



Title	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
Author(s)	Ueda, Shigeharu; Takahashi, Michiaki; Minekawa, Yoshiichi et al.
Citation	Biken journal : journal of Research Institute for Microbial Diseases. 1970, 13(2), p. 111-116
Version Type	VoR
URL	https://doi.org/10.18910/82806
rights	
Note	

The University of Osaka Institutional Knowledge Archive : OUKA

<https://ir.library.osaka-u.ac.jp/>

The University of Osaka

STUDIES ON FURTHER ATTENUATED LIVE MEASLES VACCINE

I. ADAPTATION OF MEASLES VIRUS TO THE CHORIOAL- LANTOIC MEMBRANE OF CHICK EMBRYO AND CLINICAL TESTS ON THE STRAIN¹

SHIGAHARU UEDA, MICHIAKI TAKAHASHI, YOSHIICHI
MINEKAWA, TAKEO OGINO, NORIMOTO SUZUKI, KOICHI
YAMANISHI, KOICHI BABA and YOSHIOMI OKUNO

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

TAKEO KONOBE, TAKAHIRO SASADA and KEISUKE TAKAKU

Department of Measles Vaccine, Kanonji Institute of the Research Foundation for Micro-
bial Diseases of Osaka University, Kanonji, Kagawa

(Received February 28, 1970)

SUMMARY Further attenuation of live measles vaccine virus was achieved by adap-
tation of measles virus to the chorioallantoic membrane of chick embryo. A good
antibody response was observed in all children vaccinated with this virus. About
half the children vaccinated showed no clinical reactions.

INTRODUCTION

Live, attenuated measles vaccine of the Toyo-
shima strain adapted to the amniotic membrane
of chick embryos is not widely used because it
causes vaccination measles, with fever in espe-
cially high frequency (Okuno et al., 1960;
The Japanese Committee for the Study of
Measles Vaccine, 1963). Accordingly this
measles was modified by the KL method
(Okuno et al., 1965a, 1965b; The Japanese
Committee for the Study of Measles Vaccine,
1965; Ueda et al., 1966). Killed measles

vaccine (K) and live measles vaccine (L) were
licensed in 1966 in Japan. Children re-
ceiving KL showed good persistence of im-
munity against measles (Ueda et al., 1969).

Studies were made on the possibility of using
live measles vaccine alone. Taniguchi and his
associates prepared live measles vaccine from
the chorioallantoic membrane of chick embryos
(CAM) and tested it in healthy children.
The clinical reactions of these children were
mild or undetectable, but the quantitation of
the vaccine virus was difficult (Taniguchi et al.,
1956).

This paper presents results on the adaptation
of measles virus to CAM and on the clinical

1. A summary of these results was reported at the
16th Annual Meeting of the Japanese Virologists
in Fukuoka, October, 1968.

use of the CAM adapted strain.

MATERIALS AND METHODS

1. Measles Virus

1) Toyoshima strain

Toyoshima strain isolated by Dr. K. Toyoshima (1959) was adapted to the amniotic membrane of chick embryos (Am) and was attenuated by 90 passages in Am.

2) Tanabe strain

Tanabe strain isolated by Dr. M. Mutai (Research Institute of Yakult, Inc.) using primary monkey kidney (MK) cell cultures was subjected to 3 passages in MK cell cultures, 8 passages in primary human embryonic kidney cell cultures and 90 passages in Am.

Small scale clinical test on the Am-adapted Tanabe strain revealed that clinical reactions and antibody responses of children vaccinated with it were similar to those caused with the Am-adapted Toyoshima strain (unpublished data).

2. CAM

Seven-day old CAM in an artificial air sack was used for propagation of attenuated measles virus. Chick eggs free from avian leucosis virus were imported from the Kimber Farm, USA.

3. Preparation of Test Vaccine

CAM was inoculated with 0.2 ml of the Toyoshima strain or Tanabe strain ($10^{4.5}$ TCID₅₀/0.1 ml, each). It was then incubated for 5 to 7 days at 36 C and harvested and a 20% homogenate was made with phosphate buffered saline. The supernatant fluid obtained by low speed centrifugation of the homogenate was clarified by treatment with activated charcoal. The clarified fluid was mixed with stabilizer and antibiotics and was stored at -70 C. Before use as vaccine, the safety, identity, potency and other qualities of the fluid were checked following the national regulations on live measles vaccine.

4. Cells

Established human amnion (FL) cells were used for titration of virus and neutralizing antibody.

