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STUDIES ON THE COMBINED USE OF KILLED AND LIVE MEASLES VACCINES
II. ADVANTAGES OF THE INHALATION METHOD

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SUMMARY The combined use of killed and live (K & L) measles vaccine was found to be improved by adopting the method of inhalation, in place of injection, of live vaccine.

As a result, even with quite a high antibody titer due to inoculation of killed measles vaccine, a further increase was obtained after inhalation of live vaccine.

INTRODUCTION

Under the present conditions the combined use of killed and live measles vaccines seems to afford the best method for mass immunization against measles. In this method, pre-treatment with killed vaccine reduces the clinical reactions induced by subsequent inoculation of live vaccine, and does not affect the antibody response.

However, there is a great difference in the response of different individuals. The antibody titers observed following inoculation with killed vaccine were very variable while the clinical reactions to live vaccine varied from severe to negligible. Generally speaking, when the antibody titer following inoculation with killed vaccine was high, the clinical reactions following subsequent inoculation with live vaccine were low, and vice versa, (OKUNO *et al.*, 1965).

Thus an attempt was made to develop a practical method for use of live vaccine which would maintain a high antibody titer following inoculation with killed vaccine and would also evoke a sufficiently large antibody response. For this purpose, the inhalation method, which had previously been employed for inoculation of live vaccine, was tested for use in the combined method with killed and live (K & L) vaccines.

It was found that the inhalation method always caused an increase in antibody titer irrespective of the degree of the antibody response following a single inoculation of killed vaccine. Similar results had not previously

been obtained with any method for injection of live vaccine. Thus, while a high antibody titer was maintained following inoculation of killed vaccine after a subsequent injection of live vaccine there was generally no elevation of the antibody titers. However, the special feature of the inhalation method with live vaccine was that a general increase in antibody titer was observed.

This paper gives experimental data on the live vaccine inhalation method.

MATERIALS AND METHODS

1. *Vaccine*

The following three killed vaccines used.

A. Plain fluid vaccine $4^{2.28}$

B. Plain vaccine concentrated with ammonium sulfate $4^{2.85}$

C. Adjuvant vaccine $4^{3.18}$
(Precipitated with aluminium phosphate)

The vaccines employed were prepared at the Kanonji Institute of the Research Foundation for Microbial Diseases of Osaka University. Vaccines A, B and C were used in the field trials of the Committee for the study of measles vaccine as No. 1, 2 and 3 respectively in 1964.

Biken vaccine Lot 17 was used as the live vaccine.

2. *Test of potency of inactivated vaccines*

The antigen extinction limit (AEL) of the vaccine was determined by the mouse potency test with a 3 week interval between intraperitoneal inoculation and bleeding (TOYOSHIMA *et al.*, 1965).

The AEL values obtained were corrected for the results of simultaneous tests with reference vaccine and geometric mean values of triplicate tests were taken as the potencies of the vaccines.

3. *Administration method*

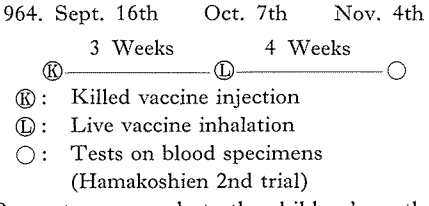
Killed vaccines A and B were injected subcutaneously in 1.0 ml doses and killed vaccine C was injected into three groups in doses of 0.5, 0.3, and 0.15 ml, respectively.

The live vaccine was always given by inhalation using a routine type compressor (Nissho-type) with a newly devised nebulizer.

4. *Vaccination schedule*

One hundred and forty-five children without any history of measles living in the H housing project were used as test subjects. Three weeks after one of the three kinds of killed vaccine had been injected into the children, live vaccine was inoculated by inhalation.

Neutralizing (NT) antibody was measured in blood specimens on the day of inoculation with killed vaccine (pre K), on the day of inoculation with live vaccine (post K), and four weeks after inoculation with live vaccine (post L). The schedule is as follows:



Ⓚ: Killed vaccine injection
Ⓛ: Live vaccine inhalation
○: Tests on blood specimens
(Hamakoshien 2nd trial)

Requests were made to the children's mothers so that they might describe clinical data in detail as to the pertinent items.

