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STUDIES ON FURTHER ATTENUATED LIVE MEASLES VACCINE

III. SELECTION OF LESS REACTIVE VARIANTS OF CAM MEASLES VACCINE VIRUS

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SUMMARY CAM measles vaccine virus was adapted to chick embryo fibroblasts (CEF). When injected into healthy children subcutaneously, it caused a febrile reaction of over 37.5 C in 50 to 60% of the children.

Selection of less reactive variants of CAM measles vaccine virus cultured in CEF was attempted by picking out plaques on CEF. Nine clones were tested clinically and serologically on healthy children. The incidence of a febrile reaction of over 37.5 C varied from 36 to 81% with different clones. All the clones were highly immunogenic.

INTRODUCTION

The previous papers of this series reported the development of CAM measles live virus vaccine and it's immunogenicity (Ueda et al., 1970a, b). CAM measles vaccine caused fewer clinical reactions than our former vaccine from virus adapted to chick amnion, and it was highly immunogenic when injected at a dose of over 2500 $TCID_{50}$. But the incidence of febrile reactions ranged from 40 to 78% in several clinical tests.

As discussed previously, this might be due to heterogenicity of the vaccine virus, so attempts were made to adapt CAM measles vaccine virus to chick embryo fibroblasts (CEF). Then, selection of less reactive variants was attempted by isolation of plaques in CEF.

This report describes the results of clinical tests of CEF adapted CAM measles vaccine virus and of selected clones.

MATERIALS AND METHODS

1. *Cells*

Chick embryo fibroblasts (CEF) where used for preparation of vaccine and plaque formation of CAM measles vaccine virus.

BSC1 cells (established line of grivet monkey kidney cells) were used for plaque assay of clones of the vaccine virus. FL cells (established line of human amnion cells) were used for titration of virus and neutralizing antibody.

2. *Isolation of clones of CAM measles vaccine virus*

Monolayers of CEF in 50 ml bottles were inoculated with 0.2 ml of CAM measles vaccine virus cultured in CEF and diluted 10^{-4} fold (Exp. A) or 10^{-3} fold (Exp. B). The first overlay was with medium 199, containing 3% calf serum and 2.5% Bacto agar, and the second overlay 7 days later was with medium 199, containing 10% calf serum, 1.3% Bacto agar and neutral red diluted 1 : 12000. Three days after the second overlay 150 isolated plaques were picked up. Cultures were incubated at 36 C.

3. *Grouping of clones*

The plaque sizes of 75 clones were tested on BSC1 cells at 30, 35 and 40 C. Large (L, >2 mm), medium (M, 2–1 mm) and small (S, <1 mm) plaques were detected after 10 days incubation at each temperature. Rapp (1964) reported that attenuated measles virus formed smaller plaques on BSC1 cells than virulent virus, so clones were grouped according to the sizes of their plaques at the temperatures used for plaque assay. Table 1 shows the results of the grouping of the clones.

4. *Vaccines*

1) CEF adapted CAM measles virus vaccine (CAM-CEF measles vaccine)

CAM measles vaccine virus was passaged 3 times in CEF at 36 C. Three different batches of vaccine (a, b and c) were prepared from the fluids of CEF cultures inoculated with CAM measles vaccine virus.

2) Vaccine prepared from selected clones

Nine clones of CAM-CEF measles vaccine virus were prepared for test vaccines.

All the vaccines had virus titers of at least $10^{3.0}$ $TCID_{50}$/0.1 ml in FL cells, and were injected subcutaneously into healthy children at doses of 0.5 ml.

