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Studies on the Prophylaxis of Measles with Attenuated Living Virus

III. Inoculation Tests in Man and Monkey with Chick Embryo Passage Measles Virus

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SUMMARY

Intranasal inoculation of children with first generation chorioallantoic passage measles virus resulted in the occurrence of "vaccination measles" in about $\frac{1}{4}$ of the cases. A marked rise in antibody titer was found in these children. With virus of the second or later generations, "vaccination measles" was not observed, but there was a rise in antibody titer in some cases. These cases were immune against natural infection.

In vaccination experiments on monkeys with the second generation chorioallantois passaged virus by the inhalation and injection methods, a remarkable rise of antibody titer was observed without any clinical manifestation. Similar results were obtained with serially amnion passed measles virus.

INTRODUCTION

Inoculation tests in man and monkey with chick embryo passaged measles virus have been reported by Rake *et al.* (1940-43) and in our previous paper (1956). A reduction in virulence has been noted on chick embryo passaged virus and there was no danger of a typical infection on inoculation. Immunity against challenging and natural infection has also been observed and it has been suggested that prophylaxis of measles might be possible with living chick embryo passaged virus. Rake *et al.*, however, obtained no constant positive results due to lack of a method for evaluation of propagation of the virus in the chick embryo, and their study was not carried to completion. A method of evaluation by interference diagnosis was developed by the authors, but a virus of constant titer could not be maintained because of the difficulty in isolation measles virus directly with the chick embryo, and prolonged serial passage and prophylaxis with chick embryo material has not been entirely satisfactory.

A measles virus has been obtained by tissue culture, and chick embryo passaged virus has readily become available. The first tests were conducted with this living virus in man and in monkey by intranasal inoculation, and clinical response and antibody formation were investigated. Virus passed through the chorioallantoic route more than twice no longer showed cytopathogenicity when

back-inoculated in FL cell tissue culture. This observation substantiated the marked reduction in virulence towards man and monkey. However the capacity of conferring immunity was retained. Inoculation of man with first passage virus resulted in the appearance of some of the symptoms of measles, that was the so-called "vaccination measles" in some of the cases and a marked increase in antibody was observed.

By the method of intranasal instillation, the rise in antibody was irregular, and by analogy with live influenza virus vaccine, with which a regular rise in antibody was obtained by the intranasal inhalation method (Okuno and Nakamura, to be published), this inhalation method was used in monkeys. Although the number of case was few, uniform results were obtained.

MATERIALS AND METHODS

1. *Measles virus cultivated in the chorioallantoic cavity*

As described in the preceding paper, culture fluid of FL cells containing about $10^{3.5}$ TCID₅₀ Edmonston or Toyoshima strains of measles virus was inoculated in the chorioallantoic cavity of 7 day embryonated eggs. After 7 days incubation, the chorioallantoic membranes and fluids were harvested, homogenized and centrifuged and the supernatant was used as the first egg chorioallantois passage material (EC₁).

This material produced cytopathic changes in FL cell cultures during a prolonged observation period of 14 days and the presence of virus material was further confirmed by our interference diagnosis method.

0.2 ml aliquots of EC₁ were successively inoculated in the chorioallantoic cavity of 7 day developing chick embryos. Suspensions of chorioallantoic membrane and fluid were likewise made and the supernatants were used as the second egg passage material (EA₂).

The third egg passage material was prepared in the same manner (EA₃). After a second passage, virus material exhibited no cytopathic effects when back-inoculated in FL cells, and the presence of the measles agent was verified in every material by the interference test.

2. *Measles virus cultivated in the amniotic cavities of embryonated chick eggs*

The Toyoshima and Edmonston strains of measles virus have been serially cultivated in the amniotic cavities of 7 day developing chick embryos for many generations. The virus material used for passage consisted of a suspension of the amniotic membrane and fluid. These materials invariably presented definite cytopathogenic effects in each generation, and the interference tests were also positive in each case. The 16th passage of the Toyoshima strain (EA₁₆-Toyoshima) was employed for monkey experiments.

3. *Complement fixation test*

Tests were carried out by a modification of Kolmer's method. The details of the technique was just the same as described in our previous paper (Toyoshima *et al.* 1959).

RESULTS

1. *Vaccination experiments on children with 1st egg chorioallantois passage virus*

19 children from various areas who had no history of measles were inoculated intranasally, each with 0.5 ml of EC₁-Edmonston.

5 cases became feverish about 7 days after inoculation with temperatures of 37°C to over 39°C. This was followed by the appearance of a few exanthems on

the face. However their development on the body was hardly seen. No catarrh symptoms, such as a cough or discharge from the eyes were noticed and no Koplik fleck was ever seen. Taken together, these symptoms were presumed to be similar to a mild clinical manifestation of measles, and we designated them as "vaccination measles". One other child showed only a transient slight fever, and the other 13 cases presented no apparent symptoms. About one month after inoculation, blood samples were taken and the sera were tested for complement fixation antibodies.

