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STUDIES ON THE COMBINED USE OF KILLED AND LIVE MEASLES VACCINES
IV. FOUR YEARS FOLLOW-UP

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SUMMARY A clinical follow-up was made for 4 years on 230 children vaccinated by the combined use of killed (K) and live (L) measles vaccines and a serological follow-up was made in the third year after vaccination. Only 3 mild cases of measles occurred among these children. The protective efficacy was highest and almost complete in the KL group, intermediate in the KKL group and lowest in the KKK group.

A serological follow-up 3 years after vaccination revealed that the pattern of antibody persistence in the KL-inhalation group was more stable than in the other groups; the KL-injection group and the KKL and KKK-L groups.

INTRODUCTION

In previous papers of this series (Okuno et al., 1965a, 1965b and Ueda et al., 1966) we described the results of vaccination of 500 children with killed and live measles vaccines in Hamakoshien housing site (populations 20 thousand, in Nishinomiya city) and in Muyuen kindergarten in 1964 and 1965. The small quantity of antibody induced by killed vaccine reduced the clinical reactions to live vaccine accompanying with a increased antibody titer. Even with a high antibody titer after administration of killed vaccine, on inhalation rather than injection of live vaccine a further increase in antibody titer was observed without any clincial reaction.

A follow-up was made by postal inquiry in Hamakoshien housing site for 4 years from 1965 to 1968. Blood specimens were collected from vaccinees in Muyuen kindergarten and in Hamakoshien housing site for measles antibody analysis in the third year after vaccination. Vaccinees in Hamakoshien housing site with low neutralizing antibody titers were challenged with live measles vaccine by the inhalation method.

This paper presents the results of clinical and serological follow-up studies of the vaccinees immunized with killed and live measles vaccines.

MATERIALS AND METHODS

1. *Postal Inquiry*

Postcards were sent every year from 1965 to parents of vacciness in Hamakoshien housing site, who were generally well educated, asking whether their children had contracted measles. Parents were also asked whether there had been any measles epidemics in the area. If their children had contracted measles, they were asked to describe the doctor's diagnosis and the date of contraction and to describe the symptoms briefly.

2. *Serological Examination*

Measles antibody titrations were done following the overnight neutralizing test (Toyoshima et al., 1965). Post L sera (one month after live vaccine administration), sera 3 years after live vaccine administration (or before challenge with live vaccine) and sera after challenge with live vaccine (one month after live vaccine challenge) were stocked at −20 C before tests, and were titrated simultaneously to permit accurate comparison.

3. *Live Measles Vaccine Challenge*

Preliminary titration of sera 3 years after vaccination revealed that 5 children had low antibody titers in Hamakoshien housing site. These children were challenged with "Live Measles Vaccine BIKEN Lot C" (lyophilized chick amnion vaccine of Toyoshima strain, $10^{3.5}$ $TCID_{50}$/0.1 ml) by the inhalation method for 40 seconds. Blood specimens were again taken from 4 of them one month after challenge with live vaccine.

RESULTS

1. *Contraction of Measles after Vaccination*

A total of 230 postal inquiry cards were sent to parents of vaccinees in Hamakoshien housing site in August 1965 and 174 answers were received. The numbers of answers decreased year by year partly because parents moved from the housing site. Table 1 shows the 4 years' results. In the KL group one case of measles occurred in 1967, in the KKL group one occurred in 1966 and in the KKK group one occurred in 1967. All 3 cases were mild. One case was afebrile with exanthema of the extremities. Among all the vaccinees surveyed, cases of measles occurred in the second and third years after vaccination, but not in the first and fourth years. There were measles epidemics in Hamakoshien housing site every year but since no cases of measles occurred in the fourth year after vaccination it seems that if vaccinees are in danger of contracting measles they will do so in the second or third year after vaccination, and the remaining children will be conferred a firm immunity.

2. *Serological Analysis*

Thirty blood specimens were collected from vaccinees in Muyuen kindergarten and in Hamakoshien housing site in May 1967, and their measles neutralizing antibody titers were measured.