TABLE 1. *Plaque sizes on BSC1 cells at 30, 35 and 40 C of clones of CAM-CEF measles vaccine virus*

Plaque size at 30C	35C	40C	Clone				
L	L	L	A23[a]	A41	A43[a]	B36	
L	L	M	A32	B39	B59	B73	
L	M	M	B14				
L	M	(−)	A12[a]				
LM	LM	LM	A13	B1	B53	B101	
LM	LM	M	B67				
M	L	L	A9	A24[a]	B57	B84	
M	LM	L	A56	A62[a]	B94	B114	
M	LM	LM	A1	A16	A33	A57	B48
			B50	B109	B130	B139	
M	M	L	A4[a]	A52			
M	M	LM	A28	A40	B112	B120	
M	M	M	A6	A10	A15	A39	A59
			A64	B3	B5	B34	B42
			B47	B62	B65	B66	B75
			B88	B91	B100	B102	B115
			B117	B119	B122		
M	M	(−)	A29[a]	A38	A51	A53	B34
			B118				
S	M	M	B15	B18	B37	B83	B131
S	S	M	B7[a]				
S	M	(−)	B140[a]				

L : large (>2 mm) M : medium (2–1 mm) S : small (<1 mm) LM : mixed, L and M (−) : not visible
[a] used in clinical tests

3) ESC measles vaccine

A live measles virus vaccine further attenated in the USSR, ESC measles vaccine, was kindly supplied by Dr. M. P. Chumakov (1967) of the Institute of Poliomyelitis and Virus Encephalitides, USSR.

This vaccine was used as a control in clinical tests on the clone-vaccines.

5. *Serological examination*

Blood specimens were taken on the day of vaccination and 3 weeks later.

1) Neutralization test

Neutralizing antibody titers were measured by the overnight method (Toyoshima et al., 1965).

2) Hemagglutination-inhibition (HI) test

The HI test was done using Norrby type antigen (Norrby et al., 1962) and the Microtiter (Cooke

Engineering Co., Alexandria, VA., U.S.A.).

6. *Vaccination*

Vaccination was carried out between August and September, 1969. Doses of 0.5 ml of test vaccine were injected subcutaneously into healthy children with no history of measles aged 10 months to 6 years living with their families in Suita City, Osaka and Nishinomiya City, Hyogo.

Other materials and methods were as described in the previous papers (Ueda et al., 1970 a,b).

RESULTS

1. *Clinical reactions and serological response to CAM-CEF measles vaccine*

The results of clinical tests on 3 batches of CAM-CEF measles vaccines are summarized in Table 2. The incidence of febrile reactions of over 37.5 C was between 50 and 60%. The mean incubation period of development of a febrile reaction was 8 days after vaccination. The mean maximal axillary temperature was 38.6 C and the mean duration of the fever was 2 days. A sporadic rash appeared in 14% to 24% of the children who recieved the vaccine.

All the children showed a neutralizing antibody response and the geometric mean titer was between 2^6 and 2^7.

2. *Clinical tests with vaccines prepared from clones of CAM-CEF measles vaccine virus*

1) Serological response

As shown in Table 3, all the clone-vaccines were highly immunogenic and the geometric mean HI antibody titers were between $2^{4.3}$ and $2^{5.8}$. There was not much difference in the heights of HI antibody titers of children receiving different clone-vaccines. The geometric mean HI antibody titer of children receving ESC vaccine was $2^{6.1}$.

2) Clinical reactions

Before clinical tests on children, clones A23, A43, A24, A62 and A4 were suspected to be less

TABLE 3. *HI antibody response of children vaccinated with clone-vaccines*

Clone	Antibody response		
	Serocoversion rate (%)	G.M. Titer (log_2)	Range (log_2)
A 23	7/7[a] (100)	4.7	4–6
A 43	7/8 (87.5)	5.7	5–7
A 12	6/6 (100)	4.3	4–5
A 24	15/15 (100)	5.0	3–7
A 62	23/23 (100)	5.3	3–7
A 4	9/9 (100)	5.0	4–6
A 29	11/12 (91.7)	5.5	5–7
B 7	18/18 (100)	5.3	4–7
B140	10/10 (100)	5.8	4–7
ESC	13/13 (100)	6.1	5–7