5. Vaccination

Healthy children living at home with no history of measles were vaccinated by inhalation of vaccine for one min using a routine type compressor with a specially devised nebulizer.

6. Collection of Clinical Data

Parents were given a clinical questionnaire on the day of vaccination and were asked to take the axillary temperature one to four times daily (at 8, 13, 18 and 22 o'clock when their children had a fever) for 3 weeks. They were also asked to record other clinical observations.

7. Serological Test

Blood specimens were taken from vaccinated children on the day of vaccination and 4 weeks later. The paired sera were tested simultaneously by the overnight neutralization test (Toyoshima et al., 1965). Children who were initially seropositive (with a neutralizing antibody titer of over 2^2) were not included in the survey.

RESULTS

1. Adaptation of Toyoshima Strain to CAM

Studies were made on the possibility of adapting the Toyoshima strain of attenuated measles virus to CAM. The virus titers on serial passages in CAM and antibody responses of children receiving the strain passaged in CAM are shown in Fig. 1. The virus titers were $10^{2.5}$ TCID₅₀/0.1 ml in the first 4 passages. Then the titer dropped to $10^{1.5}$ TCID₅₀/0.1 ml in the fifth passage and no cytopathogenic effect of measles virus was detectable in FL cells after the sixth passage.

Clinical tests on the strain after those or more passages in CAM were made on groups of 5 to 10 children. With virus in the fifth passage

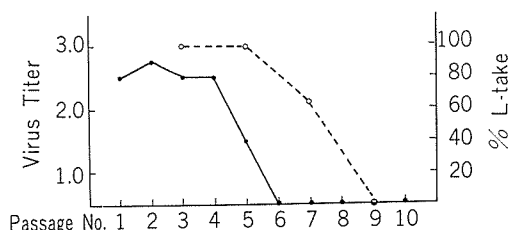


FIGURE 1. Virus titers of Toyoshima strain during passages in CAM and the seroconversion rate of children receiving Toyoshima strain passaged in CAM.

●—● virus titer in TCID₅₀/0.1 ml (log₁₀)
○-----○ percent seroconversion rate

the clinical reactions were milder than those due to Am-adapted Toyoshima strain. No virus was detected in FL cells after the sixth passage but clinical tests were continued to the ninth passage because CAM became edematous after inoculation of measles virus. Using virus in the seventh passage about half the children vaccinated showed neither symptoms nor an antibody response. With virus in the ninth passage no children showed any response.

2. Adaptation of Tanabe Strain to CAM

Adaptation of Am-adapted Toyoshima strain to CAM was not achieved, so Am-adapted, attenuated Tanabe strain was used instead. The virus titers on serial passages in CAM are shown in Fig. 2. Virus titers were low in the first few passages as with the Toyoshima strain.

After the sixth passage in CAM, the virus harvested from CAM was back-inoculated into the amniotic cavity of chick embryos. The

virus was harvested from Am and again inoculated onto CAM, but the virus titer dropped to $10^{0.85}$ TCID₅₀/0.1 ml. So, virus in the seventh passage in CAM was again back-inoculated into the amniotic cavity. After the second back-inoculation into the amniotic cavity, the virus titers became higher than those before back-inoculation into the amniotic cavity. The third back-inoculation into the amniotic cavity was done between the eleventh and twelfth passage in CAM. The virus titers, which had increased on back-inoculation into the amniotic cavity, decreased during serial passages in CAM till the twenty-third passage. After the twenty-fourth passage the virus titers became stable at the high value of about $10^{4.5}$ TCID₅₀/0.1 ml.

Two test vaccines were prepared with virus in the fifteenth and thirty-second passages, respectively, in CAM.

