BIKEN JOURNAL Vol. 13, 169-174, 1970

STUDIES ON FURTHER ATTENUATED LIVE MEASLES VACCINE

IV. CLINICAL AND SEROLOGICAL EVALUATION OF A CLONE OF CAM-CEF MEASLES VACCINE VIRUS[1]

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(Received May 30, 1970)

SUMMARY Live measles virus vaccine prepared from one of the clones of CAM-CEF measles vaccine virus was tested clinically and serologically on healthy children living with their families. A febrile reaction of over 37.5 C developed in 43.6% and of over 39.0 C in 8.3% of the children. A sporadic rash developed in 12 of the 133 children.

When the measles vaccine was injected with live mumps virus vaccine developed in our laboratory, only 27.7% of the children showed a febrile reaction of over 37.5 C and both vaccines were as immunogenic as when given separately.

INTRODUCTION

Nine clones were selected from 75 clones of CAM-CEF measles vaccine virus and tested clinically on healthy children. Some of the clones showed low reactivity (Takaku et al., 1970).

To evaluate the reproducibility of the reactions caused by the clones, a live measles virus vaccine was prepared from a clone, A4 (CAM-A4 measles vaccine), and a slightly larger field trial than previously was carried out on healthy children.

Recently, live mumps virus vaccine cultured in the amniotic cavity of chick embryos has been developed in our laboratory (Yamanishi et al., 1970). A mixture of live measles virus vaccine, CAM-A4 and live mumps virus vaccine was tested clinically and serologically on children.

1 A summary of these results was reported at the Second Annual Meeting of the Japan Measles Vaccine Research Commission in Tokyo, March, 1970.

This paper describes the results of the two field trials.

MATERIALS AND METHODS

1. *Vaccines*

1) Measles vaccine

Chick embryo fibroblasts (CEF) were infected with clone A4 of CAM-CEF measles vaccine virus and this was passaged 3 times more in CEF after cloning. Then live measles vaccine was prepared from the culture fluid (CAM-A4 measles vaccine). The lyophilized vaccine in one dose ampule was reconstituted with 0.5 ml of distilled water and this had a titer of $10^{3.8}$ $TCID_{50}$/0.1 ml. A dose of 0.5 ml was injected subcutaneously.

2) Mumps vaccine

The Urabe strain of mumps virus was attenuated by serial passages in the amniotic cavity of chick embryos. Lyophilized live mumps vaccine was prepared from the amniotic fluid. The vaccine titer was $10^{5.5}$ $TCID_{50}$/0.1 ml in human embryonic kidney cells (Yamanishi et al., 1970).

Equal volumes of CAM-A4 measles vaccine and live mumps virus vaccine were mixed just before injection and 0.5 ml of the mixture was injected subcutaneously. This dose ($2.5 \times 10^{3.8}$ $TCID_{50}$) of measles vaccine did not affect the clinical reactions and antibody responses of children receiving live mesles virus vaccine, as reported previously (Ueda et al., 1970b).

2. *Vaccinees*

Vaccinees were 10 month to 6 year old children with no history of measles or mumps living in their homes in two housing sites in Suita City, Osaka and Nishinomiya City, Hyogo, respectively. The age distribution of the children receiving CAM-A4 measles vaccine alone is shown in Table 1.

Blood specimens were collected on the day of vaccination and 3 weeks later. Children who were seropositive before vaccination were not included in the survey.

Other materials and methods were as described previously (Takaku et al., 1970; Ueda et al., 1970a, b).

RESULTS

1. *Vaccination with CAM-A4 measles vaccine*

1) Serological response

Antibody titers were measured by the HI test. Comparison of neutralizing antibody titers and HI antibody titers with those of sera obtained in other field trials is shown in Fig. 1 and 2. The HI antibody titers corresponded well with the neutralizing antibody titers, but were about half the latter.

