

Enzyme-linked Immunosorbent Assay on Japanese Encephalitis Virus

VI. Antibody response in human vaccinees

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Abstract: Antibody response in human vaccinees immunized with formalin-inactivated and purified Japanese encephalitis (JE) vaccine was examined by enzyme-linked immunosorbent assay (ELISA). Immunoglobulin (Ig-) ELISA antibody response in primary vaccination was relatively low, compared with marked response in booster immunization. On the other hand, antibody response as measured by the IgM-ELISA was only slight and observed in small number of vaccinees with the maximum titer below 100. The results substantiate the significance of IgM-ELISA in the serodiagnosis or seroepidemiological studies on JE to detect natural infections by JE virus.

Key Words: ELISA, Japanese encephalitis virus, vaccine, antibody

INTRODUCTION

We have been applying the ELISA to measure antibody titers against JE virus in patients as well as in inhabitants of JE-endemic or nonendemic areas in order to set up diagnostic criteria for the disease by the ELISA (Igarashi et al., 1981; Bundo et al., 1981; 1982). During these studies, we found that some sera in the endemic area showed significantly high Ig-ELISA titers and also that small number of healthy inhabitants possessed some degree of IgM-ELISA titers over 100. Since vaccination against JE has been performed as a prophylactic measure to prevent the disease, the antibody levels in healthy inhabitants should partly be due to the vaccination besides natural infections

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in endemic areas. It became necessary to measure the types of immunoglobulins as well as the antibody levels in vaccinees in order to know the nature of antibodies detectable by the ELISA, in relation to diagnostic criteria and detection of inapparent natural infections. Accordingly, we examined ELISA antibody levels in human sera of primary and booster vaccination in Hokkaido (JE-nonendemic area) and in Okinawa (JE-endemic area), and sera from adults with previous exposure to natural infections or JE vaccination.

MATERIALS AND METHODS

Test sera

A. Vaccinees in Hokkaido: Details of vaccination schedule, description on the vaccinees and antibody responses as determined by the neutralization (N) test were reported by Katsurada (1968a) and Kanamitsu et al., (1969). The vaccinees were the first and the second grade students in a Junior High School in Wakkanai City in the age of 12-14 years old. They did not have previous vaccination histories and did not have detectable antibodies against JE virus by the neutralization (N) test. The vaccines were made of the Nakayama strain of JE virus and Lot 1 vaccine was purified by alcohol-protamine method (Nakamura, 1971), while Lot 2 and 3 by ultracentrifugation (Takaku et al., 1968). The vaccines were given subcutaneously using 1 ml for each immunization. Test group No. 1 (20 students) received a single shot with Lot 3 vaccine and they were bled (1) at the time of vaccination, (2) 1 month, (3) 1 year, and (4) 2 years after the vaccination. Test group No. 2 (8 students) received a single shot with either Lot 1 or 2 vaccine and boosted 1 year later with Lot 3. They were bled (1) at the time of primary vaccination, (2) 1 month, (3) 5 weeks, (4) 4 months, (5) 1 year, (6) 13 months, and (7) 2 years after the primary vaccination.

B. Vaccinees in Okinawa: Details of vaccination schedule and results of antibody assay by the hemagglutination-inhibition (HI) and N tests were described by Ura et al. (1974; 1977). Vaccinees were 14 students of 5-13 years old living in Naha City, and did not show detectable HI and N antibodies and did not have previous vaccination histories. However, possibility of natural infection cannot be excluded, because Okinawa is one of the JE-endemic areas. Two successive injections with 1-2 weeks interval of commercial JE vaccine were given as primary vaccination. The vaccine had passed national standard (Ministry of Health and Welfare of Japan, 1956) and was used in volume of 1.0 ml. Booster immunization was given 10 months after the primary vaccination. Vaccinees were bled (1) at the time of primary vaccination, (2) 2 months after the primary vaccination, (3) 1 month, (4) 3 years, and (5) 8 years after the booster vaccination.

