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NEUTRALIZING ANTIBODY RESPONSES TO JAPANESE ENCEPHALITIS VACCINE IN CHILDREN

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SUMMARY Two shots of the current Japanese encephalitis (JE) vaccine were given to children and their immune responses to the Nakayama strain (the vaccine strain) and two wild strains (JaGAr-01 and E-50) of JE virus were examined by neutralizing (N) antibody titrations.

Seventy vaccinees had no N antibody to JE virus before the first vaccination and were bled one month after the second vaccination. The N antibody responses to the JaGAr-01 and E-50 strains were found to be similar and to be less than that to the Nakayama strain after the second vaccination: the geometric mean titers (GMT) of N antibodies to the JaGAr-01 and E-50 strains (as logarithms) were 1.87 and 1.75, respectively, while the GMT to the Nakayama strain was 2.89. The seroconversion rates to the Nakayama, JaGAr-01 and E-50 strains were 70/70 (100%), 69/70 (99%) and 68/70 (97%), respectively, after the second vaccination.

Twenty-seven of the 70 vacciness were also bled before the second vaccination. Most of them showed a considerably high N antibody response against the Nakayama strain and only one vaccinee failed to show seroconversion after the first vaccination. However, the antibody response to the E-50 strain appeared to be rather low and 9 of 25 vaccinees did not show any seroconversion. Similarly 3 of 25 failed to show any seroconversion against the JaGAr-01 strain.

These results indicate that at the initial immunization two shots, at least, of the current JE vaccine are necessary to stimulate effective immune responses to wild strains of JE virus.

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INTRODUCTION

Since the early 1970's, cases of Japanese encephalitis (JE) have been decreasing in Japan. Vaccination of school children in Japan may be one reason for this decrease. On the other hand, the positive rate of antibody to JE virus in swine sera from slaughter-houses has been found to be more than 50% in many prefectures and JE virus has been isolated from *Culex tritaeniorhyncus* mosquitoes in the field in the epidemic season of JE in south-western parts of Japan (Igarashi et al., 1981). Moreover, during the last 10 years, JE has been a major problem in public health in some Asian countries, such as Thailand and India, where JE vaccine has not been used extensively.

Previously we reported (Susilowati et al., 1981) the immune responses induced by JE vaccine in comparison with the neutralizing (N) antibody responses to the homologous Nakayama strain and a wild strain of E-50, which had been isolated from wild *C. tritaeniorhyncus* in clone C6/36 of Singh's *Aedes albopictus* cell culture (Igarashi, 1978). The work was done because (1) the Nakayama strain used in the vaccine might differ in antigenicity from wild strains and (2) no data have been reported on the N antibody responses to newly isolated wild strains induced by the current JE vaccine. However, the results obtained in the work were not clear, because the study was made on adults (20–60 years old) most of whom had a positive N titer to JE before vaccination. Furthermore, in the previous study, JE vaccine was administered as a single booster dose, whereas two shots of JE vaccine are usually given at the time of initial immunization in Japan. Therefore, in this work we used the standard immunization procedure and we examined the immune responses of children who had no N antibody before vaccination.

This paper describes the N antibody responses of the children to the Nakayama, JaGAr-01 and E-50 strains of JE virus after the first and second vaccinations.

MATERIALS AND METHODS

1. *Vaccination and bleeding*

Purified inactivated vaccine (Takaku et al., 1968) was obtained from the Research Foundation for Microbial Diseases of Osaka University (Lot No. 285, 294 and 340, in the liquid state). The schedule of vaccination and bleeding of the vaccinees is shown in Fig. 1. In all 119 children of 1 to 11 years old were given two shots of JE vaccine (0.5 ml for 1–3 year olds and 1.0 ml for older children) with an interval of one month between shots. The children were bled by cubital puncture 3 times: once before the first vaccination (pre-vaccination serum), once before the second vaccination (post-vaccination serum I) and finally one month after the second vaccination (post-vaccination serum II), as shown in Fig. 1. Administration of the vaccine and taking