TABLE 1. *Protective efficacy of combined killed and live measles vaccines (Results of 4 years postal inquiry in the Hamakoshien housing site)*

Vaccine	No. surveyed in 1965	1965 Case/Answer (%)	1966 Case/Answer (%)	1967 Case/Answer (%)	1968 Case/Answer (%)
KL	149	0/114 (0)	0/108 (0)	1/ 91 (1.1)	0/ 73 (0)
KKL[a]	49	0/ 37 (0)	1/ 32 (3.1)	0/ 29 (0)	0/ 20 (0)
KKK	8	0/ 6 (0)	0/ 6 (0)	1/ 5 (20.0)	0/ 2 (0)
KL-L	7	0/ 5 (0)	0/ 5 (0)	0/ 4 (0)	0/ 1 (0)
KKK-L	17	0/ 12 (0)	0/ 10 (0)	0/ 9 (0)	0/ 7 (0)
Total	230	0/174 (0)	1/161 (0.6)	2/138 (1.4)	0/103 (0)

Vaccination; between May and Oct., 1964 (a: *between Dec., 1964 and Feb., 1965*)

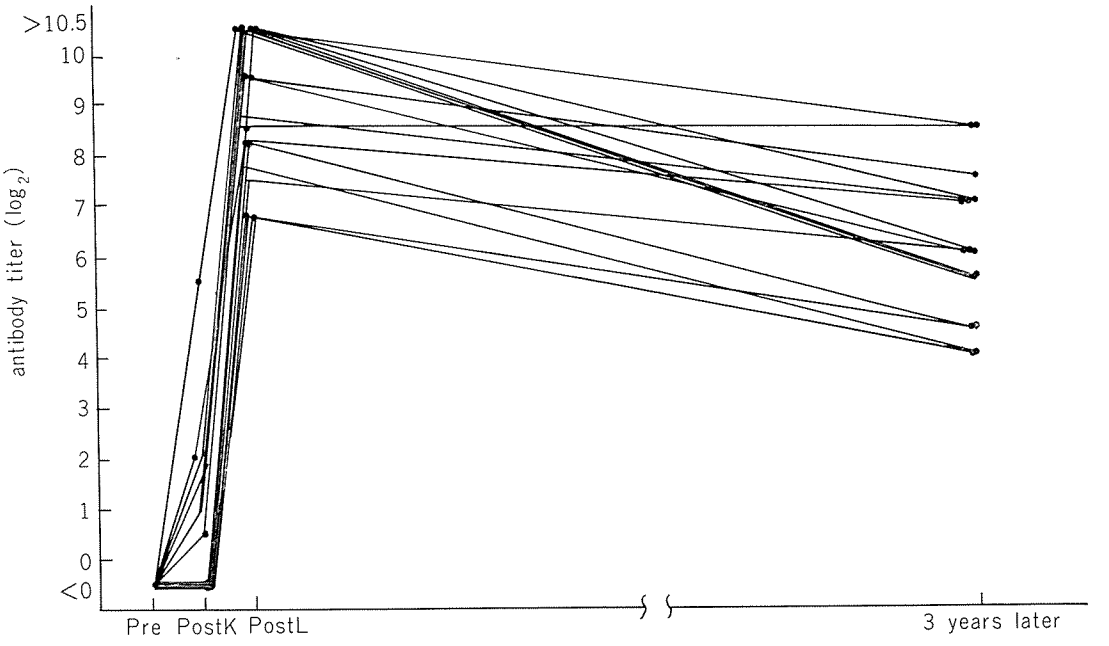

FIGURE 1. *Measles neutralizing antibody titers of* 15 *children* 3 *years after immunization with KL-inhalation.*

1) KL-inhalation group

As shown in Fig. 1, all 15 vaccinees immunized with a single dose of killed vaccine followed by live vaccine inhalation a month later had neutralizing antibody titers over 2^4. The patterns of persistence of antibody in this group was stable, and there was only a 4 fold reduction in the mean titer. No vaccinee showed an increased titer.

