OUKA
The University of Osaka
Institutional Knowledge Archive

Title	Studies on the Prophylaxis of Measles with Attenuated Living Virus IV. Inoculation Tests in Children with Chick Embryo Passage Measles Virus in 1960
Author(s)	Okuno, Yoshiomi; Sugai, Toshiji; Toyoshima, Kumao et al.
Citation	Biken's journal : journal of the Research Institute for Microbial Diseases. 1960, 3(4), p. 293-300
Version Type	VoR
URL	https://doi.org/10.18910/83077
rights	
Note	

The University of Osaka Institutional Knowledge Archive : OUKA

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

The University of Osaka

BIKEN'S JOURNAL 3, 293-300 (1960)

Studies on the Prophylaxis of Measles with Attenuated Living Virus
IV. Inoculation Tests in Children with Chick Embryo Passage Measles Virus in 1960

YOSHIOMI OKUNO, TOSHIJI SUGAI, KUMAO TOYOSHIMA, MICHIAKI TAKAHASHI, TOMOYOSHI YAMAMURA, SEICHIRO HATA, TAKASHI MIKI, KANZEN NAKAMURA, SHIGEHARU UEDA AND NOBUHARU KUNITA

Department of Virology, The Research Institute for Microbial Diseases, Osaka University, Osaka
(Received for publication, November 30, 1960)

SUMMARY

Considerably large scale field trials in an isolated village and a group of apartment houses were carried out with our egg-passaged measles virus. The inhalation method was employed throughout.

Second and later generation chorioallantoic passaged virus caused no signs of illness, but no uniform antibody response was obtained. Furthermore the result varied markedly from batch to batch of virus preparation.

On the contrary, modified measles virus obtained by passage in the chorioallantoic cavity after 26 serial passages in the amniotic cavity produced a high level of measles antibody in nearly all the vaccinated children. Vaccination, however, was followed by clinical responses, that is "vaccination measles," as we reported previously. The symptoms of "vaccination measles" were a considerably high fever and rash, but with no symptoms of catahrr or Koplik spots, and the symptoms were milder than those of typical measles.

INTRODUCTION

Our previous paper (Okuno *et al.*, 1960c) reported on inoculation tests in man and monkeys with chick embryo passaged measles virus.

The first generation of chorioallantois passaged virus material represented so-called "vaccination measles" in some of the inoculated children with remarkable antibody response.

On the contrary, no clinical symptoms were observed with second or later generation virus material, being followed by poor antibody response.

In monkeys, both the second chorioallantois passaged and 26th amniotic passaged measles virus preparations elicited remarkable antibody responses without appreciable clinical manifestations when administered in a large dose by the inhalation or the injection method.

Consequently in the present study, larger scale field trials were carried out with chorioallantoic passaged and modified amniotic passaged measles virus preparations. When a definite dose of modified amniotic passaged virus was given to children by the inhalation method, nearly all children showed symptoms of "vaccination measles,"

as reported in our previous paper (Okuno *et al.*, 1960c), and these were followed without exception by a strong antibody response.

MATERIALS AND METHODS

1. *Measles virus passaged in the amniotic cavity and subsequently in the allantoic cavity*

As described in the previous paper (Okuno *et al.*, 1960b), the Toyoshima strain of measles virus was cultivated serially in the amniotic cavity.

Introduction of passaged virus material from each generation into tissue cultures of FL cells, resulted in typical cytopathic changes indicative of multiplication of the measles virus. Infectivity titration of passaged virus was occasionally made using FL cells and a titer of 10^{-4}/0.2 ml $TCID_{50}$ was consistently obtained.

After the 26th passage, a new collateral serial cultivation in the chorioallantoic cavity was also attempted. In this passage, both the amniotic and chorioallantoic membrane were harvested and mixed with chorioallantoic fluid. The mixture was homogenized and centrifuged at 3,000 rpm for 15 minutes and the supernatant was used for serial passage. The 6th generation thus chorioallantoic passaged measles virus was used in the field trial.

Virus derived from this new passage line also showed definite cytopathic effects on FL cells, and a titer of 10^{-4}/0.2 ml $TCID_{50}$ was obtained in occasional titrations.

The interference with mumps virus was also tested in the allantoic cavity of chick embryos and positive results were consistently obtained.