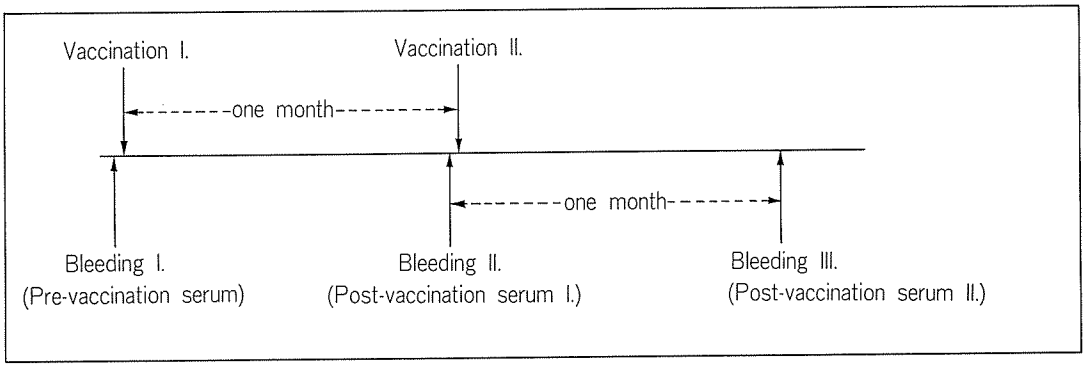

FIGURE 1. Schedule of vaccination and bleeding of vaccinees.

