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A FIELD TRIAL WITH AN IMPROVED JAPANESE ENCEPHALITIS VACCINE IN A NONENDEMIC AREA OF THE DISEASE

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SUMMARY The antibody responses to inactivated Japanese encephalitis (JE) vaccine of junior high school students and aged persons living in an area where JE was not endemic were examined. In all, 10 lots of vaccine prepared by different methods and differing in potency and immunotype were tested. The magnitude of the response to a primary inoculation with Nakayama vaccine was closely correlated with the potency of the vaccine. The response to a single injection with vaccines of the JaGAr immunotype was invariably poor, even when they had as high a potency as that of the Nakayama vaccine. However, two successive injections with JaGAr type vaccine resulted in a greatly enhanced response.

A booster dose of vaccine had a marked effect on the antibody response, irrespective of the previous history of JE vaccination and the immunological status before the booster dose. The increased antibody level remained high enough for 1 or 2 years after vaccination to prevent natural infection with not only homologous but also heterologous JE viruses to the vaccine. The magnitude of antibody response of aged persons was similar to that of high school students. The physico-chemical properties of the antibody produced by the vaccine and the side reactions caused by vaccination were also studied.

INTRODUCTION

Inactivated Japanese encephalitis (JE) vaccine has been greatly improved in recent years. The trichloracetic acid (TCA)-precipitable nitrogen in the mouse brain type vaccine was reduced to less than 0.02 mg/ml, without impairment of the immunizing potency of the vaccine (Takaku et al., 1968a; Nakamura et al., 1969). This vaccine does not cause allergic encephalomyelitis as shown both clinically (Okinaka et al., 1967) and neuropathologically (Egashira et al., 1966; Shiraki, 1966), Currently, an inactivated cell culture vaccine for JE has been developed (Darwish and Hammon, 1966a; Lee et al., 1967; Kaneko and Inoue, 1967; Kaneko, 1968). This vaccine contains virtually no mouse brain substances responsible for allergic side reaction.

The serologic response of man to the improved JE vaccine has also been studied. In Japan, however, most studies have been

performed in Honshu, where cases of JE occur every summer. In this endemic area naturally occurring JE infection affects the serologic response following vaccination, and so complicates interpretation of results. In association with the Committee on Japanese Encephalitis Vaccine (CJEV), organized in 1966 with Dr. Kawakita as chairman, we studied the antibody response to the vaccine of high school students living in the northern part of Hokkaido, the only place in Japan known to be free from JE (Miura and Kitaoka, 1955). Some results have already been reported (Katsurada, 1968a, b). Further results are described in the present paper.

MATERIALS AND METHODS

1. *Subjects*

About 500 junior high school students of 12 and 13 years old, living all their life in the northern part of Hokkaido were chosen as subjects. They had not lived in an endemic area of JE or travelled into the area during the epidemic season of the disease. None of them showed neutralizing antibody to JE virus at a serum dilution 1: 2.5, at the time of vaccination. A group of persons of 60 years old or more, living in an asylum in the middle part of Hokkaido, were also included in the subjects to compare their antibody responses to the vaccine with the responses of the students.

2. *Vaccine*

A total of 10 lots of JE vaccine prepared by different methods and differing in potency and immunotype were tested. Of these, 5 were prepared from mouse brains infected with Nakayama-NIH strain of JE virus, and the other 5 were prepared from viruses of the JaGAr immunotype. Two of the latter were mouse brain type vaccine while the other 3 were the cell culture type. Details of these vaccines are summarized in Table 1.

3. *Administration of vaccine and collection of serum*

One group of subjects was inoculated once subcutaneously with a dose (1 ml) of vaccine, and the other twice with one dose each with an interval of 1 or 4 weeks between injections. One year after the primary vaccination, most groups were given a booster dose which was immunologically homologus or heterologous to the first dose. Two further groups were boosted in the following ways: one

TABLE 1. *Vaccine used*

Vaccine		Starting material	Method of purification	TCA-N[k] (μg/ml)
Nakayama	lot 1[a]	Mouse brain	Alcohol-protamine	8.9
	2[b]	do.	Ultracentrifugation	3.0
	3[c]	do.	do.	7.0
	149[d]	do.	do.	4.0
	164[e]	do.	do.	1.5
JaGAr type				
JaGArOl	lot 2[f]	Mouse brain	Ultracentrifugation	17.0
	4[g]	do.	do.	4.8
Mukai	lot 1[h]	Hamster kidney	Membrane filtration	13.6
JaOH	lot 3[i]	Monkey kidney	do.	8.0
	4[j]	do.	do.	16.0

a; Experimental vaccine produced by Nippon Institute for Biological Science
b, f, g, i, j; Experimental vaccine produced by Research Foundation for Microbial Diseases of Osaka University
c, d, e; Commercial vaccine produced by Research Foundation for Microbial Diseases of Osaka University
h; Experimental vaccine produced by Takeda Chemical Industries, LTD.
k; Trichloracetic acid-precipitable nitrogen

was given a booster dose 2 years after the primary vaccination, and the other was given two booster doses 1 and 3 years respectively after the first vaccination. Serum samples were collected at intervals from the subjects, as described in the text.

