BIKEN JOURNAL Vol. 15, 173-177, 1972

DEVELOPMENT OF EXTREMELY ATTENUATED LIVE MEASLES VIRUS VACCINE (CAM-EX)

SHIGEHARU UEDA, MICHIAKI TAKAHASHI, TAKASHI KURIMURA, YOSHIICHI MINEKAWA, NORIMOTO SUZUKI, KOICHI YAMANISHI, KOICHI BABA and YOSHIOMI OKUNO

Department of Virology, Research Institute for Microbial Diseases, Osaka University, Yamada-kami, Suita, Osaka

TAKAHIRO SASADA, KOZABURO ONISHI, TAKEO KONOBE and KEISUKE TAKAKU

Kanonji Institute of the Research Foundation for Microbial Diseases of Osaka University, Kanonji, Kagawa

YOSHITSUGU YAMADA, YO TANAMI and FUMITSUGU KUSANO

Department of Bacteriology and Department of Public Health, Shinshu University Medical School, Matsumoto, Nagano

YASUSHI HAYAKAWA and TAKASHI KUROSE

Department of Pediatrics, Tsukaguchi Branch Hospital of Hyogo Prefectural Amagasaki Hospital, Amagasaki, Hyogo

(Received April 6, 1972)

SUMMARY Measles virus (Tanabe strain) was further attenuated by a combination of adaptation of the virus in chorioallantoic membranes of chick embryos, plaque cloning in chick embryo fibroblast (CEF) cultures and serial passages at low temperature in CEF. During this further attenuation a variant was obtained which caused extremely few febrile reactions but had sufficient immunogenicity.

In recent clinical tests in 1971, vaccine from this variant of the Tanabe strain of measles virus caused a febrile reaction in only about 10% of the children who received a single injection. Of the 401 children who were initially seronegative, 47 had a slight fever one week after vaccination. A neutralizing antibody response was detected in 341 of the 350 children (97.4%) from whom serum specimens were collected one month after vaccination, and the geometric mean titer of these sera was $2^{3.6}$.

INTRODUCTION

In the last ten years there has been much effort to attenuate measles virus. In 1960 three kinds of live attenuated measles virus vaccine were simultaneously developed in Japan, U.S.

A. and U.S.S.R. (Okuno et al., 1960; Enders et al., 1960; Smorodintsev et al., 1960).

Schwarz succeeded in further attenuation of measles virus in 1962 (Schwarz, 1962), and later further attenuated live measles virus vaccines were developed in several countries (Smorodintsev et al., 1965; Hendrickse et al., 1966; Chumakov, 1967; Hilleman et al., 1968; Ueda et al., 1970 a, b). Vaccination against measles has been done in many countries, but the further attenuated live measles virus vaccines used still cause a febrile reaction in at least about half the children vaccinated.

In our laboratory, work on attenuation of measles virus has been continued since Okuno and his group developed an attenuated live measles virus vaccine. A combination of methods were used for attenuation of measles virus (Tanabe strain): namely, adaptation in chorioallantoic membranes (CAM) of chick embryos (Ueda et al., 1970 a), plaque cloning in chick embryo fibroblast (CEF) cultures (Takaku et al., 1970) and serial passages at low temperature in CEF. As a result the CAM-70 further attenuated live measles virus vaccine was developed in 1971. This caused a febrile reaction in about 30% of the children given the vaccine (Okuno et al., 1971).

To study the limit of attenuation possible for measles virus retaining immunogenicity, we selected a temperature-sensitive variant from the seed virus of the CAM-70 measles virus vaccine, again by serial plaque cloning in CEF cultures at 36 C and passed it in CEF cultures at low temperature. A preliminary clinical test with this variant showed that it caused less febrile reaction in children than the vaccine of the parent virus. This report presents the results of clinical tests with the new vaccine made with the variant.

MATERIALS AND METHODS

1. *Vaccine*

A temperature-sensitive variant was selected from the seed virus of the CAM-70 measles virus vaccine by serial plaque clonings in CEF cultures at 36 C. This variant was passed in CEF cultures 3 times at 26 C to obtain a higher viral yield than that possible at 36 C. The first test vaccine was made of the variant at this passage level. A clincial test with this revealed that the incidence of a febrile reaction was less than with the vaccine of the parent, CAM-70 (Table 1, Lot No. K-72).

To examine the genetic stability of the variant, it was passed 10 to 20 times more in CEF cultures at 26 C. Six test vaccines were made of the virus at passage levels of 10 to 20 in CFF cultures.

All test vaccines were lyophilized in one dose ampoules, giving over $10^{3.5}$ $TCID_{50}$/0.1 ml when reconstituted with 0.5 ml of distilled water.