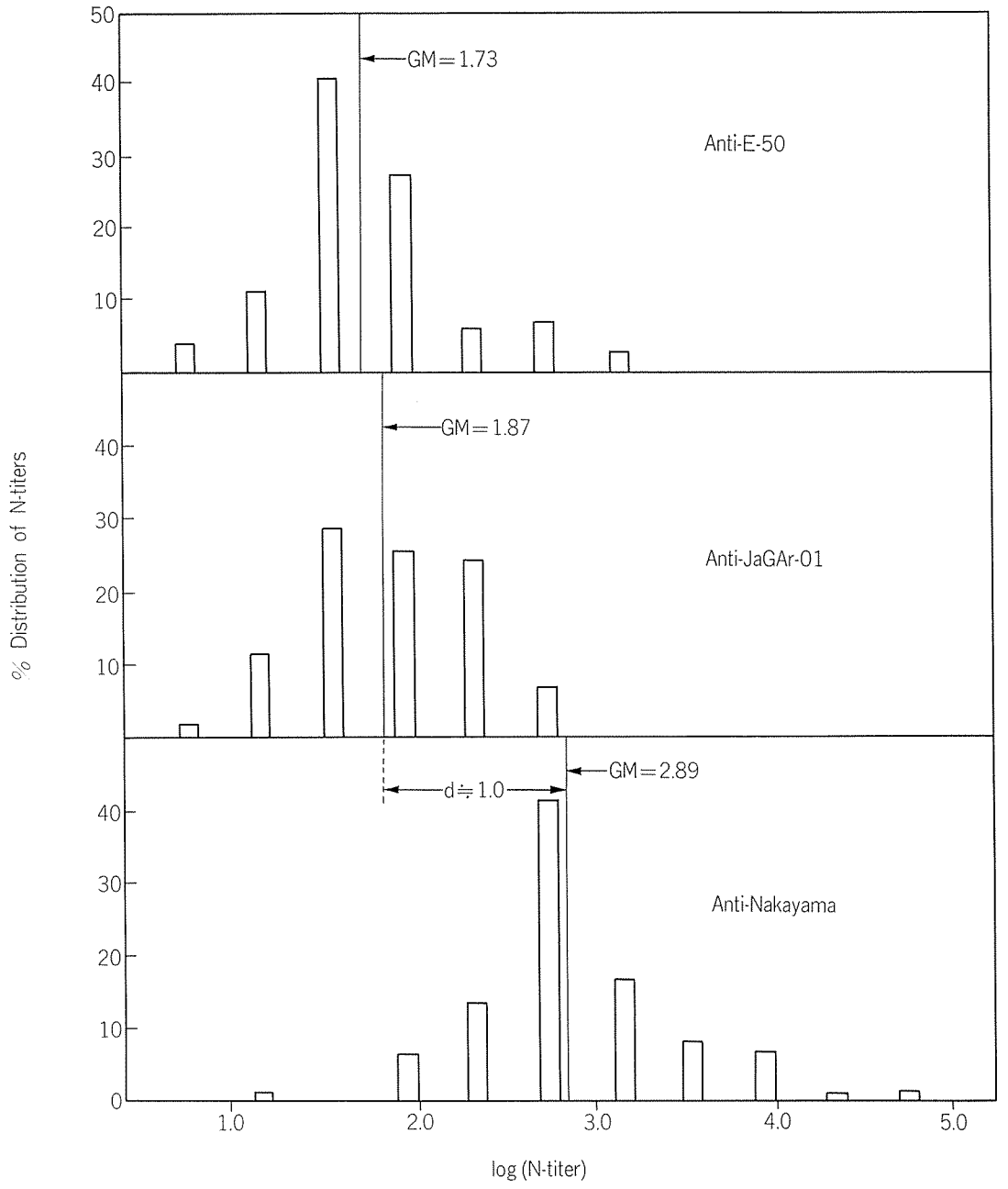

FIGURE 2. Distribution of N antibody titers against three strains of JE virus in post-vaccination serum II.

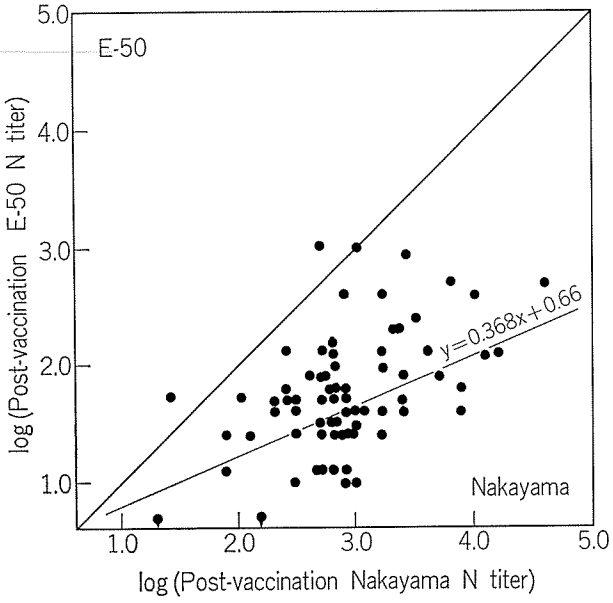

FIGURE 3. Correlation between N antibody titers of post-vaccination serum II against the Nakayama and E-50 strains.

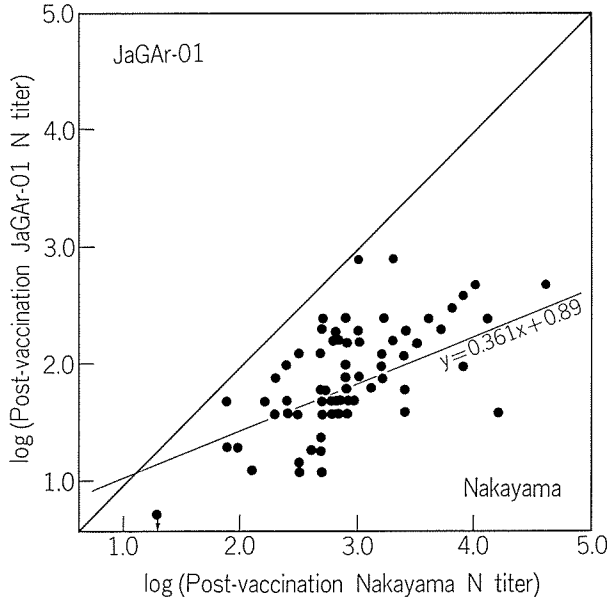

FIGURE 4. Correlation between N antibody titers of post-vaccination serum II against the Nakayama and JaGAr-01 strains.

of blood samples were carried out in the Department of Pediatrics, School of Medicine, Osaka University. The serum specimens collected were brought to the Serum Bank of the Research Institute for Microbial Diseases, Osaka University and stored in a nitrogen container until tests.

2. *Viruses*

The Nakayama strain (the vaccine strain) and the JaGAr-01 and E-50 strains of JE virus were used for neutralization tests on the sera of the vaccinees. The Nakayama and JaGAr-01 strains have been serially passaged in mouse brain and virus stocks were prepared from 10% homogenates of infected suckling mouse brain in Eagle's minimal essential medium. The homogenates were clarified by centrifugation at 10,000 rpm for 30 min and supernatants were stored in sealed ampoules at −70 C until use. The E-50 strain was isolated from *C. tritaeniorhyncus* mosquitoes in the field using clone C6/36 cell cultures in 1979, at Mihara City, Osaka, and was kindly supplied by Dr. N. Ueba, Osaka Prefectural Institute of Public Health. The virus was propagated in C6/36 cell cultures 4 times and infected culture fluids were harvested and kept in sealed ampoules at −70 C until use.