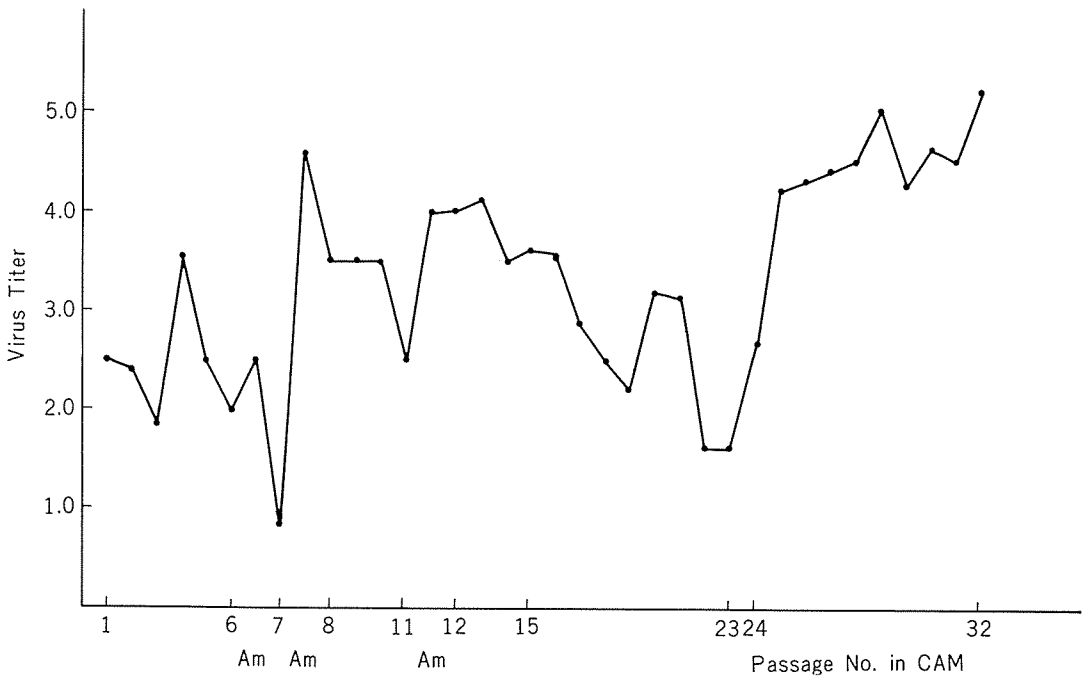


FIGURE 2. Virus titers of Tanabe strain during passages in CAM. Virus titer, expressed in TCID₅₀/0.1 ml (log₁₀). Am, back-inoculation of CAM passaged virus into the amniotic cavity.

3. Clinical Tests with Tanabe Strain in the 15th passage in CAM (CAM 15)

Vaccination was carried out in March, 1968.

1) Antibody response

The antibody responses of 11 of the 25 seronegative children were tested using paired sera. The seroconversion rate was 11/11 (100%). The neutralizing antibody titers ranged between $2^{7.5}$ and $2^{10.5}$. The geometric mean antibody titer was $2^{9.7}$.

2) Clinical reactions

The main clinical reactions due to the test vaccine were a febrile reaction and rash. Malaise and loss of appetite were observed in children with high fever, but these symptoms were in general mild. Catarrh symptoms were negligible.

As shown in Table 1, all the children developed a fever of over 37.5°C, but only 6 of the 25 children (24.0%) had a fever of over 39.5°C. The mean incubation period was 8.9 days. The mean maximal temperature of the children with fever was 38.8°C and the mean duration of fever was 2.6 days. A mild rash was observed in 10 of the 25 children (40.0%).

TABLE 1. Incidence of fever after vaccination with CAM 15

Maximal temperature (C)	No. of children	%
≥39.5	6	24.0
38.5-39.4	9	36.0
37.5-38.4	10	40.0
≤37.4	0	0
Total	25	100

4. Clinical Test with Tanabe Strain in the 32nd passage in CAM (CAM 32)

Vaccination was carried out in July, 1968.

1) Antibody response

The antibody responses of 14 of the 17 seronegative children were tested using paired sera. Seroconversion was observed in all the

children. Their neutralizing antibody titers ranged between $2^{6.5}$ and $2^{12.5}$ and the geometric mean titer was $2^{8.4}$.

2) Clinical reactions

Results on development of a febrile reaction are shown in Table 2. Nine of the 17 children (52.9%) had no fever. Only 2 of the 17 children (11.8%) had a fever of over 39.5°C. The mean incubation period was 9.7 days. The mean maximal temperature of the children with fever was 38.6°C and the mean duration of the fever was 2.2 days. No children developed a rash.

TABLE 2. Incidence of fever after vaccination with CAM 32

Maximal temperature (C)	No. of children	%
≥39.5	2	11.8
38.5-39.4	4	23.6
37.5-38.4	2	11.8
≤37.4	9	52.9
Total	17	100

DISCUSSION

Further attenuation of live, attenuated measles vaccine virus has been achieved in other countries (Schwarz, 1964; Smorodintsev et al., 1965; Chumakov, 1967; Hilleman et al., 1968). In Japan, Taniguchi et al. (1956) observed much milder clinical reactions than normal when they inoculated children with live measles virus cultured in the chorioallantoic cavity of chick embryos. However, unfortunately the virus disappeared during serial passages in the chorioallantoic cavity.