2) KL-injection group

The antibody levels of 6 vaccinees immunized with a sigle dose of killed vaccine followed by live vaccine injection a month later are shown in Fig. 2. Though the pattern of antibody persistence was less stable than that in the KL-inhalation group, neutralizing antibody of over $2^{1.5}$ was detected in all cases.

3) KKL group

Sera were collected from 5 children vaccinated with 2 doses of killed vaccine followed by live vaccine by injection or inhalation at monthly intervals. The pattern of antibody persistence was similar to that in the KL-injection group, as shown in Fig. 3. In this group there was no difference in the pattern of antibody persistence after injection and inhalation of live vaccine, as observed in the KL group.

4) KKK-L group

The children vaccinated with 3 doses of killed vaccine at intervals of 2 weeks between May and June 1964 inhaled live vaccine in October 1964. Four of them were available for serological follow-up. Fig. 4 shows the patterns of persistence of their antibody titers which were also less stable and were similar to those shown in Figs. 2 and 3.

3. *Clinical and Serological Responses to Live Vaccine Challenge*

Of 30 vaccinees available for antibody analysis 5 with low neutralizing antibody titers in May

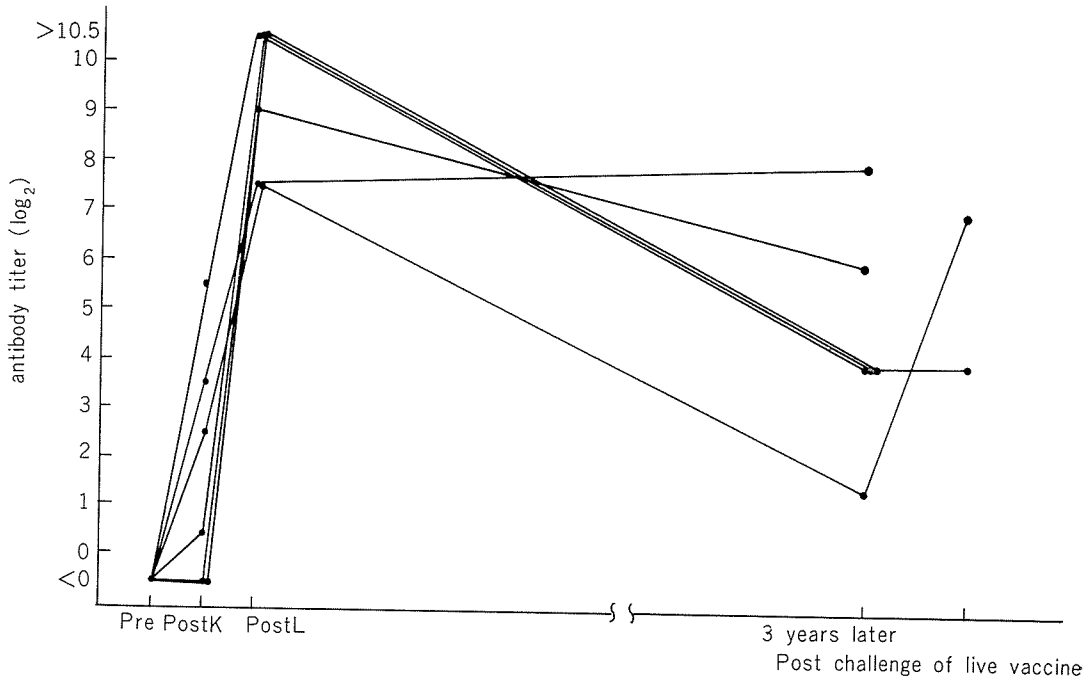

FIGURE 2. *Measles neutralizing antibody titers of 6 children 3 years after immunization with KL-injection. One case showed a booster response to live vaccine challenge.*

1967 were challenged with live vaccine, BIKEN Lot C, by the inhalation method. None of the 5 children showed any clinical reaction. Blood specimens were collected from 4 of the 5 children. The booster responses are shown in Figs. 2 and 3. Three children with antibody titers below 2^3 responded to live vaccine and their antibody titers increased after one month. The other child with an antibody titer of 2^4 resisted challenge with live vaccine.