C. Adults with previous vaccination histories or probable exposure to natural infections: Eighteen adults between 23 to 64 years old were given a single subcutaneous shot of

purified JE vaccine (Kanonji Institute, Research Foundation for Microbial Diseases of Osaka University) with 1 ml volume. Seventeen of the vaccinees were the residents in Kyushu island and one in Shikoku island, both JE-endemic areas. Fifteen of the vaccinees had previous vaccination histories against JE, and 2 Okinawa residents did not, however, one of the 2 showed previous HI antibody titer of 1:20. Vaccination history of 1 Nagasaki resident is unknown. They were bled (1) at the time, and (2) 8 days after the vaccination.

ELISA: Indirect micromethod of Voller et al. (1976) was used with modifications as described (Igarashi et al., 1981; Bundo et al., 1981; 1982). Test sera were diluted 1:100 or 1:1000 and reacted with purified JE vaccine (Takaku et al., 1968) which had been coated to microplate wells as antigen. Color reaction of each test serum was compared with those of standard series of positive serum with known titer, and the titer of test serum was calculated by a computer system (Morita et al., 1982).

Hemagglutination-inhibition (HI) test: The method of Clarke and Casals (1958) was used with modification to microtiter system (Sever, 1962).

Statistical methods: The methods described by Snedecor (1952) were followed.

Reagents: Peroxidase-labelled anti-human IgG (heavy and light chains) was used to measure immunoglobulin (Ig-) ELISA titer, and peroxidase-labelled anti-human IgM (μ -chain specific) for IgM-ELISA. These enzyme conjugates were obtained from Cappel Laboratories, Pa. USA. Formalin-inactivated and purified JE vaccine concentrate and commercial vaccine products were kindly supplied by the Kanonji Institute, Research Foundation for Microbial Diseases of Osaka University. *o*-Phenylene diamine dihydrochloride was the product of Wako Pure Chemicals Co. Osaka.

RESULTS

ELISA antibody response in Hokkaido vaccinees: The result is summarized in Fig.1 and the changes in geometrical mean titer (GMT) is shown by continuous lines. The GMT of Ig-ELISA after the primary vaccination was 213 in test group No. 1, and 220 in test group No. 2, showing relatively low level of antibody response as determined by the Ig-ELISA. On the other hand, the titer after the booster immunization was 2627, showing significant antibody response. In contrast, IgM-ELISA titer in most of the vaccinees remained at low level of less than 25, with a single case of the titer 37 after the primary vaccination in group 2 and another case with titer of 32 after the booster immunization. Fig 2 shows antibody response in each individual before and after the primary or booster immunization. Twelve out of the 20 vaccinees in group 1 showed 2-fold or more increase in the Ig-ELISA titer by the primary vaccination, and four of them showed 4-fold or more increase (Fig. 2A). However, none of them showed increase in their IgM-ELISA titers. While in group 2, only a single case out of the 5 vaccinees showed