2. *Measles virus passaged in the chorioallantoic cavity*

As described previously, measles virus of Toyoshima strain was passaged in the chorioallantoic cavity of 7 day eggs. The infected chorioallantoic membrane-fluid mixtures were homogenized and centrifuged at 3,000 rpm for 15 minutes and the supernatant was used for the passage. As the virus material thus passaged was noncytopathogenic for tissue culture cells after the second generation, strict quantitative measurement of the virus dose was difficult. The second and third generation passaged virus were employed in the field trials.

3. *Subjects for vaccination*

An isolated village and a group of apartment houses in a city were chosen for the field trial.

Children having no history of measles received vaccination with their parents' consent. These children were between 6 months and more than 12 years of age. After vaccination, house-to-house checks by physicians were made for reactions.

4. *Method of vaccination*

The inhalation method was exclusively employed for vaccination, as it had been proved in vaccination experiments with attenuated living influenza virus (Okuno *et al.*, 1960a) that this method was simple and gave a more uniform antibody response than the instillation method. In practice, the virus suspension was sprayed out of a nebulizer fitted with a compressor at a speed of approximately 0.1 ml per minute. The children breathed this for a definite time.

5. *Serological examination*

Blood was collected immediately after and one month after vaccination, and pairs of serum were tested by the following serological reactions.

i) *The complement fixation test*

The complement fixation test was carried out as described previously (Toyoshima *et al.*, 1960)

ii) *The neutralization test*

The same volume of two-fold serial dilutions of inactivated serum and diluted virus solution con-