DISCUSSION

In our experience, an attack of measles is followed by life long immunity, though details of the mechanism of persistence of immunity are not clear. The observation of Panum (1939) in the Faroe Islands showed that measles immunity persisted for at least 65 years without re-exposure to the disease. The data of Black and Rosen (1962) in Thahiti and of Bech (1960) in Greenland showed stable persistence of measles antibody following natural infection. Our seroepidemiological survey in Thailand (1967) and that of Enders-Ruckle et al. in West Germany (1965) revealed that nearly 100 per cent of adults had detectable measles antibody. These data suggest that persistence of measles antibody following natural attack is life long. A booster infection, however, may be important in maintenance of antibody in areas where measles epidemics occur. As we reported elsewhere (Ueda et al., 1969), booster infections of measles were observed in mothers with low neutralizing antibody titers when their children had measles. Krugman et al. (1965) and Sokes et al. (1961) reported similar phenomena in children. It, now, seems that 1) measles immunity following natural infections persists for many decades without re-

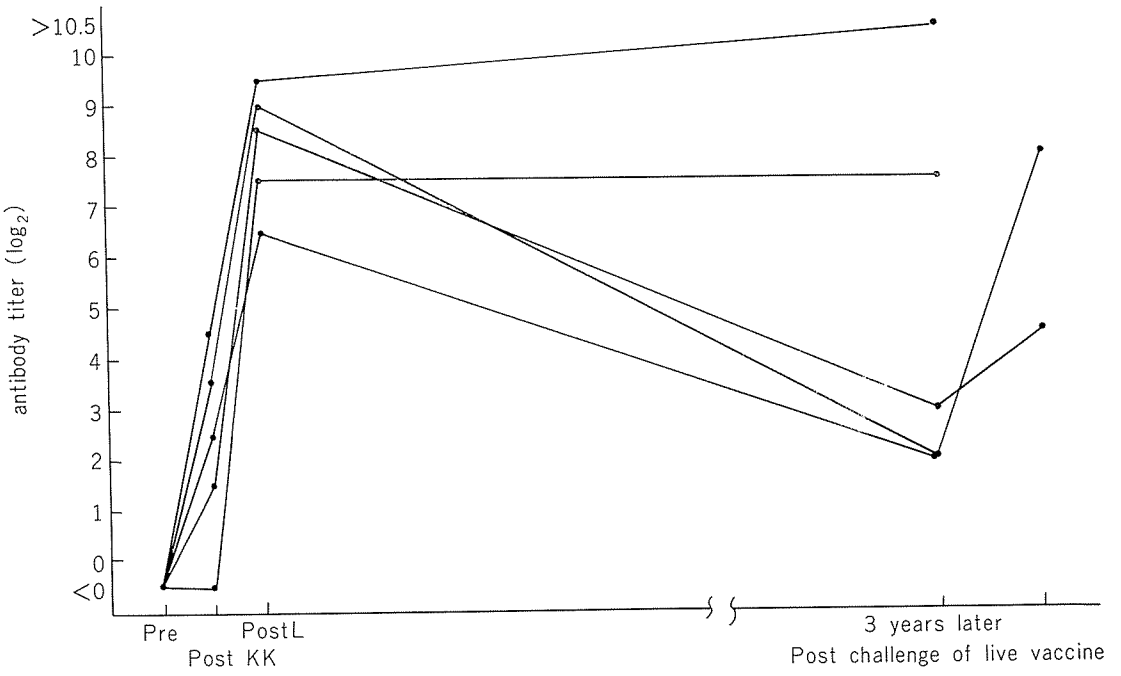

FIGURE 3. *Measles neutralizing antibody titers of* 5 *children* 3 *years after immunization with KKL.* 2 *cases showed booster responses to live vaccine challenge.*

infection, even on re-exposure, and 2) measles antibody titers are increased by booster infections in areas with epidemics, and so measles antibodies are detectable in almost every adult.