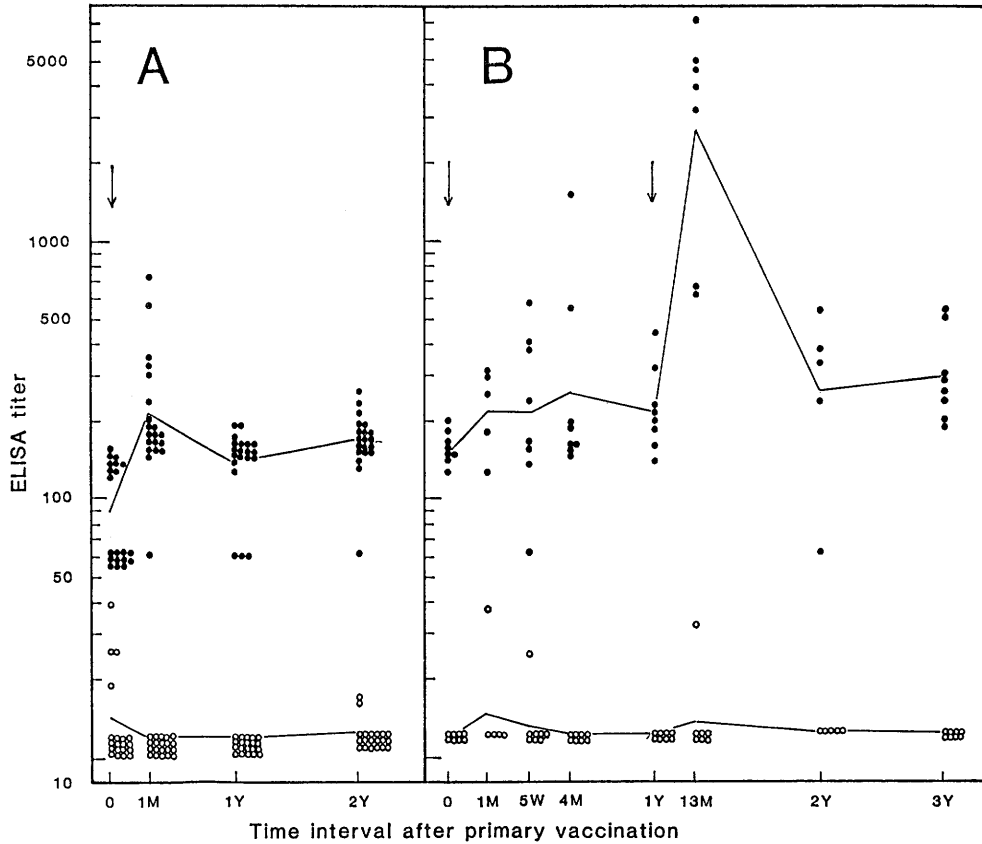


Fig. 1. Changes in the ELISA antibody titers in Hokkaido vaccinees. Vaccinees of test group No. 1 (A), and No. 2 (B) were immunized and bled, and their sera were tested by the Ig-ELISA (\bullet), and IgM-ELISA (\circ) as described in the Materials and Methods. Continuous lines represent changes of GMT.

more than 2-fold increase in the Ig- and IgM-ELISA titers (Fig. 2B). However, all the 7 vaccinees showed more than 2-fold increase in their Ig-ELISA titers after the booster vaccination, and five of them more than 4-fold increase, and one out of the 5 increased in its IgM-ELISA titer more than 2-fold (Fig. 2C).

ELISA antibody response in Okinawa vaccinees: The result is summarized in Fig. 3, and changes in GMT were shown by continuous lines. The GMT of Ig-ELISA after the primary vaccination was 496, which increased to 2100 after the booster immunization. The result is consistent with those observed with Hokkaido vaccinees. Slight increase in the Ig-ELISA titers from 4 years to 9 years after the vaccination may partly be due to natural inapparent infections. On the other hand, the level of IgM-ELISA titer was relatively low throughout the observation period, with 3 cases showing slight increase 2 months after the primary vaccination, and the highest titer was 54, which

remained at the level of 36 one month after the booster. All the other vaccinees showed IgM-ELISA titer less than 25 throughout the observation period. Fig. 4 shows antibody response of each vaccinees in the primary or booster immunization. Nine out of the 13 vaccinees showed 2-fold or more increase in their Ig-ELISA titer after the primary vaccination, and 7 of them showed 4-fold or more increase (Fig. 4A). While three of the 7 cases showed 2-fold or more increase in their IgM-ELISA titer and two of them showed 4-fold increase (Fig. 4). Fig 4B shows antibody titer in each vaccinee 2 months after the primary vaccination and 1 month after the booster vaccination. Eleven of the 13 vaccinees showed more than 2-fold increase in their Ig-ELISA titers and eight of them more than 4-fold increase. In contrast, none of them showed significant increase in their IgM-ELISA titers.