TABLE 1 *Febrile reactions in groups A, B and C following live vaccine inoculation*

Killed vaccine group	No. vaccinated	No. Exam.	No. of subjects with fev. (%)	Children who had febrile reaction			
				Average Max. Temp.	>39.0°C (%)	>39.5°C (%)	Duration of fev. (days)
A	27	23	19(83)	38.9	8(35)	3(13)	2.6
B	26	20	14(70)	38.8	7(35)	5(25)	2.0
C	92	63	31(49)	38.4	5(8)	3(5)	1.7
(0.5)	20	13	5(38)	38.7	1(7)	1(7)	1.8
(0.3)	29	19	8(42)	38.1	1(5)	0(0)	1.6
(0.15)	43	31	18(58)	38.5	3(10)	2(6)	1.7
Total	145	106	64(60)		20(19)	11(10)	

RESULTS

1. *Clinical reactions*

Inoculation with killed vaccine did not have any notable adverse effects. The clinical reactions following live vaccine inoculation subsequent to killed vaccine inoculation are summarized in Table 1.

In general, the combined use of killed and live vaccine caused less clinical reaction than live vaccine alone, though it did cause a fever of more than 39.0°C in some children.

In general when live vaccine was inoculated after injection of killed vaccine (A, B or C) children receiving vaccine A had the severest clinical symptoms, followed by those receiving B and C, which indicates the order of the potencies of these vaccines. Though no marked difference in the potencies of B and C was observed in mice B had a much lower potency than C in humans.

The extent of the inhibition of clinical symtoms in children receiving killed vaccine C was not very great, but depended on the size of the inoculum.

2. *Antibody response*

Blood specimens were taken from 55 of the 145 children inoculated with killed and live vaccines. The clinical reactions and NT antibody titers found are shown in Tables 2 and 3. In general, the antibody titers after inoculation with killed vaccine were low, while those after inoculation with live vaccine were very high and there was as good an antibody response as after inoculation with live vaccine alone.

TABLE 2 *Results of field trials on killed and live measles vaccines—1*

K vaccine group	Name	Body weight	Clinical symptoms after L vaccine inoculation		NT antibody titers (log_2)		
			Fever	Rash	Pre K	Post K	Post L
	H. I.	10.0	−	−			9.5
	M. F.	20.0	−	−			>10.5
	Y. T.	14.5	−	−	<0	<0	9.0
	K. F.	15.3	−	−	<0	2.5	>10.5
	K. H.	15.5	−	−	<0	3.0	>10.5
A	K. H.	14.0			<0	2.5	
	H. T.	14.5			<0	<0	9.0
	H. K.	17.0	+	−	<0	<0	8.0
	S. T.	11.0	+	+	<0	0.5	>10.5
	S. M.	14.0	+	−	<0	0.5	
	M. Y.	18.0	+	+	<0		8.5
	K. S.	16.0	+	−		1.5	10.0
	K. T.	12.0	−	−			9.5
	Y. S.	17.0	−	−			10.0
	A. K.	16.0	+	−	<0	<0	
B	A. S.	14.0	−	−			>10.5
	T. A.	18.5	+	+	<0	<0	>10.5
	K. S.	12.0	−	−			>10.5
	S. M.	17.0			<0	<0	>10.5
	K. T.	13.0			<0	<0	
	Y. H.	14.1	−	−			>10.0

(Home-dwelling group, 1964, Fall, Hamakoshien)

It should be noted that children who had a post K NT antibody titer of over 2^5 showed no clinical reaction but they all showed a marked increase in antibody titer following live vaccine inoculation.

This was not observed in the previous experiments on the effect of injection of live vaccine.

In one series of the present experiments (OKUNO *et al.*, 1965), out of 31 children with antibody titers of over 2^2, many of the 15 children who received injection had no take of live vaccine but all 16 cases subjected to inhala-