The results are summarised in Table 1. Children who developed any of the symptoms described above, showed without exception a great increase in antibody titer, and it was noteworthy that in 2 cases, in whom no clinical symptoms were observed, a considerable rise of titer was also demonstrated. These antibody titers were comparable to those of the children demonstrated in our previous report who recovered from a natural infection of measles (Toyoshima *et al.* 1959).

Table 1. Result of Intranasal Vaccination on Children with the First Generation Egg Chorioallantois Passage Measles Virus of Edmonston Strain

Initial of child	Age	"Vaccination measles"	Complement fixing antibody	
			Before vaccination	1 month after vaccination
I. S.	3	+	< 4	256
T. Y.	4	+	< 4	256
S. N.	5	+	< 4	256
H.	5	+	< 4	256
K. K.	4	+	< 4	256
T. K.	5	+ ?	< 4	32
Y. Y.	6	—	< 4	64
A.	6	—	< 4	256
I. F.	2	—	< 4	< 4
H. K.	1	—	< 4	< 4
T. K.	3	—	< 4	< 4
K. Y.	2	—	< 4	< 4
M. K.	2	—	< 4	< 4
M. K.	4	—	< 4	< 4
M. N.	3	—	< 4	< 4
I. S.		—	< 4	< 4
T. T.	3	—	< 4	< 4
Y. N.		—	< 4	< 4
H. O.	5	—	32	32

+ ? : Uncertain whether the clinical manifestations were due to vaccination.

Table 2. Comparison of Symptoms of "Vaccination Measles" and Typical Measles

Symptoms	"Vaccination Measles"	Typical Measles
Fever	+	+
Exanthem	± (generally confined to face)	+
Catarrh (Bronchus; Eye)	—	+
Koplik fleck	—	+
Antibody Response	+	+

2. *Vaccination experiments on children with the second and third egg chorioallantoic passage virus*

Vaccinations were made with the second and third chorioallantoic passage virus (EC₂-EC₃-Edmonston). Blood samples were taken immediately before and one month after intranasal vaccination from 3 of the 6 children vaccinated, and the antibody for measles virus was titrated by the complement fixation test.

The blood of 3 of 8 children in another group who have been vaccinated

Table 3. Result of Intranasal Vaccination on Children in a Welfare Facility with second and third Generation Egg-Chorioallantois passed Measles Virus of Edmonston Strain

Initial of child	Vaccinated virus material	Clinical symptoms	Complement fixing antibody		Infection with measles which prevailed about 30 days after vaccination
			Immediately before vaccination	1 month after vaccination	
Y.M.	EC ₂ -Edmonston	—	< 4	< 4	+ (31/VII)
M.O.	"	—	< 4	< 4	+ (9/VIII)
T.T.	"	—	< 4	32	—
S.M.	"	—	N.T.	N.T.	—
I.T.	"	—	N.T.	N.T.	—
Y.I.	"	—	N.T.	N.T.	—
S.M.	EC ₃ -Edmonston	—	< 4	< 4	+ (11/VIII)
T.M.	"	—	< 4	< 4	+ (1/VIII)
T.T.	"	—	< 4	8	—
I.K.	"	—	N.T.	N.T.	+ (18/VII)
M.Y.	"	—	N.T.	N.T.	+ (18/VII)
S.S.	"	—	N.T.	N.T.	—
Z.M.	"	—	N.T.	N.T.	—
T.U.	"	—	N.T.	N.T.	—

Vaccinated on July 18. N.T. : Not tested

with EC₃-Edmonston was tested for antibody in the same way. While in neither group, symptoms attributable to vaccination were observed for more than one month after inoculation, an increase in antibody titer was detected in one case in each group.

About 30 days after vaccination, a natural infection of measles took place in that area. Out of 14 children who have been vaccinated, 6 developed measles showing typical symptoms. The other 8 children were resistant to the disease in spite of frequent contact with infected children during the epidemic.

2 children who showed a positive antibody response by the serum test did not develop measles, while 4 children in whom no increase was observed presented apparent symptoms.

3. *Vaccination experiments on monkeys with egg passage measles virus*

4 pairs of *cynomolgus* monkeys were inoculated intranasally with second generation egg chorioallantois passed virus (Edmonston and Toyoshima strain) and amnion passed virus (EA₁₄ mixed with EA₁) and tissue culture fluid of Toyoshima strain virus respectively.

No monkeys showed either definite clinical manifestations or changes in blood cells.

The antibody response to measles virus was determined by the complement fixation test and it turned out that all the monkeys had a considerable titer even before inoculation and no decisive result was obtained.

