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STUDIES ON FURTHER ATTENUATED LIVE MEASLES VACCINE
VII. DEVELOPMENT AND EVALUATION OF CAM-70 MEASLES VIRUS VACCINE

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SUMMARY The CAM-A4 further attenuated live measles vaccine virus was successfully adapted to grow in primary chick embryonic fibroblast cultures at 26C. The live measles virus vaccine prepared from the cold-adapted variant caused less febrile reaction in healthy children than the original vaccine when tested clinically. Of 206 children who were initially seronegative 65 (31.6%) developed a febrile reaction of over 37.5C one week after a subcutaneous injection of the newly developed vaccine. The vaccine elicited as good antibody responses as the original vaccine.

INTRODUCTION

There have been many reports on use of the cold adaptation procedure in selecting attenuated variants of several viruses for development of live virus vaccines for clinical use. The relationship between the ability to grow at a lowered temperature and the virulence has been studied with poliovirus (Plotkin et al., 1961), Japanese B encephalitis virus (Rohitayodhin and Hammon, 1962), measles virus (Hozinski et al., 1965) and influenza virus (Maassab, 1967).

The further attenuated live measles virus vaccine, CAM-A4 strain was prepared from a clone selected by plaque isolation at 36C (Takaku et al., 1970) and showed similar evidence of attenuation to the Schwarz strain of one of the further attenuated live measles vaccines prepared in America (Japan Measles Vaccine Research Commission, 1970; Ueda et al., 1970b). But the CAM-A4 strain still induced a ferbrile reaction in half the children tested when injected subcutaneously. So, attempts were made to adapt the strain to grow at a lowered temperature in primary chick embryo fibroblast cultures (CEF).

This report shows the results of a clinical

test on the cold-adapted variant of the CAM-A4 further attenuated live measles vaccine virus.

MATERIALS AND METHODS

1. *Seed virus for vaccine preparation*

Clone A4 of the CAM-CEF live measles vaccine virus (Takaku et al., 1970; Ueda et al., 1970b) was passaged 8 times in CEF at 26 C. This strain easily became adapted to grow in CEF at a lowered temperature.

A preliminary clinical test on the strain after two passages at the lower temperature in CEF showed that it caused fewer febrile reactions in healthy children than the parent virus.

2. *Vaccine*

A live measles virus vaccine was prepared from the cold-adapted strain of CAM-A4 measles vaccine virus after 8 passages at 26 C in CEF. This new vaccine was designated " CAM-70 " measles virus vaccine.

The lyophilized vaccine in an one dose ampoule was reconstituted with 0.5 ml of distilled water and had a titer of $10^{3.1}TCID_{50}$/0.1 ml. For vaccination a dose of 0.5 ml was injected subcutaneously.

3. *Vaccinees and vaccination*

The vaccinees were 9 months to 6 years old home-dwelling children with on history of measles in Suita City, Osaka and Nishinomiya City, Hyogo.

Vaccination was carried out between June, 1970 and January, 1971.

4. *Hemagglutination-inhibition (HI) test*

Blood specimens were collected on the day of vaccination and one month later. The HI antibody titers were measured by the micro-method (Ueda, 1971a). Data on children who were immune before vaccination were excluded in evaluating the results of this clinical test.

5. *Survey of clinical reactions*

A chart for clinical records (body temperature, rash, eye and nasal discharges, cough, diarrhoea, appetite, general condition etc.) was given to the parents on the day of vaccination, and the parents were asked to fill in the clinical record on their children every day for 3 weeks after vaccination. Axillary temperatures were taken once or twice a day and 4 times (8 a.m., and 1, 6 and 10 p.m.) when children developed a fever. If necessary, one of the authors was called in.

Other procedures used were as described previously (Ueda et al., 1970a).

RESULTS

1. *Clinical evaluation*

Table 1 shows the age distribution of the 206 children who were initially seronegative. About 90% of the immunized children was under 3 years old.

Sixty-five of the 206 children developed a fever 5 to 12 days after vaccination. The distribution of the days of onset of the febrile reaction is shown in Fig. 1, the median being day 7.

