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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

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SUMMARY The combined use of inactivated (killed) and live measles vaccines, (i.e., inoculation with inactivated vaccine prior to live vaccine in order to afford a certain extent of immunity) is considered the safest and best method for mass immunization in Japan.

However, in this method there is the problem that while on the one hand, when less immunity is obtained with killed vaccine the clinical reactions to live vaccine are not much reduced, on the other hand, when too high an immunity with few clinical reactions is obtained there is an increase in the number of children who do not take live vaccine, and are very poorly immunized by the live vaccine.

The optimal range of the neutralizing antibody titer after inoculation with killed vaccine to obtain favorable effects with live vaccine was found to be around 2^2–2^3.

INTRODUCTION

The disadvantage of inoculation with live measles vaccine is that it causes marked clinical reactions, represented by fever. The present authors reported at the Conference of the Society of Japanese Virologists in 1959 that such reactions were not due to a mild form of measles but to so-called " vaccination measles " (OKUNO *et al.*, 1960) in which there is slight catarrh and a rash but which is not communicable and we indicated that this should be distinguished from natural measles.

However, live measles vaccine alone seems to have been employed with favorable effects. It has an excellent immunizing effect and it causes

no complications, pneumonia or brain disturbances.

Immunization with live measles vaccine alone may be applicable on an individual basis to children who are physically well, but it does not seem advisable to use it on a mass basis. Hence the combined use of live measles vaccine and killed vaccine (KARELITZ *et al.*, 1962 and KRUGMAN *et al.*), has been advocated in a number of countries including the U.S.A. and Japan. This combination is considered to minimize the clinical response without reducing the immunizing potency. Experience on the combined use of live and killed vaccine has shown that the clinical reactions due to live measles vaccine are great when the antibody response following immunization with killed measles vaccine is low. On the contrary there is little or no clinical response in the presence of a high antibody titer, and sometimes live measles vaccine does not take.

Accordingly, the following experiments were performed to determine the optimal titer following administration of a certain potency of killed measles vaccine, at which live measles vaccine should be inoculated.

MATERIALS AND METHODS

Experiment 1

As killed vaccine, plain fluid vaccine was employed. The Tanabe strain, isolated by MUTAI (1959) was cultivated in monkey kidney cells in 199 medium. The resulting culture fluid was not concentrated and the virus in it was inactivated with 1 : 4000 formalin ; for mice the ED_{50} was $4^{1.5}$.

As live vaccine, Biken lot 16 was employed. Its virus titer was $10^{4.0}$ TCD_{50}/0.1 ml to FL cells. It was given by inhalation 14 days after inoculation with killed vaccine.

The experiment was begun on April 14, 1964 with 18 home-dwelling children in I city and 60 in M settlement in Osaka, all of whom had no previous history of measles.

Experiment 2

Vaccine used : Killed vaccines with various potencies were employed.

A. Plain vaccine (Mouse $ED_{50} = 4^{1.7}$)

B. Concentrated plain vaccine was concentrated by centrifugation (Mouse $ED_{50} = 4^{0.8}$)

C. Concentrated plain vaccine was concentrated by ammonium sulfate (Mouse $ED_{50} = 4^{2.1}$)

D. Aluminium phosphate precipitated vaccine (Mouse $ED_{50} = 4^{2.5}$)

As live vaccine, Biken lot 16 was injected subcutaneously.

The experiment was made on 120 children on May 25th 1964 in H housing project.

Both killed and live measles vaccine were prepared at the Kanonji Institute of the Research Foundation for Microbial Diseases of Osaka University.

Vaccination schedule :

Sub-groups

May 25th 1964		June 8th		June 22nd		July 23rd
	2 Weeks		2 Weeks		4 Weeks	
Ⓚ	———	S	———	Ⓛ	———	○
Ⓢ	———	K	———	Ⓛ	———	○
Ⓚ	———	K	———	Ⓚ	———	○

K : Killed vaccine injection
S : Salk vaccine injection used as control
L : Live vaccine injection
○ : Blood specimens taken

In the above sub-groups three combinations were made with respect to K.

In Experiments 1 and 2, 1.0 ml of killed vaccine was given by subcutaneous injection.

RESULTS

Experiment 1

The symptoms manifested were generally much slighter than those following inoculation with live vaccine alone. Because of the difficulty in checking clinical reactions in detail, clinical data on the children in the settlement were not considered.

The home-dwelling children (Table 1) showed fewer obvious clinical reactions corresponding with their higher antibody titers following immunization with killed vaccine. They generally showed a marked increase in neutralizing antibody following inoculation with live vaccine by the inhalation method.