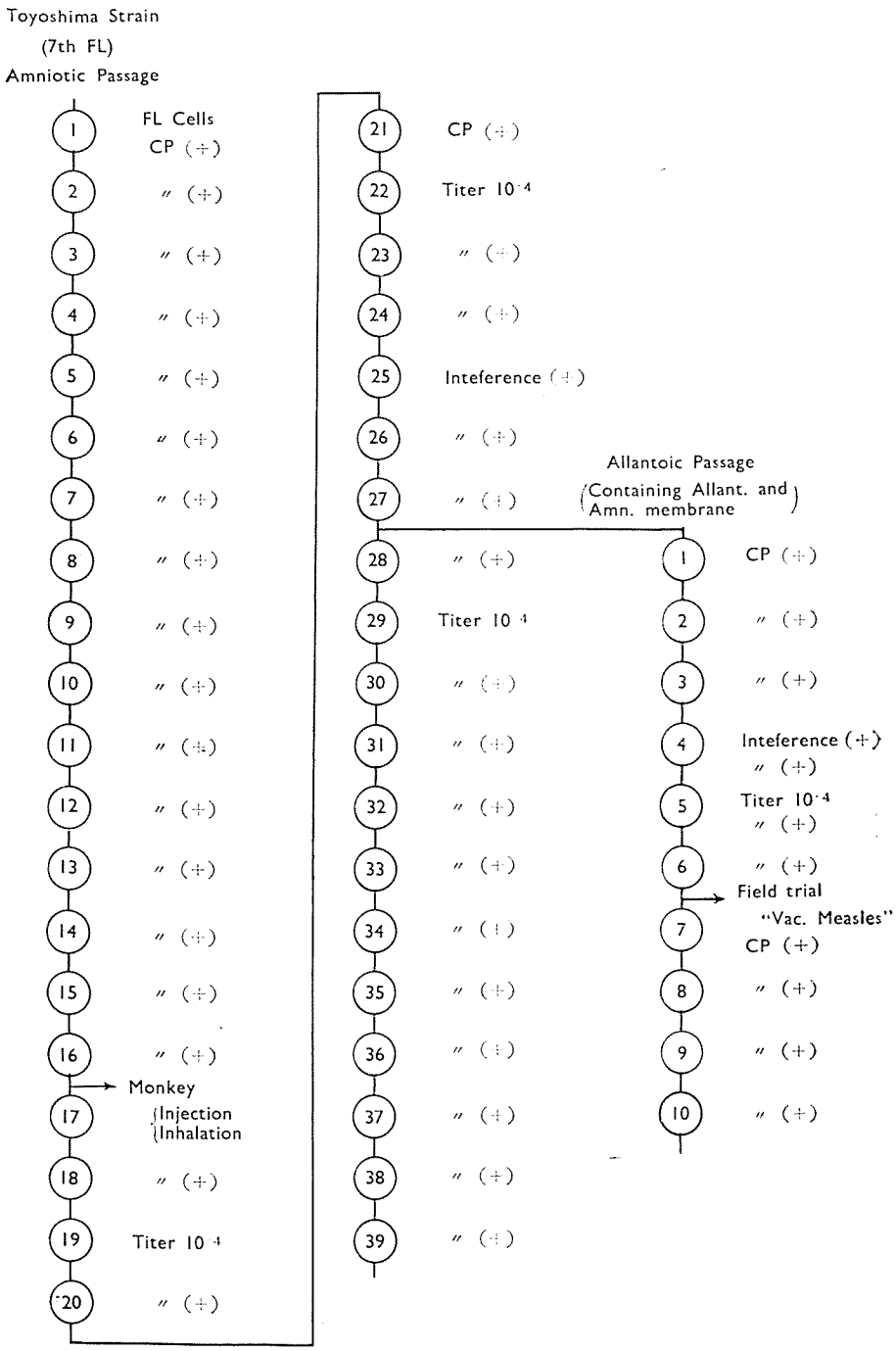


Table 1. Serial cultivation of measles virus in the amniotic and chorioallantoic cavities of embryonated eggs.

taining approximately 100 $TCID_{50}$ were mixed and incubated for 1 hour at room temperature. The mixture was introduced into FL cell culture and the final reading for cytopathic change was made on the 14th day.

RESULTS

1. *Vaccination experiments on children with 2nd or 3rd egg chorioallantois passaged virus*

Vaccination was carried out in an isolated village where no epidemic or endemic of measles had occurred during the past few years. 937 children were vaccinated with the 2nd or 3rd egg chorioallantois passaged measles virus by the inhalation method for 20 to 120 seconds. Sera of 166 children were examined for the complement fixation reaction, and unexpectedly 80 samples proved to have measles antibody (>4) prior to vaccination. Details of the epidemiological study will be published in this journal later. Of the cases having no complement fixation antibody, 51 serum specimens were collected 1 month after vaccination and examined.

Three different batches of virus were employed for vaccination. The first batch was the same that was used in the experiments on monkeys (Okuno *et al.*, 1960c), and the other was produced later.

None of the children vaccinated showed any clinical symptoms with any virus preparation.

Table 2. Vaccination of Children with Chorioallantois Passaged Measles Virus of Toyoshima Strain (Inhalation Method)

Virus Preparation		Inhalation Time (seconds)	Number of Cases giving Antibody Response (C.F. Titer: >4)	Number of Cases not giving Antibody Response (C.F. Titer: <4)
Passage Series	Generation			
I	2 nd	20	5 (8. 64. 64. 128. 256*)	4
Total			5	4
II	2 nd	20	4 (4. 4. 4. 16*)	38
		40 ~ 120	3 (8. 8. 8*)	3
	3 rd	20	0	2
		40 ~120	1 (4*)	7
Total			8	50

* Each figure expresses the complement fixing antibody titer in each case.

As shown in Table 2, with the first batch of virus, 5 of the 9 children showed a good antibody response. The result was not so good as that in monkeys reported previously, perhaps because of the shorter inhalation time used. The other batch of virus material evoked a poor antibody response, only 8 out of 58 children giving a positive serum test with low antibody titer.

2. *Vaccination with amniotic and subsequently chorioallantoic passaged measles virus*

Virus material of the 6th generation of the chorioallantoic passage line derived from the 26th generation of amniotic passage virus was employed for vaccination of 22 children in a group of apartment houses.

These children were between 7 months and 4 years 9 months, the average age being one year nine months. Following vaccination, all but two children developed fever after an incubation period of 6 to 11 days. The maximum fever range was 37.5 to 40°C, and the mean of the maximum fever was 39.1°C. The duration of fever was 1 to 6 days, the mean being 2.8 days.

A rash was observed in 8 of the 19 children who had fever. This developed on the face 2 or 3 days after the onset of fever, and later the trunks and extremities were

Table 3. Clinical Response in 22 Children Vaccinated with Modified Amniotic Passaged Measles Virus.