Immunity following measles vaccination is probably maintained in the same way as after natural measles infection. In the present survey of children vaccinated with killed and live measles vaccines, measles antibodies of children in the KL-inhalation group were maintained very stably. Antibodies in the children in the KL-injection, KKL and KKK-L groups were less stable than in the KL-inhalation group, but high levels of antibodies were detected in all cases. Some children showed increased antibody titers 3 years after vaccination without having suffered clinically detected measles. Experimental booster responses without any clincial reaction were observed, as shown in Figs. 2 and 3. These results may account for the occurrence of only 3 mild cases of measles among 230 vaccinees surveyed for 4 years after vaccination with a combination of killed and live vaccines, as shown in Table 1. Krugman et al. (1965) reported that the pattern and persistence of antibody response was similar following natural infection and vaccination with a single injection of live vaccine and that one inoculation of potent live vaccine would be followed by life long immunity. Brody et al. (1966) reported that two years after vaccination, in spite of the booster effect of natural measles, the titers among those who received inactivated vaccine plus live vaccine had fallen to low or undetectable levels in more than half of the vaccinees, suggesting that this combination might not produce lasting protection. In our present

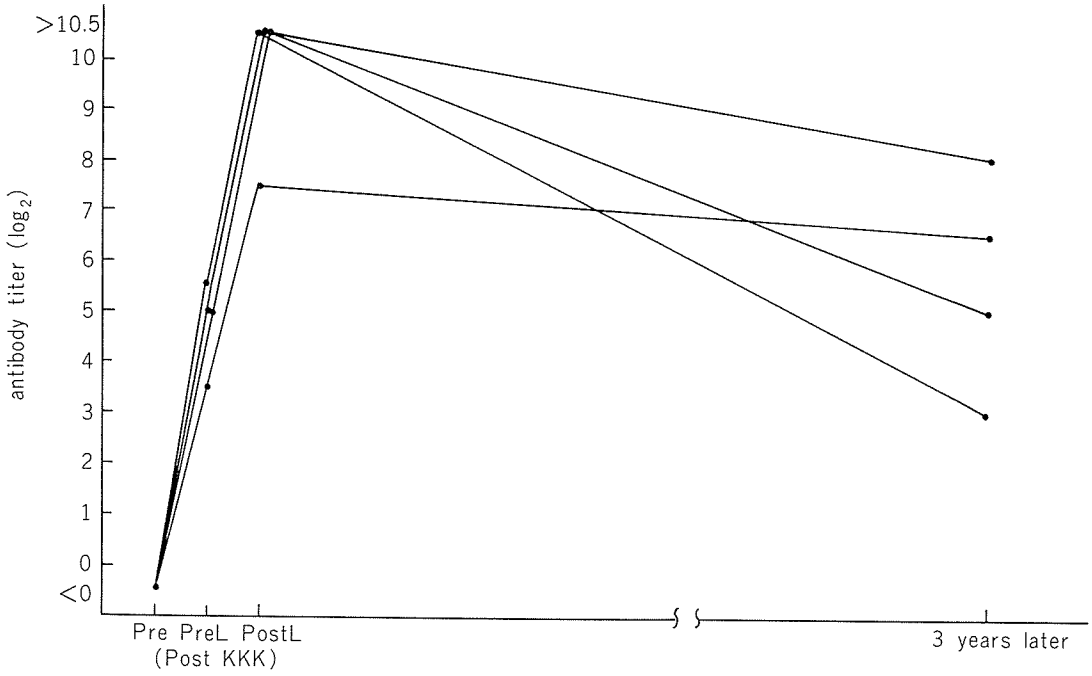

FIGURE 4. *Measles neutralizing antibody titers of* 4 *children* 3 *years after immunization with KKK-L. The interval between KKK and L was* 4 *months.*

study, however, the patterns of antibody persistence of children were stable, especially in the KL-inhalation group and life long immunity will be conferred by KL-inhalation without vaccination measles just as by live vaccine alone with vaccination measles.

The difference between the antibody persistence in the KL-inhalation group and in the KL-injection group may depend on the difference in the method of administration of live vaccine. Inhalation as a method of administration of live measles vaccine or other live virus vaccines against respiratory infections should be re-evaluated in view of antibody persistence and local immunity.

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