ELISA antibody response in booster immunization to adults with previous vaccination or probable natural infections: The result is shown in Fig. 5 and changes in GMT by continuous lines. The GMT of Ig-ELISA at the time of vaccination was 764, which increased to 2802 after the immunization (Fig. 5A). showing similar response observed in booster immunization in Hokkaido or Okinawa. On the other hand, changes in IgM-

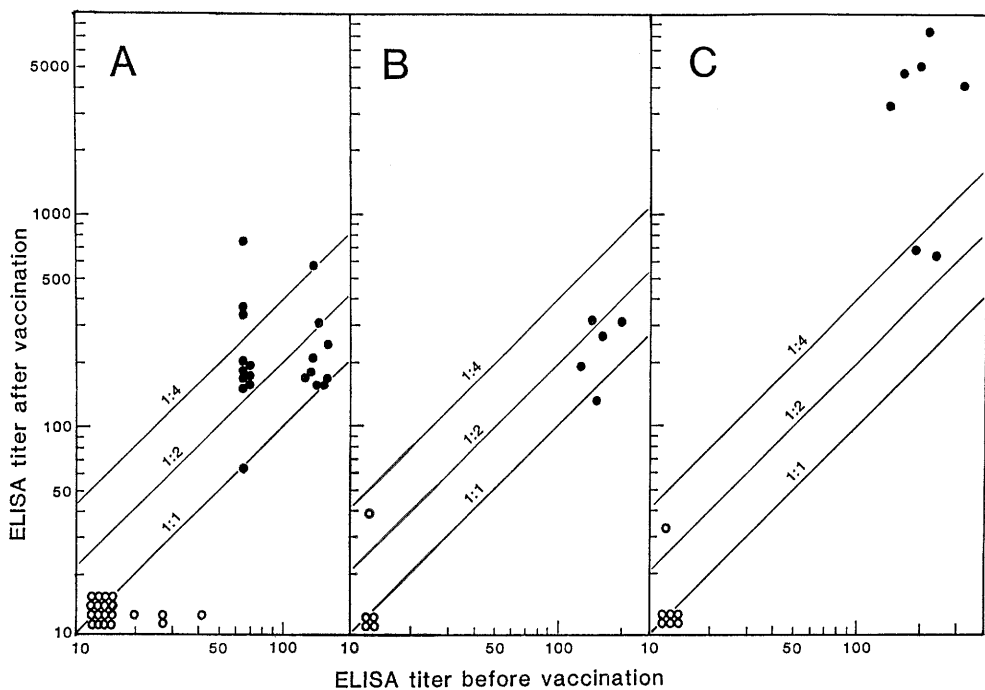


Fig. 2. ELISA antibody response in each Hokkaido vaccinee. Ig-ELISA (\bullet), and IgM-ELISA (\circ) titers of each individual at the time of primary and 1 month after the primary vaccination of group No. 1(A); at the time and 1 month after the primary vaccination of group 2(B); and at the time and 1 month after the booster immunization of group 2 (C) were compared.

ELISA titer were relatively slight with the GMT from 18 to 29 following immunization, and the maximum titer was 67 (Fig. 5B). Change of the HI-GMT was from 5.8 to 18 by the immunization (Fig. 5C). Changes in the antibody titers in each vaccinee is shown in Fig. 6. Thirteen of the 15 vaccinees showed more than 2-fold increase in their Ig-ELISA titers and seven of them 4-fold or more increase. On the other hand, only 4 vaccinees showed 2-fold or more increase in their IgM-ELISA titers and two of them 4-fold or more increase (FIG. 6A). The antibody response examined by the HI is shown in Fig. 6B, and eleven of the 13 vaccinees examined showed 2-fold or more increase and seven of them 4-fold or more increase.