4. *Antibody titration*

In principle, antibody was titrated for virus homologous to that of vaccine administered. However, the antibody of subjects inoculated with Mukai vaccine was titrated against JaGArOl virus. The 50% plaque reduction method recommended by the CJEV was employed for titration (Takaku et al., 1968a). In this method, serial 4-fold dilutions of inactivated serum are mixed with an equal volume of solution containing about 200 PFU of virus. After incubation at 37 C for 2 hr, 0.4 ml of the mixture is inoculated into a 70 mm-diameter Petri dish containing a 24 hr-culture of primary chick embryo fibrobalsts (CEF). As controls 10 dishes containing CEF cell monolayers are inoculated with mixtures of virus and phosphate buffered saline (PBS) containing 5% normal calf serum. As positive serum controls dishes are inoculated with mixtures of virus and rabbit immune serum with a known antibody titer against JE virus. After incubation at 37 C for 90 minutes under CO_2, Earle's medium (containing 1% agar, 0.5% lactalbumin hydrolysate and 0.1% yeast extract) is poured into the dishes to form the first overlay. A second overlay is made 2 days later, and plaques are usually counted the following day. Unless stated otherwise, a dilution of serum 1 : 10 was taken as the lowest limit of a positive result. The neutralizing antibody of old people was titrated by the microtitration technique of Sullivan and Rosenbaum (1967) using suspension of PS cells and carboxymethyl cellulose as an overlay (Salim, 1967).

5. *Gel filtration*

A column (1.8 × 80 cm) of Sephadex G-200 (Pharmacia) was prepared by the method of Flodin and Killander (1962) with slight modifications. Two ml of serum sample were applied to the gel, and the column was eluted with PBS, pH 7.3, under hydrostatic pressure at a flow rate of 11 ml/hr. Fractions of 2 ml of effluent were collected. The protein concentration of the fractions was estimated from the absorption at 280 mμ, and their virus-neutralizing activity was measured as described above.

6. *Treatment of serum with 2-mercaptoethanol (2-ME)*

The method of Svehag (1964) was used. Serum samples were mixed with an equal volume of 0.2 M 2-ME. After incubation at 4 C for 24 hr, the mixture was exhaustively dialysed against PBS. As a control the same serum sample was treated in the same way but without 2-ME.

RESULTS

1. *Effects of cell culture passage of JE virus on its ability to react with antibody*

We found that while the antibody responses of high school students to primary injection with Nakayama vaccine was as expected, the response to JaGAr immunotype vaccines was very poor even when vaccines of as high potency as that of Nakayama were injected. Recently, using currently isolated JE virus, Ozaki and Kumagai (1969) found that the neutralizing antibody titer of a given serum was much greater when measured using virus which had been serially propagated in PS cell cultures. This finding is very relevant to our work since the viruses we used for antibody titration had different histories of passage after isolation (Okuno et al., 1968; Takaku et al., 1968b). Accordingly, the possible effect of serial passage of JE virus in cell cultures on its ability to react with antibody was studied. Two viruses, Nakayama-NIH and JaGArOl, which had been maintained by intracerebral passage in mice were serially propagated in CEF cell cultures with transfer at 2 day intervals. Nakayama virus was serially propagated also in primary pig kidney (PK) cell cultures in a similar way. Anti-Nakayama and -JaGArOl hyperimmune rabbit sera and human sera of different origins were titrated for neutralizing antibody against the original viruses and those after serial passage. Antibody titration was performed on cultures of cells of the same kind as those employed for viral passage. Results are shown in Tables 2 and 3. The antibody titers of some sera were similar with the original virus and that after passage in cell cultures,

TABLE 2. *Variation in antibody titers of various sera as measured with Nakayama viruses with different histories of passage in cell cultures*

Serum and history		Exp. 1		Exp. 2		Exp. 3	
		Original[a]	CEF (8)[b]	Original	CEF (10)	Original	PK (14)[c]
Rabbit	Hyperimmune to Nakayama virus	4	16	4	16	4	16
Human 1	Received Nakayama vaccine	160	640	160	640	—	—
2	do.	640	2560	640	640	—	—
6	do.	—	—	—	—	160	640
3	Naturally infected with JE	160	160	40	160	—	—

a Nor passaged in cell culture
b 8th passage in chick embryo cells
c 14th passage in primary pig kidney cells