3. *Neutralization test*

The N antibody titers of the sera from vaccinees were determined by focus reduction neutralization tests applying peroxidase-anti-peroxidase staining method (Okuno et al., 1978). For the test, BHK21 cells cultured on Lab-Tek 8 chamber slides (Miles, Ill., U.S.A.) were used. Serum specimens were heat-inactivated at 56 C for 30 min before the test.

RESULTS

1. *N antibody titers one month after the second vaccination (post-vaccination II)*

Post-vaccination serum II (cf. Fig. 1) was obtained from 70 vaccinees who had had no N antibody to JE virus before the first vaccination. In these 70 specimens of post-vaccination serum II the distribution patterns of N antibody titers to E-50 and JaGAr-01 strains appeared similar, but the peak of the distribution pattern of titers to the Nakayama strain was shifted to higher titers, as shown in Fig. 2. The geometric mean titers (GMT) of the N antibodies to the E-50 and JaGAr-01 strains

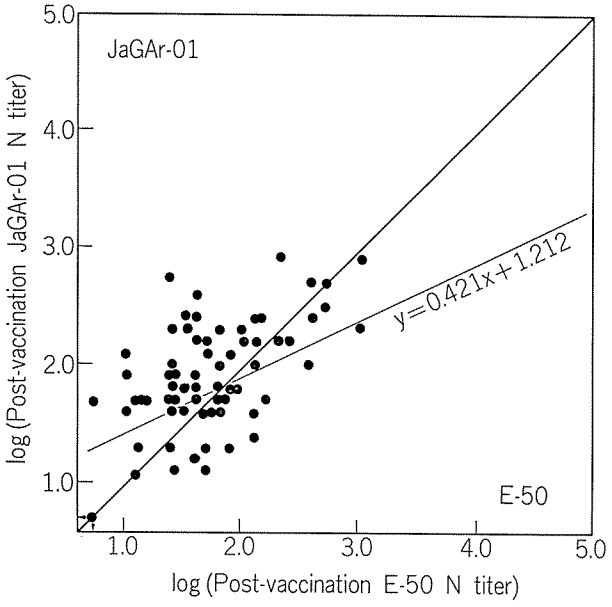

FIGURE 5. Correlation between N antibody titers of post-vaccination serum II against the E-50 and JaGAr-01 strains.

were 1.75 and 1.87, respectively, and that to the Nakayama strain was 2.89. All the vaccinees showed seroconversion to the Nakayama strain, but 2 of 70 (3%) and 1 of 70 (1%) vaccinees did not show seroconversions to the E-50 and JaGAr-01 strains, respectively (Fig. 2).

2. *Correlation of the N antibody titers of the post-vaccination serum II*

The correlation of the titers of the post-vaccination serum II to the Nakayama and E-50 strains (r=0.4611) is shown in Fig. 3. The distribution is markedly shifted toward the Nakayama strain, indicating that the N antibody responses induced by the JE vaccine are much stronger to the homologous strain than to the wild E-50 strain. Similarly in Fig. 4, showing the correlation between the titers to the Nakayama and JaGAr-01 strains (r=0.5188), the distribution pattern appears to be shifted toward the Nakayama strain, indicating that the N antibody response to the Nakayama strain is stronger than that to the JaGAr-01 strain. Figure 5 shows the correlation between the N antibody titers to the E-50 and JaGAr-01 strains (r=0.4485). Though the distribution pattern seems to be shifted toward the JaGAr-01 strain, the shift is not so distinct as that to the Nakayama strain in Figs. 3 and 4.

The correlations shown in Figs. 3, 4 and 5 were analyzed statistically. As shown in Table 1, the correlations of the N antibody titers to the three strains after the second vaccination were all statistically significant, while those of the titers to three strains after the first vaccination were all not significant.