TABLE 1. *Age of children receiving " CAM-70 " measles vaccine*

Age	9–11 mo	1 yr	2 yr	3 yr	4–6 yr	Total
No.	28	90	42	23	23	206
%	13.6	43.7	20.4	11.2	11.2	100

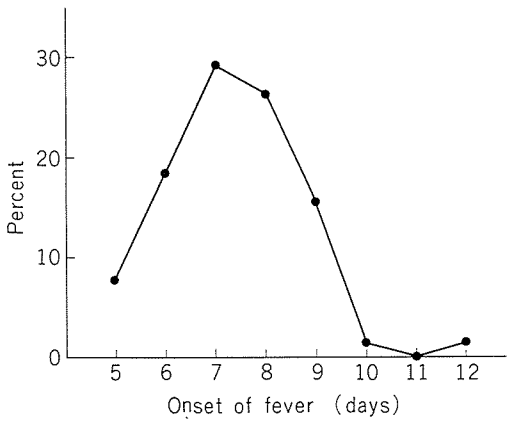

FIGURE 1. *Distribution of days of onset of febrile reactions following immunization with CAM-70 measles virus vaccine.*

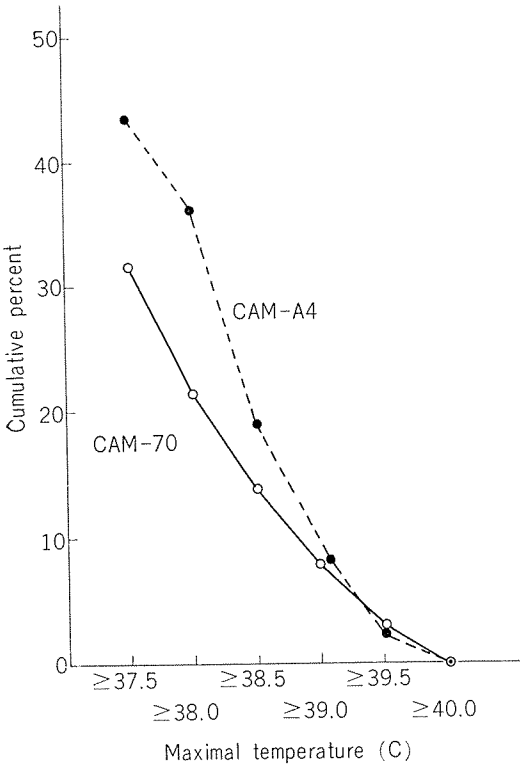

FIGURE 2. *Comparison of the cumulative percent of maximal temperatures following vaccination with CAM-70 and CAM-A4 measles virus vaccine.*

In Fig. 2 the cumulative percent of maximal temperatures of children who developed a ferbrile reaction, is compared with that in previous clinical tests with the original, CAM-A4 vaccine (Ueda et al., 1970b). The incidence of a ferbrile reaction of under 39.0 C was considerably less with the new vaccine, " CAM-70 ".

The cumulative percent of the duration of a fever of over 37.5 C is shown in Fig. 3. Of the children who developed a ferbrile reaction 90% recovered within at most 3 days.

The main clinical reactions other than a febrile reaction were a rash and febrile convulsions. A sporadic rash developed in 20 of the 206 children (9.7%) following febrile reactions (Table 2), and the rash disappeared in a day or two without pigmentation. Febrile convulsions occurred in a 19 month old girl when her body temperature rose to 38.0 C on the 8th day after vaccination.

Other clinical reactions such as malaise, anorexia or catarrh were observed in some children who developed febrile reactions, but they were so mild that the children recovered as soon after their temperature dropped.

TABLE 2. *Clinical reactions and HI antibody responses of children receiving " CAM-70 " measles vaccine*

Clinical reactions

Febrile reaction					Rash	Convuls.
≥37.5C	≥39.0C	Inc	Max. T.	Dur. F.		
65/206 (31.6%)	16/206 (7.8%)	7.4 days	38.4 C	1.6 days	20/206 (9.7%)	1/206 (0.5%)

HI antibody response

Seroconversion rate	G. M. titer (log_2)	Range (log_2)
163/164 (99.4%)	5.1	3–8

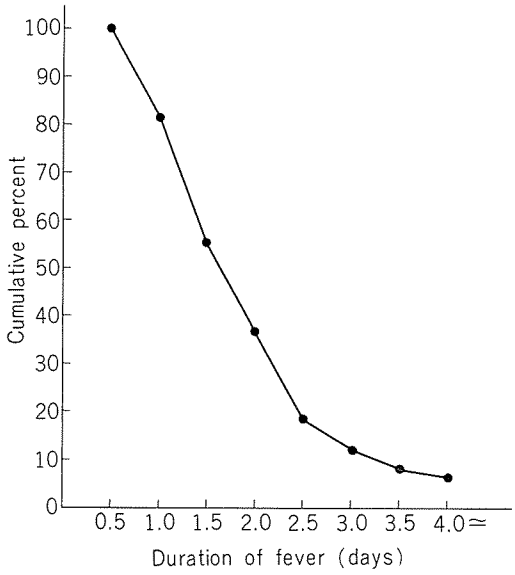

FIGURE 3. *Cumulative percent of the duration of a fever following immunization with CAM-70 measles virus vaccine.*