a No. of children showing antibody response/No. of children vaccinated

TABLE 2. *Summary of results of clinical tests with CAM-CEF measles vaccine*

Vaccine	Neutralizing Antibody		Febrile Reaction				Rash
	Seroconversion rate	G.M. Titer (log_2)	≥37.5C	Inc.[a]	Max. T.[b]	Dur. F.[c]	
a	22/22[d] (100%)	6.2	15/26[d] (57.7%)	7.9	38.6	1.8	2/15[d] (13.3%)
b	24/24 (100)	7.1	17/35 (48.6)	8.2	38.6	2.3	4/17 (23.5)
c	9/9 (100)	7.0	7/12 (58.3)	8.2	38.7	1.8	1/7 (14.3)

a mean period before febrile reaction (days)
b mean maximal temperature (C)
c mean duration of fever (days)
d No. of cases/No. of children vaccinated

attenuated than clone B7. Clones A12, A29 and B140 were thought to be temperature sensitive variants which might be the most attenuated of the clones, judging from their plaque sizes at 40 C. But, as summarized in Table 4, the grade of the main clinical reactions (febrile reaction, development of a rash and convulsions) due to these clone viruses was not closely correlated with their plaque sizes on BSC1 cells.

The incidence of febrile reactions of over 37.5 C varied from 35.7% with clone A23, to 81.3% with clone A29, and that of over 39.0C varied from 0% with clone A23 to 37.6% with clone A29. The mean incubation period of development of febrile reactions was 7 days with all the clones. The mean maximal temperature varied from 38.2 C with clone B140 to 38.8 C with clones A12 and A29. The mean duration of a fever of over 37.5 C varied from 1.0 day with clones A4 and B7 to 2.4 days with clone A12.

None of the 12 children receiving clone A43 developed a rash. With the other clones the incidence of a sporadic rash varied from 9.1% of the children with clone A24 to 33.4% of the children with clone A12. Convulsions occurred in only one child who received clone A62.

With ESC vaccine, 8 of 16 children (50.0%) developed a fever of over 37.5 C and 2 of them (12.5%) had a high fever of over 39.0 C. The mean maximal temperature was 38.4 C. A rash appeared on 6 of 16 children (37.6%).

Curves of the cumulative percentages of the maximal temperatures of children receiving different test vaccines are shown in Fig. 1. Clone A29 caused the highest incidence of a high fever. Clones A43 and A62 frequently caused a febrile reaction. Clones A4, A12, A23, A24, B7 and B140 caused a febrile reaction less frequently and their curves were very similar. The curve of ESC vaccine was similar to those of clones A4, A12, A23, A24, B7 and B140.

TABLE 4. *Summary of clinical reactions to clone-vaccines*

Clone	Febrile reaction					Rash (%)	Convulsions (%)
	≥37.5C (%)	≥39.0C (%)	Inc.[a] (days)	Max. T.[b] (C)	Dur. F.[c] (days)		
A 23	5/14[d] (35.7)	0/14[d] (0)	6.8	38.4	1.1	3/14[d] (21.4)	0/14[d] (0)
A 43	9/12 (75.0)	1/12 (8.3)	7.1	38.3	1.4	0/12 (0)	0/12 (0)
A 12	7/15 (46.7)	2/15 (13.3)	6.6	38.8	2.4	5/15 (33.4)	0/15 (0)
A 24	9/22 (40.9)	2/22 (9.1)	7.7	38.6	1.2	2/22 (9.1)	0/22 (0)
A 62	28/46 (60.9)	8/46 (17.4)	7.0	38.6	1.3	5/46 (10.9)	1/46 (2.2)
A 4	7/17 (41.2)	2/17 (11.8)	7.3	38.4	1.0	2/17 (11.8)	0/17 (0)
A 29	13/16 (81.3)	6/16 (37.6)	6.7	38.8	1.5	3/16 (18.7)	0/16 (0)
B 7	8/20 (40.0)	1/20 (5.0)	7.5	38.4	1.0	2/20 (10.0)	0/20 (0)
B140	8/18 (44.4)	1/18 (5.6)	7.4	38.2	1.4	3/18 (16.7)	0/18 (0)
ESC	8/16 (50.0)	2/16 (12.5)	8.1	38.4	1.5	6/16 (37.6)	0/16 (0)

a mean period before febrile reaction
b mean maximal temperature
c mean duration of fever of over 37.5C
d No. of cases/No. of children vaccinated