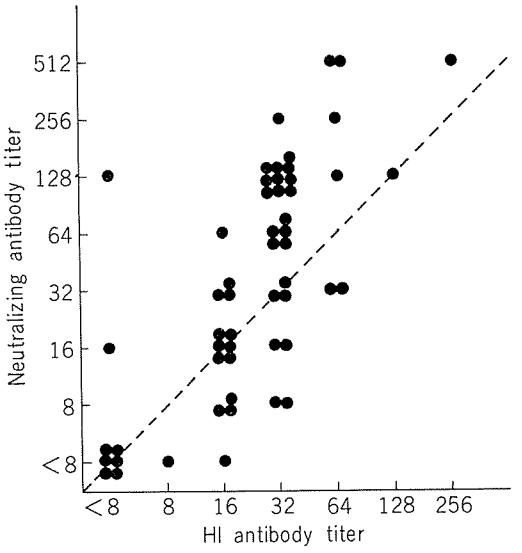

FIGURE 1. *Comparison of neutralizing antibody titers and HI antibody titers (I).*

TABLE 1. *Age distribution of children receiving CAM-A4 measles vaccine*

	Age	10–11mth	1yr	2yr	3yr	4yr	5yr	6yr	Total
Tested serologically	No.	7	41	29	12	7	6	2	104
	%	6.7	39.4	27.4	11.5	6.7	5.8	1.9	100
Observed clinically	No.	8	56	32	15	8	8	6	133
	%	6.0	42.1	24.0	11.3	6.0	6.0	4.5	100

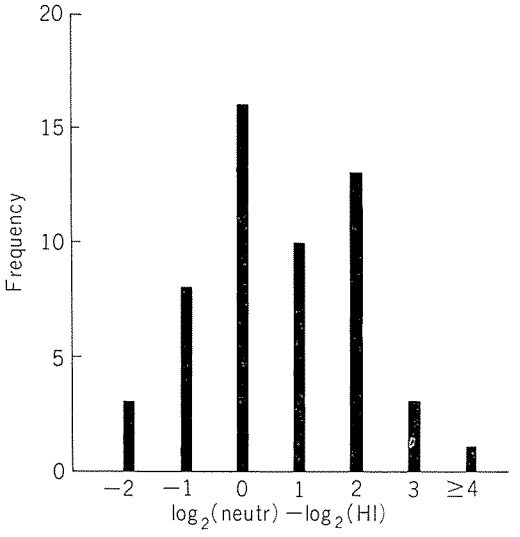

FIGURE 2. *Comparison of neutralizing antibody titers and HI antibody titers (II).*

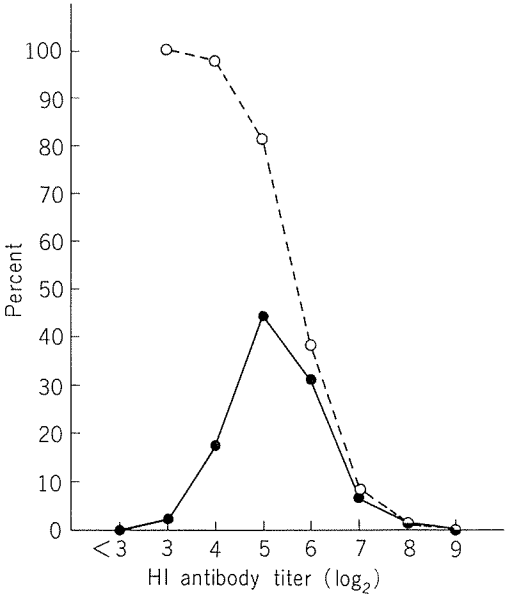

FIGURE 3. *Distribution (●——●) and cumulative percent (○------○) of HI antibody titers in children receiving CAM-A4 measles vaccine.*

Three weeks after vaccination the HI antibody titers of all the 104 children who received the vaccine had increased by over 2^3. The distribution of HI antibody titers is shown in Fig. 3, the peak being at 2^5, and most children having titers between 2^4 and 2^6. As shown in Fig. 4, the antibody titers of children who developed a febrile reaction were slightly higher than those of other children.

The mean antibody titers of children in the different age groups are shown in Table 2, not much difference being found between the groups.

2) Clinical reactions

The chief clinical reactions were a febrile reaction and development of a sporadic rash.