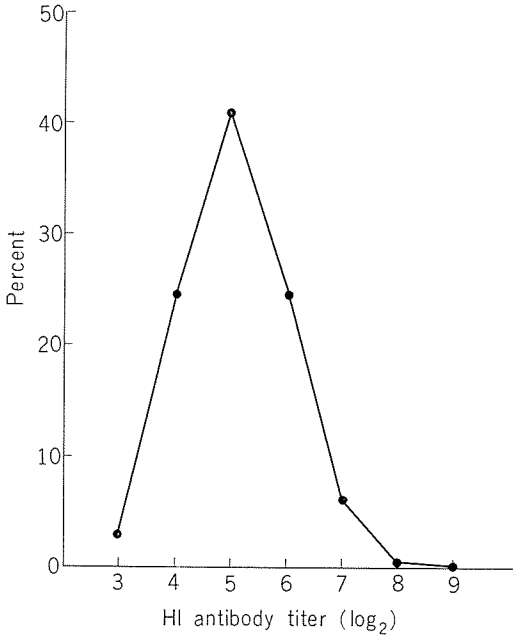

FIGURE 4. *Distribution of HI antibody titers of children one month after immunization with CAM-70 measles virus vaccine.*

2. *Serological evaluation*

Paired sera from 164 of the 206 children were examined for the HI antibody titers. Of the 164 children 163 (99.4%) responded to the vaccine and their HI antibody titers had risen 2^3 or more one month after the vaccination (Table 2).

Fig. 4 shows the distribution of the HI antibody titers. Of the children who responded to the vaccine 90% showed titers of between 2^4 and 2^6, and the median titer was 2^5. There was no difference between the HI antibody titers following immunization with the new "CAM-70" vaccine and with the original "CAM-A4" (Ueda et al., 1970b).

DISCUSSION

CAM measles virus vaccine, which had been further attenuated by adaptation to grow in the chorioallantoic membranes of developing chick embryos and purified by plaque isolation in CEF at 36 C (Takaku et al., 1970; Ueda et al., 1970a), caused febrile reactions in half the children vaccinated. Though it showed similar evidence of attenuation to Schwarz vaccine in comparable field trials carried out in 1969 by the Japan Measles Vaccine Research Commission (Japan Measles Vaccine Research Commission, 1970; Ueda et al., 1970b), this incidence of febrile reactions was much higher than that following vaccination by the KL method (Okuno et al., 1965, Ueda et al., 1966).

From studies on the effects of live virus vaccines in man, cold adaptation of viruses was reported to be effective in separating attenuated variants from virulent ones. Selected cold-adapted variants of poliovirus, Japanese B encephalitis virus, measles virus and influenza virus lost their virulence in appropriate experimental animals, such as monkeys and mice, or

lost their ability to grow at high temperature (Plotkin et al., 1961 ; Rohitayodhin and Hammon, 1962 ; Hozinski et al., 1966 ; Maassab, 1967).

CAM measles vaccine virus was readily adapted to grow at 26 C in CEF without stepwise lowering of the incubation temperature. This may be because the vaccine virus had previously been purified by plaque isolation. No animals are suitable for testing the virulence of measles virus. Monkeys are the only animals which can be infected with measles virus, but they show few symptoms of infection. So, the cold-adapted variant of CAM measles vaccine virus was tested clinically in healthy children after confirming that it was safe by tests in cynomolgus monkeys, as with the original vaccine.

As presented in this report, the incidence of a febrile reaction due to the new " CAM-70 " vaccine decreased to about 30%, whereas the original vaccine caused a febrile reaction in 50% of the children. However, there were no differences between the two vaccines in the mean values of the febrile reaction such as the maximal temperature or duration of fever in children who developed a febrile reaction. It is, at present, unknown why the intensity of the febrile reaction was not different, but it may be the result of that children may have the variety of threshold against a febrile reaction.

The antibody responses of the children receiving the " CAM-70 " measles vaccine were satisfactory for the following reasons: 1) the sero-conversion rate was over 99%, 2) the geometric mean HI antibody titer, measured by the micromethod, was 2^5, and this titer would be around 2^7–2^8 if measured by the neutralization test (Ueda et al., 1971a), 3) a follow-up study on the children immunized with the " CAM-A4 " measles vaccine showed that neutralizing antibodies induced by one injection of live measles virus vaccine were stable, as reported in the associated paper (Ueda et al., 1971b).

Development of this cold-adapted live measles virus vaccine " CAM-70 " which causes fewer clinical reactions than CAM-A4 and other strains and is highly immunogenic in healthy children, should facilitate much more prevention of measles than the present.

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