TABLE 3. *Variation in antibody titers of various sera as measured with JaGAr01 viruses of different passage numbers in CEF cell cultures*

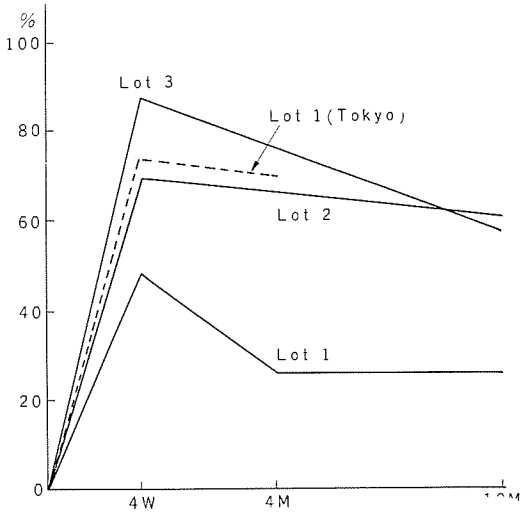
Serum and history		Exp. 1		Exp. 2	
		Original	CEF (13)	Original	CEF (13)
Rabbit	Hyperimmune to JaGAr01 virus	16	64	16	32
Human 4	Received Nakayama vaccine	40	160	80	160
5	do.	—	—	320	640

For explanation see Table 2.

however, the titers of most sera were 2- or 4-fold higher with virus after passage. Accordingly, antibody in sera of subjects with all vaccines of the JaGAr immunotype, except JaOH, was titrated using cell culture virus as antigen while that with the Nakayama vaccine was titrated using virus propagated in mouse brains.

2. *Antibody response to primary inoculation with Nakayama vaccine*

Fig. 1 shows the antibody responses to primary inoculations with 1 dose of different lots of Nakayama vaccine. The responses to lots



▶

FIGURE 1. *Duration of antibody produced by single injection of different lots of Nakayama vaccine.*

2 and 3 were apparently greater than that to lot 1. Seventy per cent of the subjects who received lot 2 and 88% of those receiving lot 3 acquired an antibody titer of 1: 10 or more after 4 weeks. Approximately 60% of the subjects in these groups were still serologically postivie 1 year later. The magnitudes of the responses to the three lots of vaccine were closely correlated with the respective potencies of the lots, as described later.

In parallel with this study, a field trial was made with Nakayama lot 1 on students at a nursing school in Tokyo who had no antibody against JE virus. The antibody responses of the students inoculated with 1 dose of the vaccine are also shown in Fig. 1 by permission of Dr. Ezaki, Director of Toyotama Hospital, who directed the trial. The response was apparently greater than that of the group in Hokkaido to this lot. This indicates that the Tokyo group contained subjects who had previously been exposed to JE virus infection, although they had no detectable antibody against the virus at the time of vaccination. The increased response may be due to immunological memory of the previous infection.

3. *Relationship between the potency of vaccine and the antibody response*

Table 4 shows the antibody responses of groups after single primary inoculation with 1 dose of different vaccines, and after two doses with 4 weeks interval between them. The table also shows the result of potency tests on the vaccines performed by the Section of Potency Testing of CJEV. In the table, ED_{50} indicates the highest dilution of vaccine at which 50% of the immunized mice were protected from an intracerebral challenge with homologous virus (mouse protection test), and $NTED_{50}$ the highest dilution of vaccine at which 50% of the immunized mice produced antibody (antigen extinction limit titer test).

The magnitudes of the responses to single injections with different lots of Nakayama vaccine were closely correlated with the respective potencies of the lots, except in the case of lot 149. A similar correlation was seen after two injections. The responses to single

TABLE 4. *Relationship between potency of JE vaccine and antibody response in man*

	4 wks after 1st vac.		1 wk after 2nd vac.		ED_{50} (4^n)	$NTED_{50}$ (4^n)	Infectivity before inactivation (10^n)
	No.	Mean titer	No.	Mean titer			
Nakayama							
lot 1	87	5.8	62	45	0.92(100)[a]	1.70	8.2 $TCID_{50}$
149	39	7.9	—	—	1.50(63)	4.50	8.6 PFU
2	71	11	61	67	1.51(100)	2.75	8.3 PFU
3	52	27	—	—	2.30(63)	4.20	8.6 PFU
164	—	—	—	—	2.85(37)	4.60	8.4 PFU
JaGAr type[b]							
Mukai lot 1	54	1.5	53	8.7	—	1.04	8.2 PFU
JaGArOl lot 2	72	2.0	70	88	3.00(13)	2.16	7.2 PFU
4	34	2.8	—	—	2.45(44)	4.50	8.4 PFU
JaOH lot 3	62	3.3	49	27	1.79(52)	3.20	8.7 PFU
4B	—	—	—	—	1.30(39)	>4.50	8.6 PFU

a Values in parenthesis indicate the LD_{50} of the virus used for intracerebral challenge.
b Antibodies of groups with Mukai and JaGArOl vaccines were titrated using CEF-adapted JaGArOl virus, as antigen, and that with JaOH was titrated using the mouse brain-adapted JaOH virus.

