus monkey testis. On the other hand, after numerous passages in the chorioallantoic cavity virus grew poorly in organ cultures of monkey testis. Thus, an attenuated strain could not be differentiated from a wild strain by the biological characteristics observed in our experiments, but might be differentiated from an over-attenuated strain which had been passaged repeatedly in the chorioallantoic cavity.

In the present study, all vaccinees showed antibody responses against mumps virus, but the antibody level following vaccination was lower than that with natural mumps. Weibel et al. (1968 and 1969) reported that the antibody induced by vaccination was low but persisted for at least three years. Further field trials and a follow up of the children who received the mumps vaccines are being planned.

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