2. *Vaccinees and vaccination*

The vaccinees were children of 9 months to 6 years of age living in Osaka, Hyogo and Nagano Prefectures. They were injected subcutaneously in the upper arm with 0.5 ml of one of the vaccines.

Vaccinations were done between July, 1971 and February, 1972.

3. *Neutralizing antibody titration (NT)*

Serum specimens were collected on the day of vaccination and one month later. The neutralizing antibody titers were measured by the micro-method of Moreau and Furesz (Moreau and Furesz, 1967) as modified by one of us (Ueda, 1971). By changing the strain of challenge virus in the NT from the Toyoshima strain (Toyoshima et al., 1959) to the Nagahata strain, the period required for reading neutralizing antibody titers was shortened from one week to 4 days.

The Nagahata strain of measles virus was isolated in our laboratory in February, 1971 from a throat swab of an 18-month old boy (Koji Nagahata) with typical measles in Toyonaka, Osaka using primary human embryonic kidney cell (HEK) cultures. It was passed 3 times more in HEK cultures and then 11 times in FL cell cultures.

4. *Survey of clinical reactions*

Clinical reactions following vaccination were surveyed as described in a previous report (Okuno et al., 1971).

RESULTS

1. *Clinical reactions*

As previously reported (Okuno et al., 1971;

Ueda et al., 1970 b), the test vaccines were also evaluated clinically by the incidence and grade of febrile reactions, rash and febrile convulsions. As shown in Table 1, the test vaccines all caused febrile reactions in only about 10% of the vaccinees. Furthermore, fever was rarely over 39.0 C. The mean time of onset of a fever was one week after vaccination. There were no essential differences between the febrile reactions caused with the various test vaccines. So, all the results may be summarized as follows: the new measles virus vaccine caused a fever of 37.5 C or over in 47 of 401 children (11.7%) and the fever was 39.0 C or over in only 4 children (1.0%). The mean maximal body temperature was 38.2 C and the mean duration of fever was 1.4 days.

Fig. 1 shows a comparison of the maximal body temperatures induced by the vaccines from the new and parent strains. From the results of field trials conducted in 1969 the CAM-A4 measles vaccine (Ueda et al., 1970 b) had been evaluated as being similarly attenuated to the Schwarz strain (Japan Measles Vaccine Research Commission, 1970). The CAM-70 measles vaccine (Okuno et al., 1971) is one of the 2 licensed further attenuated live measles virus vaccine in Japan. Fig. 1 shows clearly that the incidence and the grade of a febrile reaction with the new vaccine (CAM-EX) were much less than those observed with previously developed vaccines.

A faint rash appeared in 4% of the children, and febrile convulsions in only one of the 401 children.

TABLE 1. *Clinical reactions and neutralizing antibody responses of children receiving "CAM-EX" live measles virus vaccine*

Lot No.	$TCID_{50}$ /dose	Febrile reaction					Rash	Febrile convulsions	Neutralizing antibody response	
		≥37.5C	≥39.0C	Onset[a]	Max. T.[b]	Dur. F.[c]			Seroconversion rate[d]	G. M. titer (2^n)
K-72	$5\times10^{3.5}$	8/51[e] (15.7)	1/51[e] (2.0)	7.1	38.3	1.4	1/51[e] (2.0)	0/51[e] (0)	34/34[e] (100)	5.0
K-79	$5\times10^{3.5}$	1/5 (20.0)	0/5 (0)	(8	38.5	2.5)	0/5 (0)	0/5 (0)	5/6 (83.3)	3.8
K-79	$5\times10^{3.5}$	22/128 (17.2)	1/128 (0.8)	7.2	38.1	1.5	13/128 (10.2)	0/128 (0)	113/115 (98.3)	3.0
K-80	$5\times10^{3.5}$	3/47 (6.4)	0/47 (0)	10.7	38.0	1.5	2/47 (4.3)	0/47 (0)	47/47 (100)	4.0
K-82	$5\times10^{3.8}$	1/16 (6.3)	0/16 (0)	(8	38.8	2.0)	0/16 (0)	0/16 (0)	11/11 (100)	4.5
K-83	$5\times10^{4.0}$	6/59 (10.2)	1/59 (1.7)	9.2	38.3	1.1	0/59 (0)	0/59 (0)	44/44 (100)	4.1
K-84	$5\times10^{4.0}$	2/58 (3.5)	0/58 (0)	9.0	38.1	1.5	0/58 (0)	0/58 (0)	58/60 (96.7)	3.1
K-85 (1 : 10)	$5\times10^{2.5}$	4/37 (10.8)	1/37 (2.7)	8.5	38.5	1.1	0/37 (0)	1/37 (2.7)	29/33 (87.9)	3.1
Total		47/401 (11.7)	4/401 (1.0)	7.9	38.2	1.4	16/401 (4.0)	1/401 (0.2)	341/350 (97.4)	3.6

a mean days; *b* mean maximal temperature (C); *c* mean duration of fever (days); *d* titers of 2^1 or more; *e* No. cases/No. surveyed or tested. Percentages are shown in parentheses.