3. *Distribution of N antibody titers in age-groups*

The 70 vaccinees were divided into 4 age-groups: 4 children of $\leq$1 year old, 27 of >1–3 years old, 30 of >3–5 years old and 9 of >5 years old. The distribution patterns of N antibody titers to the three JE virus strains in these age-groups are shown in Fig. 6. Though the numbers of the vaccinees in the youngest and the oldest age-groups were too small for comparison, the numbers in the age group of >1–3 and >3–5 years old were sufficient to allow comparison of the distribution patterns of titers. As shown in Fig. 6, there is no distinct difference in the distribution patterns of titers in the age groups of >1–3 years and >3–5 years old, for the three strains of the virus. This suggests that the capacities of children in these two age-groups to show N antibody responses to JE vaccine are similar.

4. *Individual N antibody responses after the first and second vaccinations*

All three blood samples were obtained from 27 of the 70 vaccinees, as shown in Fig. 1. As shown in Fig. 7, the N antibody response of the post-vaccination serum I to the Naka-

TABLE 1. *Statistical analyses on the correlations of N antibody titers to the three JE virus strains*

N antibody titer	Correlation between	Correlation Coefficient: r	Number of paired data n	Significance level[a]: r (n-2, 0.01)	Significance of correlation
After the first vaccination	Nakayama: E-50	0.1835	16	0.6226	not significant
	Nakayama: JaGAr-01	0.1943	16	0.6226	not significant
	E-50: JaGAr-01	0.1926	16	0.6226	not significant
After the second vaccination	Nakayama: E-50	0.4611	50	0.3613	significant
	Nakayama: JaGAr-01	0.5188	50	0.3613	significant
	E-50: JaGAr-01	0.4485	50	0.3613	significant

[a] Assayed by F-distribution (Fisher & Yates, 1953)