Comparison of the rates of seroconversion or antibody response by the ELISA with

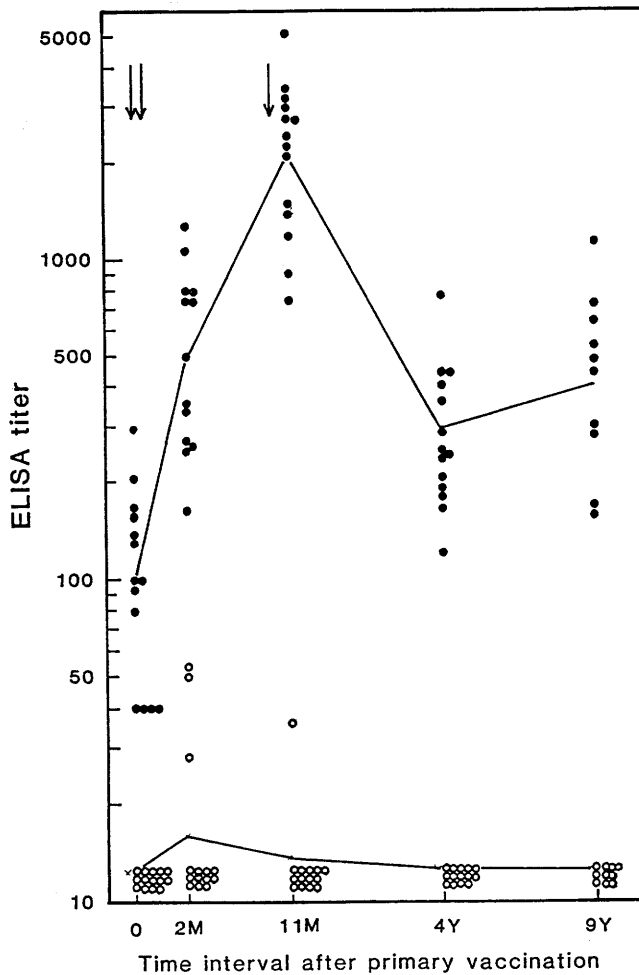


Fig. 3. Changes in the ELISA antibody titers in Okinawa vaccinees. Vaccinees were immunized and bled and their sera were tested by the Ig-ELISA (●), and IgM-ELISA (○) as described in the Materials and Methods. Continuous lines represent changes of GMT.

those by the *N*- or *HI*-tests: The antibody response of Hokkaido vaccinees was examined by the *N*-test and was reported by Katsurada previously (1968a). The data obtained by the present ELISA were compared with his data in terms of seroconversion rate or antibody response, taking the criteria of 4-fold or more increase in the titer as significant (Table 1). Nineteen out of the 20 vaccinees in group 1 showed seroconversion in their *N*-antibodies after the primary vaccination, and 4 of them seroconverted also by the Ig-ELISA, and the seroconversion of the remaining 15 was detected only by the *N*-test. Also in the case of group 2, seroconversion after the primary vaccination was detected only by the *N*-test in 2 out of the 5 vaccinees, and none of them showed 4-fold or more increase in in their Ig-ELISA titers. While, after the booster immunization, five of the 7 vaccinees showed significant antibody response both by the *N*-test and Ig-ELISA, and two of the 7 vaccinees showed antibody response only by the *N*-test and not by the Ig-ELISA. The antibody response in Okinawa vaccinees were examined by Ura et al. (1974; 1977) by the *HI*- and *N*-tests. Their data were compared with the present results by the ELISA in terms of seroconversion or antibody response and were shown in Table 2. Following primary vaccination, seven showed seroconversion as de-