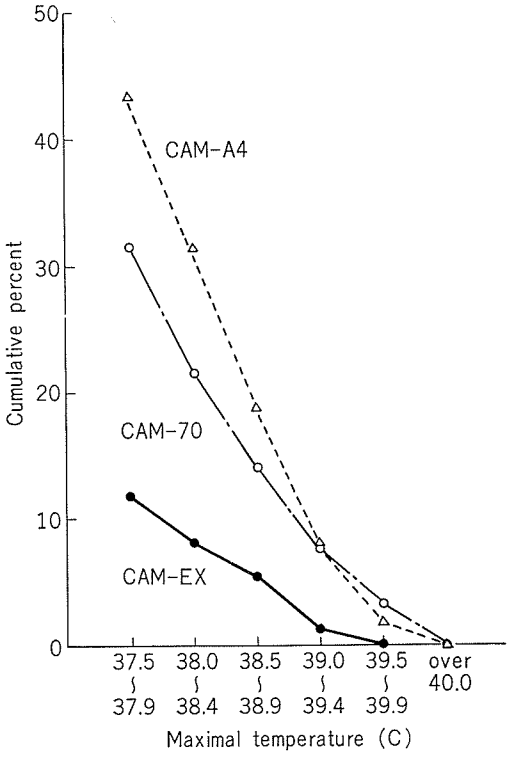

FIGURE 1. *Comparison of the cumulative percent of maximal body temperatures following vaccination with CAM-EX, CAM-70 and CAM-A4 measles virus vaccines.*

2. *Neutralizing antibody response*

Neutralizing antibodies (measured as a titer of one in 2 or over) were elicited in over 96.7% of the children one month after injection with 0.5 ml of any of the test vaccines. With only 1,500 $TCID_{50}$ of 10-fold diluted vaccine (Lot No. K-85), the seroconversion rate was 87.9% (Table 1).

Despite the good seroconversion rate, neutralizing antibody titers were rather low, the geometric mean titer being only $2^{3.6}$. Fig. 2 shows the distribution of neutralizing antibody titers. About 30% of the 350 children showed titers of one in 4 or less.

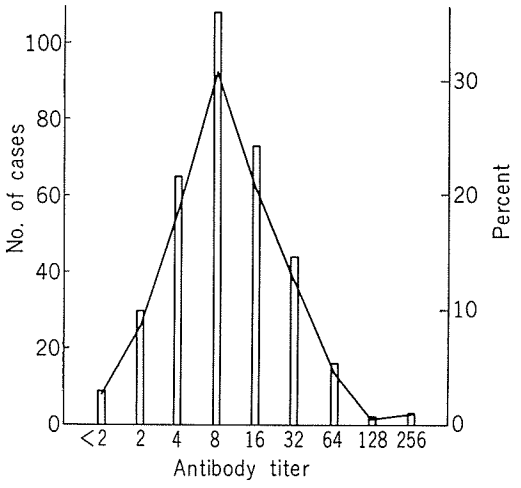

FIGURE 2. *Distribution of neutralizing antibody titers of the 350 children one month after immunization with CAM-EX measles virus vaccine.* Symbols: ▭, *No. of cases;* ●——●, *Percent.*

DISCUSSION

During attenuation of measles virus, we obtained a variant which caused a febrile reaction in only about 10% of the children vaccinated and which was genetically stable for at least 20 serial passages in CEF cultures. Judging from the incidence of febrile reactions this variant may well be at the upper limit of attenuation of measles virus possible for preparation of attenuated vaccine. So, the newly developed vaccine of this variant was designated as " CAM-EX ", meaning extremely attenuated CAM live measles virus vaccine.

The CAM-EX measles virus vaccine elicited neutralizing antibodies in over 97% of the children given a single injection. However, the antibody titers were rather low and about 30% of the children showed a titer of only one in 4 or less. Because of this, it seems that several years clinical and serological follow-up of the vaccinated children is necessary before this vaccine can be evaluated. But, if the relatively low antibody titer of the neutralizing antibodies elicited with this vaccine is found to

persist like those with other attenuated live measles virus vaccines, " CAM-EX " measles virus vaccine may be the most suitable vaccine for use in a mass vaccination to eradicate measles.