Experiment 2

Blood specimens were taken from 71 of the 120 children. The clinical reactions and neu-

TABLE 1 *NT antibody titers after KV & LV and symptoms after LV inoculation in Exp. 1*

Name	Age Y. Mo.	Antibody titer 2 wks. after KV (log_2)	Clinical response following LV inoculation				Antibody titer 3 wks. after LV (log_2)
			Incub. (days)	Max. fever (°C)	Dur. of fever (days)	Rash	
T. N.	3 2	<0	9	39.2	2.0	−	7.5
T. Y.	3 8	<0	8	38.3	2.0	+	8.25
H. Y.	3 8	<0.5	7	38.2	3.0	+	8.25
E. Y.	1 7	<0.5	8	39.9	4.0	+	9.5
Y. K.	2 9	<0.5	10	38.2	3.0	+	9.5
T. N.	3 4	<0.5	8	39.8	3.0	+	7.25
K. N.	3 5	0.75	10	37.9	0.5	+	9.75
K. K.	1 5	0.75					8.75
Y. D.	7 1	1.25	9	37.7	0.5	−	8.5
T. Y.	6 2	1.5	7	38.5	3.0	+	8.5
A. M.	1 5	2.25	9	38.2	1.0	+	≧10.5
T. M.	1 2	2.5	10	37.9	2.0	+	≧10.5
A. K.	5 2	2.5		−		−	7.25
A. M.	1 6	2.75		−		−	8.25
H. D.	3 1	3.25		−		−	9.25
T. K.	1 6	3.25		−		−	9.5
T. I.	5 7	≧3.5		−		−	7.75
A. H.	4 0	≧3.5		−		−	9.25
Y. N.	3 1	≧3.5		−		−	8.5
Mean value		1.64	8.6	37.9	2.2		8.76

April 22, 1964 KV 1.0 ml subcutaneous injection
May 6, 1964 LV 30 seconds' inhalation (Home-dwelling group, 1964)

TABLE 2 *NT antibody titers before and after KV and after LV inoculation in Exp. 1*

Name	Age Y. Mo.	NT antibody titers Pre K	Post K	Post L	Name	Age Y. Mo.	NT antibody titers Pre K	Post K	Post L
F. F.	2 5	<0.5	<0	10.5	S. M.	1 11	<0.5	<0.5	8.75
T. O.	2 2		1.5	7.75	M. G.	2 0	<0	<0	6.75
N. K.	2 5	<0	1.5	7.25	K. F.	1 10	<0	<0.5	9.25
S. N.	2 6	<0.5	<0.5	8.75	Y. O.	1 9	<0		9.5
S. O.	3 0		0.5	10.25	K. K.	1 5	<0	2.5	6.25
I. S.	2 8	<0	3.25		M. M.	1 4	<0.5	<0.5	9.25
T. K.	3 6	<0.5	<0.5	7.5	N. K.	1 4	<0	<0	8.25
C. O.	3 1	<0.5	<0.5	6.75	M. Y.	1 4	<0.5	<0	8.25
M. Y.	3 5	<0.5	<0.5	8.5	K. K.	1 4		1.75	9.5
M. M.	3 2	<0.5	<0.5	10.5	S. W.	1 3	<0	2.0	9.5

April 14, 1964 KV, April 28 LV, May 19 Blood specimen taken. (M. Settlement group, 1964)
N. B. Children with a doubtful " Negative history " of measles by the NT test or from whom it was not possible to take post L blood specimens were omitted.

tralizing (NT) antibody of the 71 children were studied.

As in Exp. 1 there was a correlation between the NT antibody titer following inoculation with killed vaccine and the clinical response (Tables 3 to 6).

Thus, when the NT antibody titer following inoculation with killed vaccine was less than 2^1, clinical reactions due to live measles vaccine were reduced to some extent. It was found that as the NT antibody titer increased above $2^{1.5}$, there were increasingly fewer clinical reactions and an increase in the number of children who showed no further antibody response following inoculation with live measles vaccine. In most children who maintained their NT

TABLE 3 *NT antibody titers after KV & LV and symptoms after LV inoculation in Exp. 2*