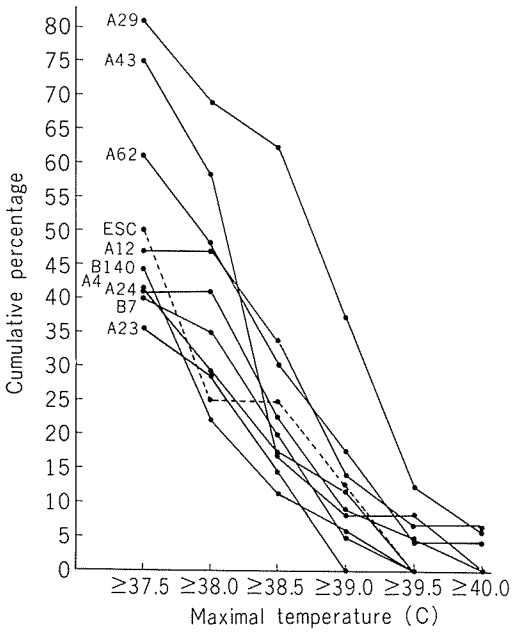

FIGURE 1. *Cumulative percentage of maximal temperatures of children receiving clone-vaccines.*
ESC measles vaccine (●------●) was used as a control in clinical tests on clone-vaccines.

DISCUSSION

The Tanabe strain of measles virus adapted to the amniotic membrane of chick embryos was attenuated further by adaptation to CAM. It caused a febrile reaction in 40 to 70% of the children in field trials, and this variation in the incidence of a febrile reaction was thought to result from contamination of the CAM vaccine material with back-mutated virus passing through the amniotic membrane (Ueda et al., 1970b).

To exclude this contamination of the CAM vaccine material, chick embryo fibroblast (CEF) cultures were used for preparation of vaccine. As shown in this paper, consistent results were obtained with 3 different batches of CAM-CEF vaccine.

However the incidence of a febrile reaction was still 50 to 60%. So, selection of more highly attenuated virus variants was attempted by picking out plaques on CEF. Mirchamsy and Rapp (1969) reported that attenuated measles virus (Schwarz strain) induced a higher titer of interferon than the unattenuated Edmonston strain, but we found it difficult to select more highly attenuated virus variants of attenuated virus by titration of interferon (unpublished data). Accordingly, plaque size on BSCl cell cultures at various temperatures was adopted as a marker of grouping and attenuation of clones of CAM-CEF measles vaccine virus. Before clinical tests, it was expected that clone A12, with a large plaque size at 30 C, small size at 35 C and no visible plaques at 40 C was a temperature sensitive mutant and might cause few reactions in children. However, clinical tests of the clones on children revealed that the plaque size on BSCl cell cultures at various temperatures was not closely related to the degree of attenuation of the measles virus.

In this study less reactive virus variants were selected by cloning of CAM-CEF measles vaccine virus and clinical tests of the clones on children. However, to develop much more highly attenuated virus vaccine the possibility of finding *in vitro* markers which are more closely related to reactivity in the human body should be studied.

Kanoh and Kawasaki (1966) reported that influenza virus induced leucocytic pyrogen on interacting with rabbit polymorphonuclear leucocytes. Furthermore, Nicols (1963) reported chromosome breakage by wild and attenuated measles virus, so if leucocytic pyrogen is induced by measles virus as well as by influenza virus it may be useful as an *in vitro* marker of the grade of attenuation of measles virus.

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