ACKNOWLEDGEMENTS

We wish to thank Dr. Toshio Kondo (Pediatric Clinic, Toyonaka, Osaka) for suppling materials for isolation of measles virus and Misses Etsuko Oda, Michiko Okada and Mieko Takiuchi for excellent technical assistance.

REFERENCES

Chumakov, M. P. 1967. Live measles vaccine from a highly attenuated strain ESC (Enders-Schwarz-Chumakov's) prepared in Inst. of Poliomyelitis and Virus Encephalitides, AMS, USSR. Trans. USSR AMS Inst. Poliomyel. and Virus Encephalit. 9: 1–67.

Enders, J. F., S. L. Katz, M. V. Milovanovic, and A. Holloway. 1960. Studies on an attenuated measles-virus vaccine. I. Development and preparation of the vaccine: Technics for assay of effects of vaccination. New Engl. J. Med. 263: 153–159.

Hendrickse, R. G., D. Montefiore, P. Sherman, and T. Peradze. 1966. Comparative trial of further attenuated measles vaccines. Brit. Med. J. 1: 779–781.

Hilleman, M. R., E. B. Buynak, R. E. Weibel, J. Stokes, Jr., J. E. Whitman, Jr., and M. B. Leagus. 1968. Development and evaluation of the Moraten measles virus vaccine. J. Amer. Med. Ass. 206: 587–590.

Japan Measles Vaccine Research Commission. 1970. A report on the evaluation of further attenuated live measles virus vaccines in Japan (in Japanese).

Moreau, P., and J. Furesz. 1967. A rapid micro tissue culture assay in BS-C-1 cells for the titration and neutralization of measles virus. Can. J. Microbiol. 13: 313–319.

Okuno, Y., T. Sugai, K. Toyoshima, M. Takahashi, T. Yamamura, S. Hata, T. Miki, K. Nakamura, S. Ueda, and N. Kunita. 1960. Studies on the prophylaxis of measles with attenuated living virus. IV. Inoculation tests in children with chick embryo passage measles virus in 1960. Biken's J. 3: 293–300.

Okuno, Y., S. Ueda, T. Kurimura, N. Suzuki, K. Yamanishi, K. Baba, M. Takahashi, T. Konobe, T. Sasada, K. Onishi, and K. Takaku. 1971. Studies on further attenuated live measles vaccine. VII. Development and evaluation of CMA-70 measles virus vaccine. Biken J. 14: 253–258.

Schwarz, A. J. F. 1962. Preliminary tests of a highly attenuated measles vaccine. Amer. J. Dis. Child. 103: 386–389.

Smorodintsev, A. A., L. M. Boichuk, E. S. Shikina, T. B. Batanova, L. V. Bystryakova, and T. V. Peradze. 1960. Clinical and immunological response to live tissue culture vaccine against measles. Acta Virol. 4: 201–214.

Smorodintsev, A. A., L. M. Boychuk, E. S. Shikina, V. N. Meshalova, L. Y. Taros, M. G. Aminova, N. D. Revenok, and D. I. Safarov. 1965. Prevention of measles by use of live vaccines in the U.S.S.R. Arch. Ges. Virusforsch. 16: 284–293.

Takaku, K., T. Sasada, T. Konobe, K. Onishi, S. Ueda, M. Takahashi, Y. Minekawa, T. Ogino, N. Suzuki, K. Yamanishi, K. Baba, and Y. Okuno. 1970. Studies on further attenuated live measles vaccine. III. Selection of less reactive variants of CAM measles vaccine virus. Biken J. 13: 163–168.

Toyoshima, K., M. Takahashi, S. Hata, N. Kunita, and Y. Okuno. 1959. Virological studies on measles virus. I. Isolation of measles virus using FL cells and immunological properties of the isolated agents. Biken's J. 2: 305–312.

Ueda, S., M. Takahashi, Y. Minekawa, T. Ogino, N. Suzuki, K. Yamanishi, K. Baba, Y. Okuno, T. Konobe, T. Sasada, and K. Takaku. 1970a. Studies on further attenuated live measles vaccine. I. Adaptation of measles virus to the chorioallantoic membrane of chick embryo and clinical tests on the strain. Biken J. 13: 111–116.

Ueda, S., M. Takahashi, T. Kurimura, T. Ogino, N. Suzuki, K. Yamanishi, K. Baba, Y. Okuno, T. Konobe, T. Sasada, K. Onishi, and K. Takaku. 1970b. Studies on further attenuated live measles vaccine. IV. Clinical and serological evaluation of a clone of CAM-CEF measles vaccine virus. Biken J. 13: 169–174.

Ueda, S. 1971. Comparison of measles antibody titers measured by the micro- and macro-methods. Biken J. 14: 155–160.