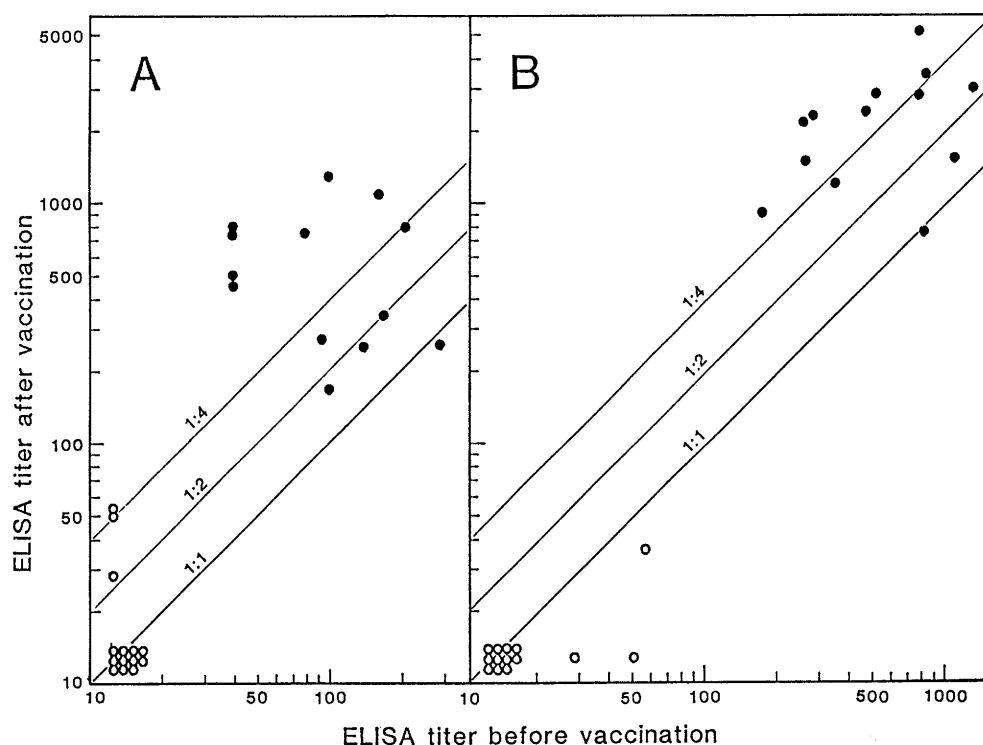


Fig. 4. ELISA antibody response in each Okinawa vaccinee. Ig-ELISA (\bullet), and IgM-ELISA (\circ) titers of each individual at the time and 2 months after the primary vaccination (A); and 2 month after the primary vaccination and 1 month after the booster immunization (B) were compared.

tected by the N-test, and four of them also by the HI, and four of the 7 by the ELISA. Two out of the 4 ELISA-positive vaccinees showed 4-fold increase in their IgM-ELISA titers. On the other hand, three vaccinees showed seroconversion by the Ig-ELISA without seroconversion in their N-antibodies. Compared with the result by the HI, only a single vaccinee showed seroconversion both by the HI- and N-tests without seroconversion in the Ig-ELISA antibodies, while four showed seroconversion in the Ig-ELISA without seroconversion in the HI antibodies. Comparison of the titers 2 months after the primary vaccination with those 1 month after the booster showed 11 vaccinees with significant antibody response by the N-test, and seven of them with significant Ig-ELISA antibody response. One of the vaccinees showed significant antibody response detectable by the HI- and Ig-ELISA and not by the N-test. Compared with the results by the HI, three vaccinees showed significant HI antibody response without showing 4-fold or more increase in their Ig-ELISA titers. Table 3 summarizes