Toyoshima Strain 7th FL Passage → 26 th Chick egg Amniotic Passage → 6 th Chick egg Allantoic Passage (containing amniotic membrane) → Children (by Inhalation)

No. of Children Vaccinated	No. of Child. with "Vaccination measles"	Mean Age (yrs.)	Range of Age (yrs.)	Incubation Period (days)
22	19	1 9/12	7/12 ~ 4 9/12	6 ~ 11

Mean day of Onset of Fever	Range of Fever	Mean Maximum of Fever	Range of Duration of Fever (days)	Average Duration of Fever (days)
8.7	37.5 ~ 40°C	39.1°C	1 ~ 6	2.8

Table 4. Antibody Response in Children Vaccinated with Modified Amniotic Passage Measles Virus of Toyoshima Strain
(6 th allantoic passage after 26 th amniotic passage: inhalation method)

Case	Age (yrs.)	Antibody Response				Clinical Signs
		C.F. Titer		Neutralization Titer		
		before	after	before	after	
1.	2 6/12	<4	16	<4	8	+
2.	3 2/12	<4	64	<4	16	+
3.	1 6/12	<4	64	<4	16	+
4.	3 6/12	<4	128	<4	32	+
5.	1 1/12	<4	8	<4	8	+
6.	1 1/12	<4	256	<4	128	+
7.	1 9/12	<4	8	<4	8	+

involved. The extent and number of these rashes, however, was less than in typical measles. None of the vaccinated children showed catahrr symptoms, such as coughs or hyperamia in the conjunctiva. No Koplik spots were detected in children who developed clinical symptoms. 7 pairs of serum specimens were tested by the complement fixation and neutralization reactions. As shown in Table 4, an increase in antibody titer was observed in all cases by both serological tests.

DISCUSSION

In our previous vaccination experiments, the first generation chorioallantois passaged measles virus of Edmonston strain was employed by the nasal instillation method and it was reported that some of the cases showed so-called "vaccination measles," the symptoms of which were considerably milder than those of typical measles. In the present study, virus material derived from 6th chorioallantois passaged measles virus of Toyoshima strain after 26 amniotic passages was tested by the inhalation method. "Vaccination measles" were observed in most cases with good antibody response. The clinical signs and symptoms observed in the two experiments were quite similar, no catahrr symptoms or Koplik spots being observed. American and Russian workers (Enders *et al.*, 1960; Katz *et al.*, 1960 a, b; Kempe *et al.*, 1960; Black *et al.*, 1960; Lepow *et al.*, 1960; Krugman *et al.*, 1960; Haggerty *et al.*, 1960; Schwarz *et al.*, 1960; Hoekenga *et al.*, 1960; Dolgin *et al.*, 1960; Smorodintsev *et al.*, 1960) recently reported that their attenuated vaccine preparation caused mild measles-like symptoms in nearly all vaccinated children. These appeared to be similar to our "vaccination measles." From these experiments, it was proved that measles virus could be attenuated to some extent.

There seems to be no fear of contact infection with our "vaccination measles" or of back-mutation of attenuated virus to the virulent type by passage through susceptible children. This can be assumed from the following evidences.

i) In the densely populated area where the vaccination experiments were performed, neither typical measles nor "vaccination measles" occured in non-vaccinated subjects following vaccination.

ii) In one family no sibling contact infection was observed. Although one child who received vaccination represented "vaccination measles," another unvaccinated child in the same family was quite healthy and had no antibody response.

iii) Though the evidence is scanty, no measles virus could be isolated from the throat washing of 2 subjects who represented "vaccination measles."

In contrast to the low occurrence of "vaccination measles" in previous vaccination experiments (about one fourth of the cases), it is striking that, in the present study, nearly all the vaccinated children represented "vaccination measles." This might be due, in some way, to a difference in the virus titer, but more probably to a difference in the method of inoculation. In the present study, the inhalation rather than the instillation method was exclusively used as it had been shown to be superior

in vaccination experiments with attenuated influenza virus.

In experiments with the second and later generation chorioallantoic passage measles virus, clinical signs and illness were scarcely observed, and no uniform antibody response could be obtained. An insufficient virus dose is considered to be one of the causes for a poor antibody response. As the chorioallantoic passaged virus is not cytopathogenic for tissue culture cells, we are not able to make an exact infectivity titration, as reported in the previous paper.

It is therefore still uncertain whether there is any essential difference in virulence between chorioallantois passaged and amnion passaged measles viruses.