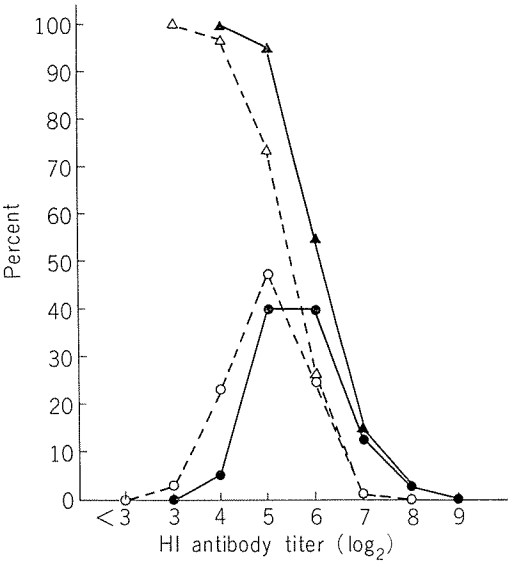

FIGURE 4. *Comparison of HI antibody titers in children with and without a fever.*
Circles show distribution of antibody titers. Triangles show cummulative percent of antibody titers. Open marks show antibody titers of children without a fever and solid marks, those of children with a fever.

TABLE 2. *Mean HI antibody titers in different age groups of children receiving CAM-A4 measles vaccine*

Age	10–11mth	1yr	2yr	3yr	4–6yr	Mean
Antibody titer (log_2)	4.9	5.5	5.1	5.2	5.1	5.3

TABLE 3. *Incubation period of development of febrile reaction*

Days after vaccination	5	6	7	8	9	10	11	12	Total
No.	2	11	29	13	2	0	1	0	58
%	3.5	19.0	50.0	22.4	3.5	0	1.7	0	100

TABLE 4. *Incidence of febrile reactions in different age groups of children receiving CAM-A4 measles vaccine*

Age		10–11mth	1yr	2yr	3yr	4–6yr	Total
≥37.5C	Cases	1/8	29/56	15/32	6/15	7/22	58/133
	%	12.5	51.8	46.9	40.0	31.8	43.6
≥39.0C	Cases	0/8	3/56	4/32	2/15	2/22	11/133
	%	0	5.4	12.5	13.3	9.1	8.3

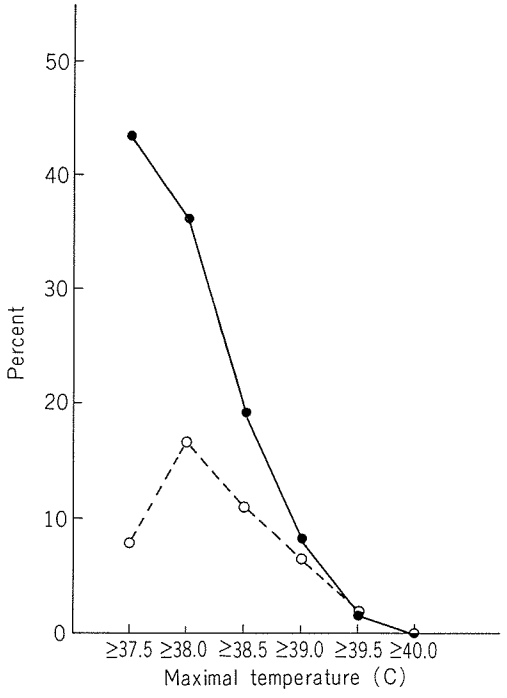

FIGURE 5. *Distribution* (○------○) *and cummulative percent* (●——●) *of maximal temperatures of children receiving CAM-A4 measles vaccine.*

Fifty eight of the 133 children developed a fever 5 to 12 days after vaccination. The incubation period of occurrence of a fever of over 37.5 C is shown in Table 3. It was observed 6 to 8 days after vaccination in more than 90% of the children.

TABLE 5. *Mean maximal temperatures in different age groups of children receiving CAM-A4 measles vaccine*

Age	10–11mth	1yr	2yr	3yr	4–6yr	Mean
Max. Temp. (C)	38.5	38.3	38.5	38.6	38.4	38.4

TABLE 6. *Duration of fever of over 37.5C*

Days	0.5	1.0	1.5	2.0	2.5	3.0	3.5	Total
No.	9	15	15	10	6	3	0	58
%	15.9	25.9	25.9	17.2	10.3	5.2	0	100

As shown in Fig. 5, 44% of the children had a fever of over 37.5 C, and 8% of the children had a fever of over 39.0 C. The peak of the distribution of the maximal temperatures of the children was between 38.0 C and 38.4 C.