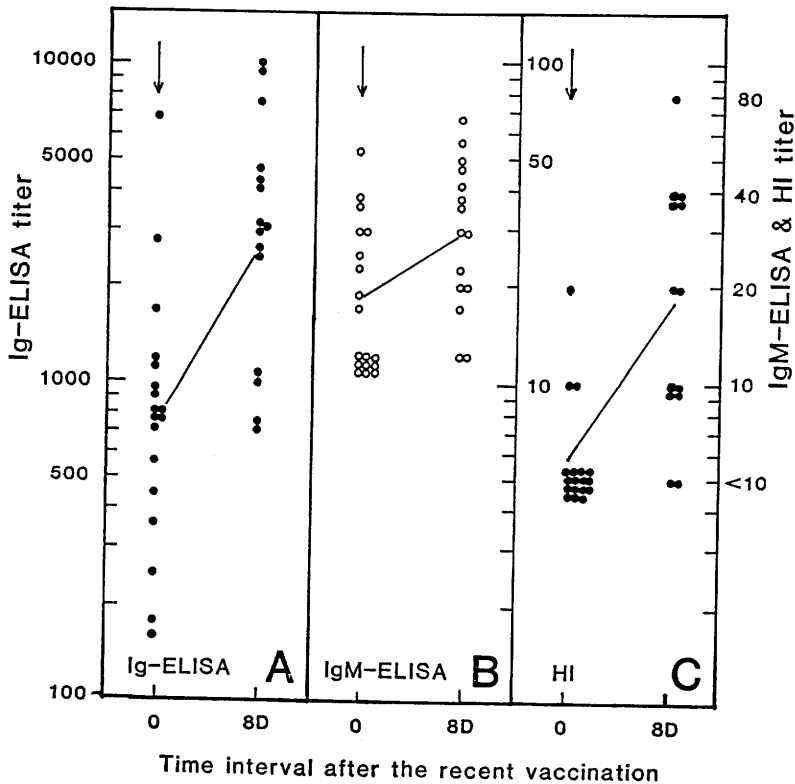


Fig. 5. Changes in the ELISA and HI antibody titers in adults with previous vaccination or exposure to natural infections and boosted with a single shot of JE vaccine. Vaccinees were immunized and bled and their sera were examined by the Ig-ELISA (A), IgM-ELISA (B), and HI (C) as described in the Materials and Methods. Continuous oblique lines represent changes in GMT.

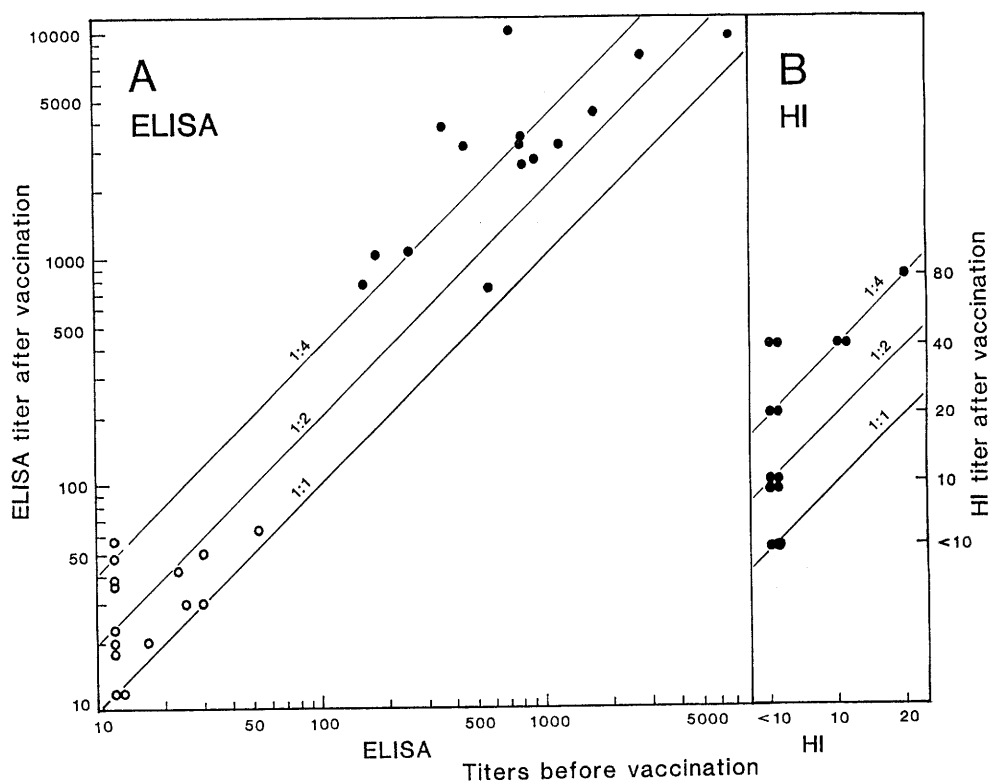


Fig. 6. ELISA and HI antibody response in each vaccinee in Fig. 5. Ig-ELISA (\bullet), and IgM-ELISA (\circ) titers in (A), and HI titer (\bullet) in (B) of each individual at the time and 8 days after the vaccination were compared.

Table 1. Seroconversion and antibody response in Hokkaido vaccinees as detected by the N-test and Ig-ELISA.

Antibody response		Number of vaccinees		
		Group 1	Group 2	
N-test	Ig-ELISA	primary	primary	booster
+	+	4	0	5
+	-	15	2	2
-	+	0	0	0
-	-	1	3	0
Total No. examined		20	5	7

+ represents those showing 4-fold or more increase in the antibody titers.