ACKNOWLEDGEMENTS

We would like to express our great thanks to Prof. Kamahora (Director of our Institute) and Prof. Seki for their useful suggestions. We are also grateful to the Department of Health, Osaka Prefecture (Director: Dr. Furuno), the Ikeda Institute for Public Health (Director: Dr. Ida, Director of the Branch Office, Dr. Morikawa and Dr. Dodo) and Dr. Kamiki of our Institute for their kind help.

REFERENCES

Black, F. L. and Sheridan, S. R. (1960). Studies on an attenuated measles virus vaccine. IV. Administration of vaccine by several routes. *New Engl. J. Med.* **263**, 165-169.

Dolgin, J., Levine, S., Markham, F. S., Cabasso, V., Weichsel, M., Ruegsegger, J. M. and Cox, H. R. (1960). Immunizing properties of live attenuated measles virus. *J. Pediat.* **57**, 36-41.

Enders, J. F., Katz, S. L., Milovanovic, M. V. and Holloway, A. (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.

Haggerty, R. J., Meyer, R. J., Leniham, E. and Katz, S. L. (1960). Studies on an attenuated measles-virus vaccine. VII. Clinical antigenic and prophylactic effects of vaccine in home dwelling children. *New Engl. J. Med.* **263**, 178-180.

Hoekenga, M. T. and Schwarz, A. J. F. (1960). Experimental vaccination against measles. II. Tests of live measles and live distemper vaccine in human volunteers during a measles epidemic in panama. *J. A. M. A.* **173**, 868-872.

Katz, S. L., Enders, J. F. and Holloway, A. (1960). Studies on an attenuated measles-virus vaccine. II. Clinical, virologic and immunologic effects of vaccine in institutionalized children. *New Engl. J. Med.* **263**, 159-161.

Katz, S. L., Kempe, C. H., Black, F. L., Lepow, M. L., Krugman, S., Haggerty, R. J. and Enders, J. F. (1960). Studies on an attenuated measles-virus vaccine. VIII. General summary and evaluation of the results of vaccination. *New Engl. J. Med.* **263**, 180-184.

Kempe, C. H., Ott, E. W., Vincent, L. S. and Maisel, J. C. (1960). Studies on an attenuated measles-virus vaccine. III. Clinical and antigenic effects of vaccine in institutionalized children. *New Engl. J. Med.* **263**, 162-165.

Krugman, S., Giles, J. P. and Jacobs, A. M. (1960). Studies on an attenuated measles-virus vaccine. VI. Clinical, antigenic and prophylactic effects of vaccine in institutionalized children. *New Engl. J. Med.* **263**, 174-177.

Lepow, M. L., Gray, N. and Robbins, F. C. (1960). Studies on an attenuated measles-virus vaccine. V. Clinical, antigenic and prophylactic effects of vaccine in institutionalized and home dwelling children. *New Engl. J. Med.* **263**, 170-173.

Okuno, Y., Nakamura, K., Yamamura, T., Takahashi, M., Toyoshima, K., Kunita, N., Sugai, T. and Fujita, T. (1960a). Studies on attenuation of influenza virus. *Proc. Jap. Acad.* **36**, 299-303.

Okuno, Y., Sugai, T., Fujita, T., Yamamura, T., Toyoshima, K., Takahashi, M., Nakamura, K. and Kunita, N. (1960b). Studies on the prophylaxis of measles with attenuated living virus. II. Cultivation of measles virus isolated by tissue culture in developing chick egg. *Biken's J.* **3**, 107-113.

Okuno, Y., Takahashi, M., Toyoshima, K., Yamamura, T., Sugai, T., Nakamura, K. and Kunita, N. (1960c). Studies on the prophylaxis of measles with attenuated living virus. III. Inoculation tests in man and monkey with chick empryo passage measles virus. *Biken's J.* **3**, 115-122.

Schwarz, A. J. F., Boyer, P. A., Zirbel, L. W. and York, C. J. (1960). Experimental vaccination against measles. I. Tests of live measles and distemper vaccine in monkeys and two human volunteers under laboratory conditions. *J. A. M. A.* **173**, 861-867.

Smorodintsev, A. A., Boichuk, L. M., Shinkina, E. S., Botanova, T. B., Bystryakov, L. V. and Perodze, T. V. (1960). Clinical and immunological response to live tissue culture vaccine against measles. *Acta Virologia* 4, 201-214.

Toyoshima, K., Takahashi, M., Hata, S., Kunita, N. and Okuno, Y. (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.