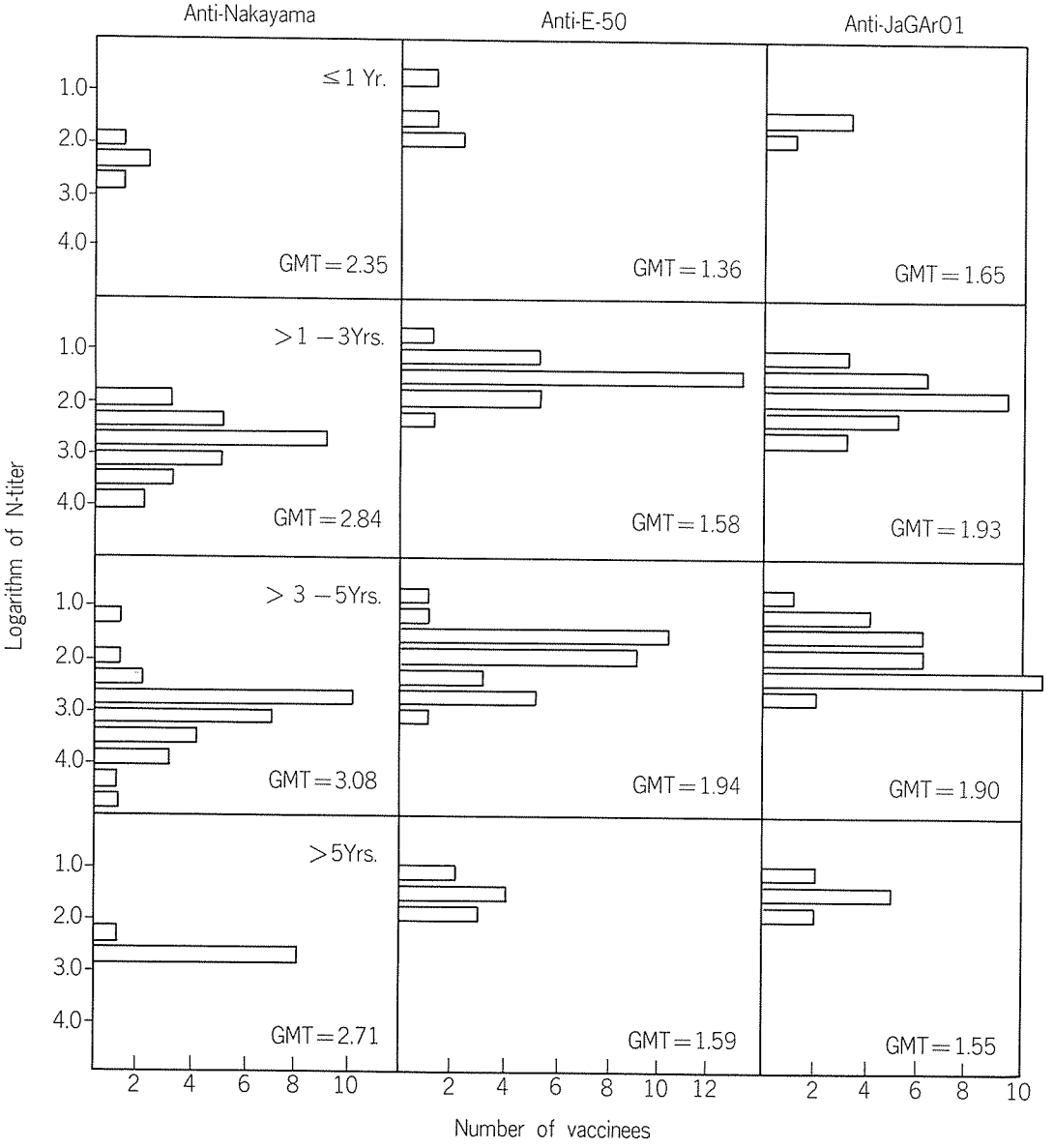

FIGURE 6. Distribution of N antibody titers to the three strains of JE virus in different age-groups.

yama strain appeared to be high (GMT=2.42), and only one vaccinee failed to show seroconversion. However, the GMT for the E-50 strain was distinctly lower (1.04), and at least 9 of 27 vaccinees showed no seroconversion. Similarly, 3 of 27 vaccinees did not show any seroconversion to the JaGAr-01 strain (GMT =1.32). However, after the second vaccination only one of 27 vaccinees remained N antibody negative to E-50, and all 27 vaccinees showed seroconversion to the Nakayama and JaGAr-01 strain. These results indicate that