In another experiment conducted later, the inhalation method, which had been found to be superior to the intranasal instillation method in vaccination experiments with the influenza virus, and the injection method were employed. Nebulizer was used for inhalation, by which about 0.1 ml of the material could be gushed out in a minute.

30 *cynomolgus* monkeys were tested for complement fixing antibody before use, and 9 monkeys with no or little antibody were selected. 2 pairs of monkeys, in which no antibody for measles was detected, were given the second generation chorioallantois passed virus (EC₂-Toyoshima), one pair by the inhalation method for 5 min. and another pair by intramuscular injection.

At the same time 5 other monkeys with a low complement fixing antibody titer (4×) were vaccinated as follows. Second generation chorioallantois passed virus (EC₂-Toyoshima) was given to 2 monkeys by inhalation and to 1 monkey by injection. The remaining 2 monkeys were vaccinated with 16th generation amnion passed virus (EA₁₆-Toyoshima) by inhalation and injection, respectively. All monkeys were bled 16 days and 1 month after inoculation, and the antibody response was examined by the complement fixing reaction.

Careful daily inspection of monkeys revealed no sign of illness for 1 month after vaccination, and no decrease of appetite or body weight was observed throughout the experimental period. Serological examination, however, showed a remarkable rise in antibody titer in almost all monkeys.

As high as 256× of the complement fixing titer was observed in the first group even 16 days after vaccination, and this high level lasted for further two weeks.

This antibody response was doubtless induced by vaccination because of the time of its appearance. No difference was observed between the result of vaccination by inhalation and by injection.

In another group of 5 monkeys, in which an appreciable antibody reaction was detected, the results were somewhat variable with the material and method of vaccination. 2 monkeys vaccinated with EC₂ by inhalation, exhibited a high level of antibody after 1 month, and a small increase was noted in 1 monkey which received injection of the same material.

Amniotic virus material induced a strong antibody response following either its inhalation or injection into monkeys.

Table 4. Vaccination Experiments on Monkeys with Egg Passed Measles Virus

Number of monkey	Vaccinated virus material	Route of vaccination	Clinical symptoms	Serum test for complement fixing antibody		
				Immediately before vaccination	16 days after vaccination	1 month after vaccination
1	EC ₂ -Toyoshima	Inhalation	—	< 4	256	256
2	"	"	—	< 4	256	256
3	"	Intramuscular injection	—	< 4	256	128
4	"	"	—	< 4	256	128
5	"	Inhalation	—	4	128	128
6	"	"	—	4	32	128
7	"	Intramuscular injection	—	4	32	32
8	EA ₁₆ -Toyoshima	Inhalation	—	4	256	128
9	"	Intramuscular injection	—	4	512	512

DISCUSSION

It was previously reported that measles virus passed through chick embryos via the allantoic route has almost no pathogenicity towards man and results showing increased resistance against natural infection have been obtained by prophylactic inoculation with this virus (Taniguchi *et al.*, 1956). The so-called Kamahora phenomenon (Kamahora *et al.*, 1955), characteristic giant cell formation and appearance of inclusion bodies, was not observed following inoculation of this virus into monkeys. In the present experiments also, virus passed more than twice in the chorioallantois, no longer showed pathogenicity against FL cells or HeLa cells, and no clinical manifestation was observed when it was inoculated intranasally or subcutaneously in man and monkeys. There was however a rise in antibody

titer and it is conceivable that the pathogenicity was altered while the infectivity and antigenicity were retained by chick embryo (chorioallantoic) culture of the measles virus.

First-passage virus, however, induced the so-called "vaccination measles" and was not suited for use as a vaccine. A rise in antibody titer with occurrence of "vaccination measles" was similar to that seen in natural infection and it is accordingly supposed that a considerably solid immunity is produced. In view of the side effects, however, it was thought that material from the the second or later chick embryo passage was better suited for preparation of the vaccine. One defect was, however, found in the vaccination experiment on children with these virus materials. This was that not all cases showed an adequate rise in antibody after intranasal vaccination. Since a consistent effect can be obtained by intranasal inhalation of living influenza virus vaccine, it was presumed that similar results could be obtained with living attenuated measles vaccine if this method of inoculation was used. The effect of this vaccine was examined in monkeys, but the results of the first experiment were indecisive as it was found that all the first 8 animals used had high complement fixing antibody levels prior to vaccination. Tests were then conducted in another group of 30 monkeys. The antibody level was examined before vaccination in these animals and 9 monkeys with either no or very low antibody level were selected. Living measles virus passed twice or more developing chick embryo was inoculated into 4 monkeys with no antibody by inhalation or by intramuscular injection and a marked rise in antibody titer was observed without any clinical manifestations.

Although standardization of the quantity and quality of the virus and the storage of the vaccine must be studied, it is believed that prophylaxis of measles might be possible by inhalation of living measles virus cultivated in the chick embryos.

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