We first used the method of Taniguchi et al. in attempts to achieve further attenuation of the Toyoshima strain of attenuated measles virus. But cultivation of the measles virus in the chorioallantoic cavity of chick embryos was not achieved. Then, we attempted to inoculate measles virus directly onto the chorio-

allantoic membrane in an artificial air sack. As described in the text, the Toyoshima strain of measles virus disappeared after the sixth passage in CAM, though clinical reactions due to the test vaccine in the fifth passage in CAM were milder than those due to the original Am-adapted vaccine.

On changing from the Toyoshima strain to the Tanabe strain, we were able to continue passage of measles virus in CAM. Back-inoculation of the virus into the amniotic cavity of chick embryos seemed to improve the cultivation of the virus in CAM.

As shown in the text, the virus titers in CAM became stable at a high level after the twenty-fourth passage in CAM. This suggests that the virus became completely adapted to CAM after the twenty-fourth passage. From com-

parison of the results of clinical tests with CAM 15 and CAM 32 vaccines, it seems that further attenuation occurred on complete adaptation of measles virus to CAM. We did not use a low temperature for further attenuation of the measles virus. A low temperature in cultivation of measles virus may be beneficial in causing further attenuation, but from our results it does not seem to be essential.

ACKNOWLEDGEMENTS

We express our gratitude to Prof. Tsunehisa Amano (Dept. of Bacteriology and Immunochemistry) and Dr. Kumao Toyoshima (Dept. of Tumor Viruses) for their valuable advice and to Dr. Masahiko Mutai (Research Institute of Yakult, Inc.) for kindly supplying the Tanabe strain of measles virus passaged in MK.

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.
- Hilleman, M. R., E. B. Buynak, R. E. Weibel, J. Stokes, Jr., J. E. Whitman and M. B. Leagus. 1968. Development and evaluation of the Moraten measles virus vaccine. J. Amer. Med. Assoc. 206: 587-590.
- 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, H. Hosai, T. Kitawaki, K. Nakamura, T. P. Chiang, S. Okabe, M. Onaka and K. Toyoshima. 1965. Studies on the combined use of killed and live measles vaccines. I. Correlation between antibody titers following injection of killed vaccine and clinical reactions after live vaccine inoculation. Biken J., 8: 73-79.
- Okuno, Y., S. Ueda, H. Hosai, T. Kitawaki, K. Nakamura, T. P. Chiang, S. Okabe, M. Onaka and K. Toyoshima. 1965. Studies on the combined use of killed and live measles vaccines. II. Advantages of the inhalation method. Biken J., 8: 81-85.
- Schwarz, A. J. F. 1964. Immunization against measles. Development and evaluation of a highly attenuated live measles vaccine. Ann. Pediat., 202: 241-252.
- Smorodintsev, A. A., L. M. Boychuk, E. S. Shikina, V. N. Meshalova, L. Y. Taros, M. G. Amonova, N. D. Revenok and D. I. Safarov. 1965. Prevention of measles by use of live vaccines in the USSR. Arch. ges. Virusforsch., 16: 284-293.
- Taniguchi, T., Y. Okuno, A. Aoyama and K. Kusumoto. 1956. Detection of measles virus in egg culture and studies on the prophylactic inoculation with living measles virus. Med. J. Osaka Univ., 6: 1013-1022.
- The Japanese Committee for the Study of Measles Vaccine. 1963. The first report on the measles vaccine. (In Japanese).
- The Japanese Committee for the Study of Measles Vaccine. 1965. The third report on the measles vaccine. (In Japanese).
- 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.
- Toyoshima, K., T. Kitawaki, K. Otsu, M. Mutai, S. Omura and N. Kunita. 1965. Studies on the serological standardization of measles virus. Biken J., 8 : 87-94.
- Ueda, S., H. Hosai, Y. Minekawa and Y. Okuno. 1966. Studies on the combined use of killed and live measles vaccines. III. Conditions for the "take" of live vaccine. Biken J., 9 : 97-101.
- Ueda, S., M. Takahashi, H. Hosai, K. Yamanishi and Y. Okuno. 1969. Studies on the combined use of killed and live measles vaccines. IV. Four years follow-up. Biken J., 12 : 69-75.