Group I

Subgroup name	Age Y.	Age Mo.	Body weight	NT antibody titers (log_2) Pre K	Post K	Post L	Clinical reactions following inoc. with LV: Max. fever	Duration at over 37.5°C (days)	Duration of rash (days)
KSL									
T. A.	3	9	17.5		<0		39.5	4	0
E. S.	3	7	15		0	10.5	39.2	3	5
N. F.	2	11	14		1.0	11.0	38.0	2.5	3
Y. S.	1	4	10			<8.5	37.5	0.5	0
A. N.	4	9	14.7			9.0	—	—	—
T. K.	1	11	11		3.0	>9.5	—	—	—
S. D.	2	9	13	<0	3.5	>7.5	—	—	—
SKL									
K. H.	4	7		<0	<0		40.6	6	7
S. H.	3	9	14	<0	<0	9.0	40.0	5	4
H. I.	1	11	11.5		<0	>10.5	39.0	3	7
H. S.	4	7	17.7		1.0	>10.5	39.8	2.5	4
J. M.	1	0	10			>9.5	39.0	3	
Y. A.	2	4	13		1.0	1.0	39.4	3	3
K. O.	1	6	11.3		1.0	3.5	—	—	—
S. H.	1	3	11		1.0	8.5	—	—	—
H. T.	2	5	11.7		>3.5	4.5	—	—	—
A. M.	2	7	13		8.5	10.5	—	—	—
KKK									
Y. N.	2	8	15.5	<0	<0	1.5			
Y. Y.	5	9	20	<0	<0	5.5			
Y. H.	2	8	12	<0	1.0	>5.5			
M. F.	1	7	10		>5.5				
E. D.	1	3			3.0				
T. M.	1	9			3.5				
Y. M.	2	5	11.6		3.5	6.0			
Y. H.	0	9	9.5		6.5	7.0			
M. S.	1	9			9.0				
J. Y.	1	0	10			>10.5			

K: Vaccine A

(1964, Spr. Hamakoshien)

titers above 2^4 there was no take of live measles vaccine by the injection method.

With regard to the interval between inoculation of killed vaccine and live vaccine, it was found that after a 4 week period there was a slightly higher antibody titer following inoculation of killed vaccine and lower fever subsequent to live vaccine inoculation than after a 2 week period.

TABLE 4 *NT antibody titers after KV & LV and symptoms after LV inoculation Exp. 2*

Group II

Subgroup Name	Age Y.	Age Mo.	Body weight	NT antibody titers Pre K	NT antibody titers Post K	NT antibody titers Post L	Clinical reactions following inoc. with LV Max. fever	Clinical reactions following inoc. with LV Duration at over 37.5°C	Clinical reactions following inoc. with LV Duration of rash
K*SL									
K. I.	1	5	11.5		<0	>12.5	39.8	2	0
K. B.	5	8	20	<0	<0	>10.5	39.7	2	3
Y. K.	2	5	13		<0	>9.5	39.5	3	3
T. F.	3	9					38.3	2	3
K. O.	3	0			1.0		38.8	2	—
Y. N.	3	2	16.5		1.0	>11.5	—	—	—
E. S.	0	8			1.5		NE	NE	NE
O. K.	2	3			1.5		NE	NE	NE
R. O.	1	2	9.7		2.5	>7.5	—	—	—
S. Y.	0	10	8		3.5	>10.5	—	—	—
SK+L									
H. I.	3	11	17.5	<0	<0.5	>10.5	—	—	—
H. Y.	0	9	7.5		0~0.5	>8.5	—	—	—
Y. I.	3	5	14	<0	1.0	>9.5	39.5	2	0
Y. F.	1	4	11.2		1.0	>9.5	—	—	—
M. O.	2	8			2.5		—	—	—
K. I.	3	2	14.5		3.5	>10.5	—	—	—
H. Y.	1	8	11		4.0	4.5	—	—	—
T. K.	1	5	12		4.5	4.5	—	—	—
K*K+K+									
T. S.	4	0	16.5	<0	4.0	5.5			
K. H.	2	3	12.5		4.5	>6.5			
A. H.	3	5	13		4.5	5.5			
S. A.	3	0	13		5.0	6.5			
K. A.	4	0	15	<0	5.0	5.5			
T. Y.	3	0	16.3	<0	5.5	6.5			
O. A.	3	0	14		5.5	7.0			
M. O	4	2	17.8	<0	6.0	7.5			
N. S.	3	8	14.5		6.5	7.5			
K. T.	1	9			6.5				
D. I.	0	10	9			>7.5			

* : B Vaccine

\+ : C Vaccine

NE : Not examined

(1964, Spr. Hamakoshien)

TABLE 5 *NT antibody titers after KV & LV and symptoms after LV inoculation in Exp. 2*
Group III