TABLE 3 *Results of field trials on killed and live measles vaccines—2*

Gr.	Name	Body weight	Clinical symptoms		NT antibody titers		
			Fever	Rash	Pre K	Pre L	Post L
	S. A.	11.5	+	+	<0	0.5	
C	N. H.	11.5			<0	1.5	
(0.5)	K. I.	19.3	−	−	<0	0.5	>10.5
	J. U.	14.4	−	−			>10.0
	K. T.	14.6	−	−		2.0	
	K. Y.	18.5	+	+	<0	2.5	>10.5
	S. N.	8.9	−	−			>10.5
	T. F.	12.5	−	−	>0.5	>5.5	9.0
	I. Y.	19.0	−	−	<0	1.0	10.0
	A. M.	20.0	−	−	<0	<0	9·5
C	Y. H.		+	−	<0	0.5	
(0.3)	Y. U.	15.0	+	−	<0	1.0	>10.5
	K. A.*	16.0	−	−	6.0	5.0	>10.5
	O. A.*	15.0	−	−	6.0	5.0	> 8.5
	S. A.*	13.5	−	−	>6.5	5.5	>10.5
	T. O.	15.2	−	−	<0	5.5	>10.0
	T. I.	15.8	+	+		1.0	>10.5
	T. M.	11.0	−	−			>10.0
	J. M.	15.9	−	−	<0	1.0	> 8.5
	K. M.	20.8	−	−	<0	1.5	> 8.5
	H. N.	20.5	+	+		3.0	
	K. K.	14.0	−	−	<0	1.5	> 9.5
	H. N.	16.8	+	+	<0	0.5	
	Y. F.	12.2	−	−			>10.5
	M. S.	11.0	−	−			>10.0
	M. N.	11.5	−	−			>10.5
	Y. Y.	15.1	−	−	<0		10.0
C	H. H.	14.5	−	−	<0	0	9.5
(0.15)	T. H.	9.5	−	−			>10.5
	T. H.	13.8	−	−			>10.5
	Y. K.	25.5			<0	<0	
	Y. K.	18.0	+	−	<0	<0	>10.5
	T. M.	19.5	+	−	<0	5.5	>10.5
	O. K.	15.1	−	−		5.0	>10.5

* KKK had already been administrated in Spring 1964

(Home-dwelling group, 1964, Fall, Hamakoshien)

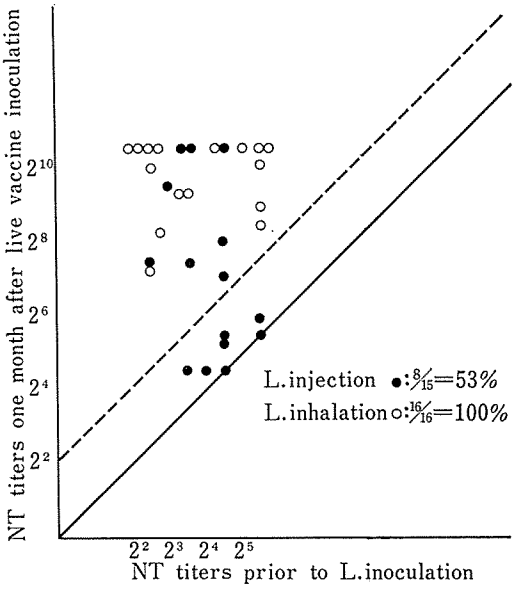


FIGURE 1 Comparison of the LV injection and inhalation methods following KV inoculation on the basis of increase in antibody titers (only $> 2^2$ listed).

tion had a take with a good antibody response (Fig. 1).

DISCUSSION

Three field trials were made of the combined use of killed and live measles vaccines in 1964. Two of these trials were made on inhalation, and one on injection of live vaccine.

It was found that while the serum antibody titer was maintained at a very high level injection of live vaccine resulted in no antibody response. However, following inhalation of live vaccine these high titers were greatly increased, reaching almost the same level as after live vaccine alone or during natural infections with measles.

The difference between the live vaccine injection method and the inhalation method was enhanced when the antibody titer following inoculation with killed vaccine increased. For example, at an NT antibody titer of 2^5 following inoculation with killed vaccine no case subjected to live vaccine injection had a take (Fig. 1) but all cases subjected to inhalation showed an increase in antibody titer. In two field trials of the inhalation method with live vaccine all 80 children showed an increase in their antibody titer following live vaccine inhalation (Tables 2, 3 and (OKUNO *et al.*, 1965)).

The question arises of why inhalation is better than injection. Probably the reason is that the NT antibody in the blood following inoculation of killed vaccine neutralizes the attenuated measles virus resulting in no stimulation of antibody producing cells after injection of vaccine. But even in the presence of NT antibody in the blood following inoculation with killed vaccine the upper respiratory tract cells seem to be infected by inhalation without disturbing the invasion and multiplication of the attenuated measles virus. Additional data are now being studied to confirm the advantages of adoption of the inhalation method for measles immunization.

It is suggested that in practice, following two injections of killed vaccine, live vaccine should be inhaled, in this way, most children will obtain sufficient immunity without developing clinical symptoms. Since the immunity thus obtained is an infection immunity, it will probably be solid and durable, but further follow-up seems necessary to confirm this point.

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