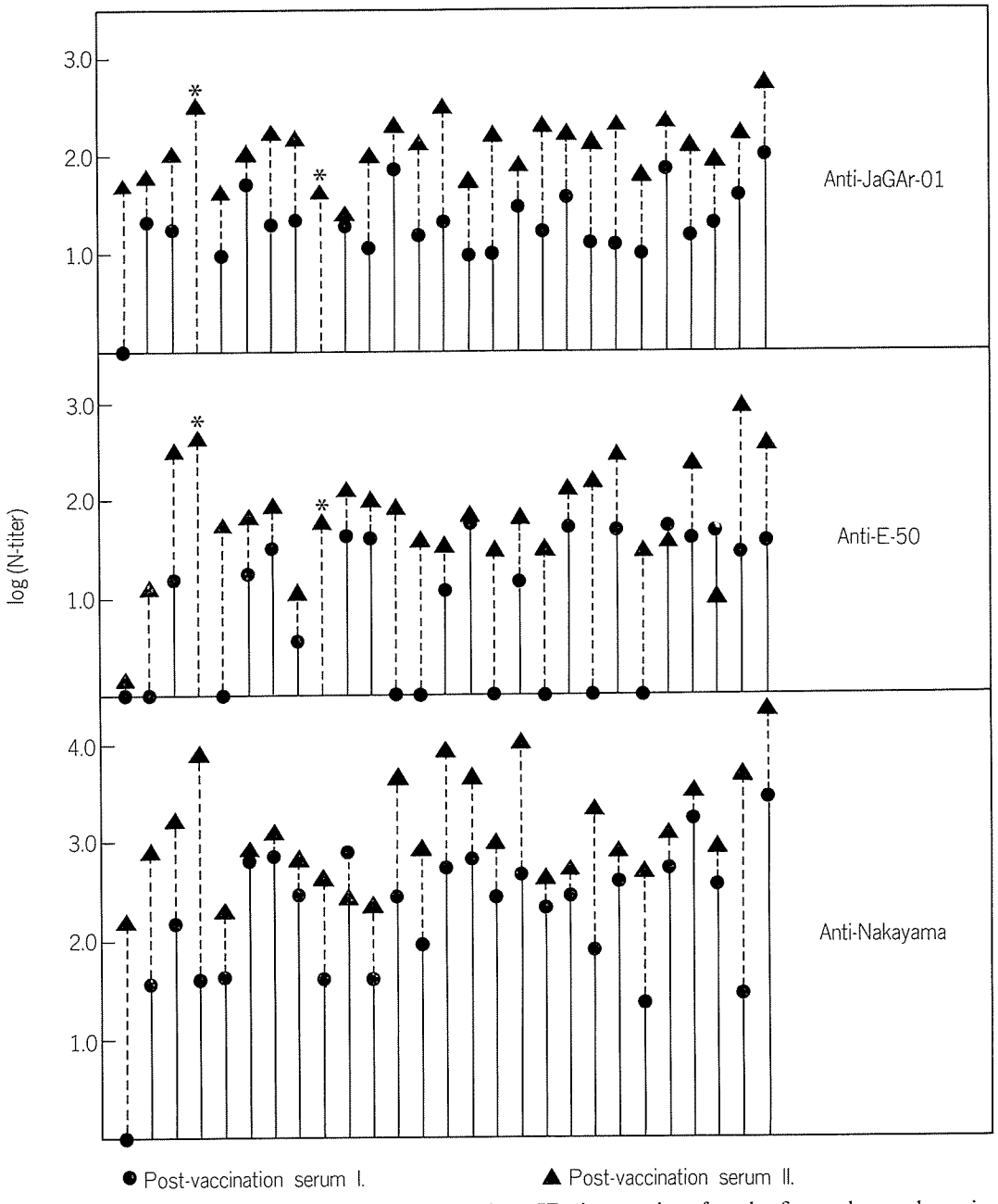

FIGURE 7. Individual N antibody responses to the three JE virus strains after the first and second vaccinations.

* N antibody titers were not determined because of shortage of serum.

two shots, at least, of the current JE vaccine are necessary to induce effective N antibody responses to wild strains of JE virus.

DISCUSSION

The pathogenesis of Japanese encephalitis (JE) is not completely clear. For instances, how does JE virus pass through the so called "blood-brain barrier"? Why do most individuals infected with the JE virus develop only inapparent infection and not show encephalitic symptoms? So far, there is no good answer to these questions. However, it has been shown that mice treated with the JE vaccine and having N antibody in the serum can survive a drastic challenge with homologous virus given by unnatural intraperitoneal or intracerebral injection. This observation suggests that the N antibody existing in the blood is able to block virus transmission to the brain through blood vessels or to inhibit growth of the virus in the brain. Therefore, the N antibody levels of human individuals induced by JE vaccine are presumably related to the capacity to withstand JE virus infection. Actually, an N antibody titer of 1:10, or 1.0 as a logarithm, to JE virus was demonstrated to be effective for survival of experimentally immunized mice challenged with the virus (Oya, 1967).

The N antibody responses induced by the JE vaccine have been studied using only "standard" JE virus of the Nakayama and/or JaGAr-01 strain, which has been passaged many times in mouse brain (Darwish et al., 1967; Kunita et al., 1968; Kanamitsu et al., 1970; Fukunaga et al., 1974; Quina et al., 1978), and the only report on the N antibody response to the wild strain of JE virus is our previous paper (Susilowati et al., 1981). However, our previous study was made on adult vaccinees many of whom already had N antibody before vaccination, and so the results were not clearcut.

In this work we studied 70 children with no N antibody to JE virus before vaccination. Most (61/70; 87%) of them were 5 years old or younger and so the JE vaccine was probably their first antigenic stimulation with JE virus antigen.

In 27 of these 70 vaccinees we examined the N antibody response after the first vaccination finding that 9 of 25 (36%) did not show any seroconversion to the wild E-50 strain. This value is consistent with the rate of 3/8 (38%) in the previous study. After the first vaccination, the N antibody response to the Nakayama strain was sufficiently high (GMT =2.42), and only one vaccinee failed to show seroconversion.

After the second vaccination, the N antibody responses to the JaGAr-01 and E-50 strains were similar, as shown by the distributions (Fig. 2) and correlations (Fig. 5) of the titers. The N antibody response to the Nakayama strain was distinctly higher than those to the JaGAr-01 and E-50 strains, as seen in Fig. 2. The GMTs, however, of the N antibodies to the JaGAr-01 and E-50 strains were 1.87 and 1.75, respectively, and the seroconversion rates were 69/70 (99%) and 68/70 (97%), respectively.

Thus the current JE vaccine seems to induce effective N antibody responses to the wild strains of JE virus when given by the two-shot procedure, because an N antibody titer of 1.0 is considered to be effective in preventing JE virus infection.

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