Subgroup Name	Age Y.	Age Mo.	Body weight	NT antibody titers Pre K	NT antibody titers Post K	NT antibody titers Post L	Clinical reactions following inoc. with LV: Max. fever	Duration at over 37.5°C	Duration of rash
KSL									
S. N.	2	9	13.3	<0			—	—	—
A. H.	2	2	13	<0	4.0	>8.5	—	—	—
K. T.	1	6	10.7		4.5	5.5	—	—	—
K. N.	2	1	11.5		4.5	5.5	—	—	—
M. K.	2	7	11.3	<0	4.5	>10.5	—	—	—
N. S.	4	7	13.5	<0	5.5	5.5	—	—	—
M. O.	2	4	10		5.5	6.0	—	—	—
SKL									
H. N.	5	0	16.3	<0	1.0	6.5	—	—	—
H. N.	6	10	18.5	<0	4.0	7.0	—	—	—
S. N.	1	7	10.5		>7.5	>9.5	—	—	—
KKK									
N. T.	2	8	12.3	<0	6.5	7.0			
K. S.	1	9	13		8.5	8.5			
K. M.	2	4	11		>8.5	9.5			
K. T.	0	10	10.5		9.5	10.0			
Y. O.	3	10		<0	11.0				

K: Vaccine D

(1964, Spr. Hamakoshien)

TABLE 6 *Relationship between NT titer following killed vaccine inoculation and fever due to live vaccine injection*

No. of children	NT antibody titers (log_2) Post K	Post L	No. of fev. reac.	Clinical symptoms: Aver. max. fev. (°C)	Aver. dur. fev. (days)
10	>1	10.2	8/10	39.2	2.8
9	1	8.0	5/9	38.0	1.0
4	1.5-2.5	7.5	0/2	—	—
5	3.0-3.5	8.5	0/5	—	—
9	4.0-5.5	6.5	0/9	—	—

(1964, Spr. Hamakoshien)

DISCUSSION

In Exp. 1, an obvious correlationship was found between the NT antibody titer following inoculation with killed vaccine and the clinical reactions following inoculation with live vaccine, i.e., the higher the antibody titer after K the less were the clinical reactions due to L vaccine. Even a single inoculation with killed vaccine resulted in a considerable suppression of clinical reactions following inhalation of live vaccine.

In Exp. 2, the clinical and antibody responses to live vaccine inoculation varied with the amount of NT antibody evoked by inoculation with killed vaccine. Thus studies were made on the optimal NT antibody titer following inoculation with killed vaccine in the method using a combination of live and killed vaccine.

A titer of below 2^1 was found to be insufficient to suppress clinical symptoms. With increase in the titer above 2^4 there was an increase in the number of children in whom injection of live vaccine caused no clinical reactions but also did not increase the antibody titer. That

is, there were a considerable number of children in whom there was no take of live vaccine.

Therefore it seems desirable to employ an amount of killed vaccine which reduces the clinical reactions, but allows a sufficiently big antibody response with live vaccine inoculation. The optimal range of antibody titer following killed vaccine inoculation seems to be rather limited and is probably around 2^2–2^3. This is based upon the idea that a few clinical reactions with live vaccine, so long as they are slight, are preferable to none at all. This range may be determined in terms of a single inoculation of killed vaccine rather than of more than two inoculations. In the present study an attempt was made to determine the optimal NT antibody titer following a single inoculation of potent killed vaccine.

There is an almost parallel relationship between the values of ED_{50} in mice following inoculation with the four kinds of killed vaccine used in Exp. 2 and their antigenicities in humans. But this is not always uniform for various kinds of vaccine. Though B vaccine was concentrated 8 fold, it still showed a very low immunogenicity in mice and children, and the killed vaccine in the SKL subgroup in Group II has been replaced by C vaccine.

Vaccine A, even in its plain form, seems to have a considerably high potency. It caused no clinical reactions in about half the children tested. But some children developed a high fever following inoculation with live vaccine. Because of the rather strong reactions of some children due to live vaccine, a two step of immunization with A vaccine in its plain form may be advisable.

D vaccine (aluminum phosphate precipitated) has a high potency. It is unsuitable for use with live vaccine injection techniques because after its injection there is frequently no take of live vaccine. The ideal inoculation method would be to give a preparation of live vaccine, which causes no clinical reactions but which greatly elevates the antibody titer. However a method such as this is inevitably handicapped by the fact that there would be no clinical markers, (e.g. clinical reactions such as fever and rashes) and so such a method could only be evaluated by measuring the serum antibody level in blood samples.

Therefore, in adopting the KL method, techniques which result in a very slight fever or rash seem to be most desirable.

A four-week interval between the inoculations of killed and live vaccine seems slightly better than a two week interval.

In Exp. 1 and 2, the antibody response to the two kinds of vaccine and the clinical reactions to live vaccine showed considerable, individual differences which were not dependent on the age or weight of the children.

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