Table 2. Serconversion and antibody response in Okinawa vaccinees as detected by the N-test, HI-test, and Ig-ELISA

Antibody response			Number of vaccinees	
N-test	HI test	Ig-ELISA	primary	booster
+	+	+	3(1)	7
+	+	-	1	3
+	-	+	1(1)	0
-	+	+	0	1
+	-	-	2	1
-	+	-	0	0
-	-	+	3	0
-	-	-	3	1
Total No. examined			13(2)	13

+ represents those showing 4-fold or more increase in the antibody titers. Figures in the parentheses shows the number of vaccinees showing 4-fold increase in IgM-ELISA

Table 3. Antibody response in adults with previous vaccination histories or natural exposure to JE virus and boosted with JE vaccine as detected by the HI and Ig-ELISA

Antibody response		Number of vaccinees
HI-test	Ig-ELISA	
+	+	2
+	-	5
-	+	3
-	-	3
Total No. examined		13

+ represents those showing 4-fold or more increase in the antibody titers.

the antibody response in adults receiving "booster" immunization with probable previous exposure to JE virus or prevaccination histories. The result of Ig-ELISA is compared with that by the HI, which was performed on 13 out of the 15 vaccinees. Seven showed significant HI antibody response, and two of them also showed 4-fold or more increase in their Ig-ELISA titers, while the response of 3 persons was significant only by the Ig-ELISA and not by the HI. These results seem to indicate that the N-test is most sensitive to detect seroconversion by the vaccination, and Ig-ELISA has comparable sensitivity as the HI.

DISCUSSION

Our data indicate that the antibody response after the primary vaccination is relatively low as examined by the ELISA, especially the response in IgM-ELISA was much less than those observed in JE patients (Bundo et al., 1981). The result is in contrast to those observed by the N- or HI-tests, since IgM antibody production was definitely shown in the vaccinees using the same serum specimens (Katsurada, 1968b; Kanamitsu et al., 1969), which is similar to those observed in JE patients (Ishii et al., 1968). These results, together with relative insensitivity of the ELISA to detect seroconversion in the primary vaccination, may suggest that the nature of anti-JE antibodies produced in the primary vaccination is different from that in natural infections, in terms of their avidity to assay antigen. Because ELISA requires repeated washing steps and thus can possibly miss to detect such antibodies with low avidity to the assay antigen, while in the HI or N-test, antigen and antibodies will remain in the same reaction mixtures (although they have been diluted) throughout the assay procedures and thus these test may possibly be able to detect such antibodies with low avidities also. These possible effects by the washing steps on the relative insensitivity of ELISA to detect antibodies against rubella virus were also observed by Minamishima et al. (personal communication) and Miyasawa et al. (1982). These problems should wait for further critical examinations. Because IgM-antibody response observed in the vaccinees was very low, with the maximum titer below 100, the result substantiate the importance or validity to detect IgM antibodies in the serodiagnosis or seroepidemiological studies on JE in order to detect natural infections. On the other hand, some vaccinees showed quite high titered Ig-ELISA antibody levels after the booster, the results of Ig-ELISA in the serodiagnosis or seroepidemiology should be interpreted carefully, especially in the areas where JE vaccination is commonly used, like in Japan. Our data indicate that when a certain test serum showed IgM antibody titer over 1:100 the subject had very probably be infected with the virus, and thus the data is diagnostic.

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日本脳炎ウイルスに対する免疫酵素測定法. VI. ワクチンによる人の抗体反応
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ホルマリン不活化日本脳炎ワクチンを免疫した人における抗体反応を, 免疫酵素測定法 (ELISA) で調べた. イムノグロブリン (Ig-) ELISA 抗体反応はワクチンの初回免疫では比較的低かったが追加免疫後は著明な反応がみられた. 一方, IgM-ELISA の抗体反応はわずかで, 少数の人においてのみ認められその最高値も100以下であった. この結果, JE の血清診断又は血清疫学の研究において, IgM-ELISA を検出した場合にはウイルスの自然感染を意味するといふことが支持された.

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