The correlation between the age of the children and the incidence of a febrile reaction is shown in Table 4. The incidence of a febrile reaction of over 37.5 C was highest in children of one year old and decreased in older children, but it was the lowest in infants of under one year old. The incidence of a fever of over 39.0 C was highest in the 3 year old group and low in the one year old group, and a high fever was not observed in babies of 10 to 11

months old. The mean maximal temperature of children with a fever was the lowest in the one year old group and highest in the 3 year old group (Table 5).

The distribution of the duration of a fever of over 37.5 C is shown in Table 6. Fever lasted for a maximum of 3 days and usually only for one or 2 days.

The results are summarized in Table 7. The seroconversion rate was 100% and the geometric mean HI antibody titers was $2^{5.3}$. The incidence of a febrile reaction of over 37.5 C was 43.6% and that of a fever of over 39.0 C was 8.3%. A febrile reaction developed after an average of 7.1 days. The mean maximal temperature was 38.4 C and the mean duration of a fever of over 37.5 C was 1.5 days. A sporadic rash developed in 9% of the children and convulsions in only one of 133 children.

TABLE 7. *Summary of antibody responses and clinical reactions of healthy chidren vaccinated with CAM-A4 measles vaccine*

HI antibody response		
Seroconversion rate (%)	G.M. titer (log_2)	Range (log_2)
104/104 (100)	5.3	3–7

Clinical reactions						
Febrile reaction					Rash	Convulsions
≥37.5C	≥39.0C	Inc.	Max. T.	Dur. F.		
58/133 (43.6%)	11/133 (8.3%)	7.1 days	38.4 C	1.5 days	12/133 (9.0%)	1/133 (0.8%)

TABLE 8. *Summary of antibody responses and clinical reactions of healthy children vaccinated with mixture of live, measles and mumps vaccines*

Measles HI antibody response		
Seroconversion rate (%)	G.M. titer (log_2)	Range (log_2)
57/57 (100)	4.9	3–7

Mumps neutralizing antibody response		
Seroconversion rate (%)	G.M. titer (log_2)	Range (log_2)
57/57 (100)	2.9	1.0–5.0

Clinical reactions						
Febrile reaction					Rash	Convulsions
≥37.5C	≥39.0C	Inc.	Max. T.	Dur. F.		
18/65 (27.7%)	4/65 (6.2%)	7.1 days	38.6 C	1.5 days	2/65 (3.1%)	0/65 (0%)

2. *Vaccination with a mixture of CAM-A4 measles vaccine and live mumps vaccine*

Of the children who received a mixture of CAM-A4 measles vaccine and live mumps vaccine by injection, 65 were initially seronegative against both measles and mumps. Paired blood specimens were taken from 57 of the 65 seronegative children.

As shown in Table 8, the seroconversion rates with both measles and mumps vaccines were 100%. The geometric mean measles HI antibody titer was $2^{4.9}$ and the geometric mean mumps neutralizing antibody titer was $2^{2.9}$.

The mean values of the maximal temperature and duration of a fever in children who showed a febrile reaction were the same as those with measles vaccine alone, but the incidence of a febrile reaction of over 37.5 C was only 27.7%. This was much less than that caused by measles vaccine alone. As Yamanishi et al. reported (1970), live mumps vaccine did not cause any kind of clinical reactions.

DISCUSSION

The clinical and serological reactivity of the vaccine prepared from clone A4 of CAM-CEF measles vaccine virus was stable, and the vaccine was less reactive clinically than the parent CAM measles vaccine virus cultured in both chorioallantoic membrane and CEF. There was little *in vitro* marker closely relating to attenuation of measles virus, but cloning of the live measles vaccine virus was effective in selecting variants which were less clinically reactive but were highly immunogenic.

Buynak et al. (1969) reported that their live mumps virus vaccine, Jeryl Lynn vaccine when injected with Moraten measles vaccine did not increase the clinical reactions of the latter. However, on injection of our measles vaccine with live mumps virus vaccine, the indidence of a febrile reaction due to live measles vaccine was much reduced. Thus the live mumps vaccine virus used in the clinical test probably interfered with the febrile reaction due to the CAM-A4 measles vaccine or the CAM-A4 measles vaccine might be more attenuated than the Moraten measles vaccine.

Antibody responses of the children to the two vaccines were similar to those observed when these vaccines were given separately. Thus, immunization against both measles and mumps in a single injection seems beneficial.

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