injections with vaccines of the JaGAr immunotype were invariably poor, even though some lots had as high potency as that of Nakayama vaccine. Two injections with all vaccines of the JaGAr immunotype, except Mukai, greatly increased the antibody, but the magnitude of the response was not correlated with the potency of the vaccines. The difference in the responses to Nakayama and JaGAr type vaccines is clear in the table. The mean antibody titer after a single injection of Nakayama lot 3 was about 10 times more than that after an injection of JaGArOl lot 4, although the two vaccines had a similar potency. The difference remained even after two injections of the same vaccines.

4. *Duration of antibody after primary vaccinations*

The above results show that the antibody response is enhanced by two primary injections of the same vaccine. Next, the duration of antibody after the primary vaccinations and the effect of the time interval between the first and second vaccinations on the response were studied. Table 5 shows the results. More than 90% of the subjects injected twice with Nakayama lots 1 and 2 acquired antibody titer of 1: 10 or more. Sixty per cent of the subjects with lot 1 and 88% of those with lot 2 still had high antibody levels 1 year later. Results with JaGAr immunotype vaccines were similar to those with Nakayama vaccines. More than 95% of those receiving two injections became seropositive. Antibody was detectable in 88%(54/64) of subjects vaccinated 1 year previously with JaGArOl, and in 79% (39/49) of those vaccinated 3 months previously with JaOH. The maximum percentage of seropositive persons in the group vaccinated with Mukai was 68%(51/75), and decreased to 29%(9/31) one year later. The time interval between the first and second vaccinations had little effect on the response.

5. *Antibody response to booster vaccination*

Groups which have received a primary vaccination 1 year previously were boosted with a further dose of vaccine homologous to that used for the primary vaccination. In addition, one group received a booster injection of Nakayama vaccine 2 years after the primary injection of the same vaccine, and the another group received two booster injections 1 and 3 years after the primary vaccination. Results are shown in Table 6 and Fig. 2. A booster vaccination had a marked effect on the antibody response. Thus, irrespective of the previous history of JE vaccination or the potency and immunotype of the vaccine used as booster, the antibody titer of most groups rose on an average to a height of 1: 1,000 or more after 4 weeks. The effect of the booster vaccination was more striking when examined on individuals. Many subjects developed an antibody titer of 1: 40,000 or more. In groups

TABLE 5. *Antibody response to primary vaccination in two doses*

Vaccine		Time interval	No.	Mean titer after 2nd vaccination					
				1 W.	4 W.	3 M.	4 M.	11 M.	12 M.
Nakayama	Lot 1	1 M.	62	45	—	21	—	10	—
	Lot 2	1 M.	61	67	—	34	—	20	—
Mukai	Lot 1	1 W.	75	—	14	—	7.5	—	3.5
		1 M.	53	8.7	—	4.3	—	—	—
JaGArOl	Lot 2	1 W.	43	—	60	—	25	—	23
		1 M.	70	88	—	31	—	13	—
JaOH	Lot 3	1 M.	49	27	—	11	—	—	—

TABLE 6. *Comparisons of booster antibody responses in groups with different histories of JE vaccination*

Primary vaccination	No.	Time interval	Booster	Mean antibody titer				
				0[a]	1 W.	4 W.	1 Y.	2 Y.
Nakayama								
Lot 1, once	21	1 Y.	Lot 3 Nakamaya	4.1	—	2060	166 (53)[b]	27 (18)
twice	56			10	—	1360		
Lot 2, once	22			9.6	—	3900	441 (51)	57 (23)
twice	53			20	—	2790		
Nakayama								
Lot 1 or Lot 2 followed by Lot 3, as booster	18	2 Y.	Lot 149 Nakayama	34	292	1079	—	—
Lot 3, once	13			3.5	460	867	—	—
JaGArOI								
Lot 2, twice	31	1 Y.	Lot 4 JaGArOI	17	2300	2190	—	—
Mukai								
Lot 1, twice	31			3.5	—	528	—	—

a Immediately before booster vaccination

b Values in parenthesis indicate the number of subjects tested.

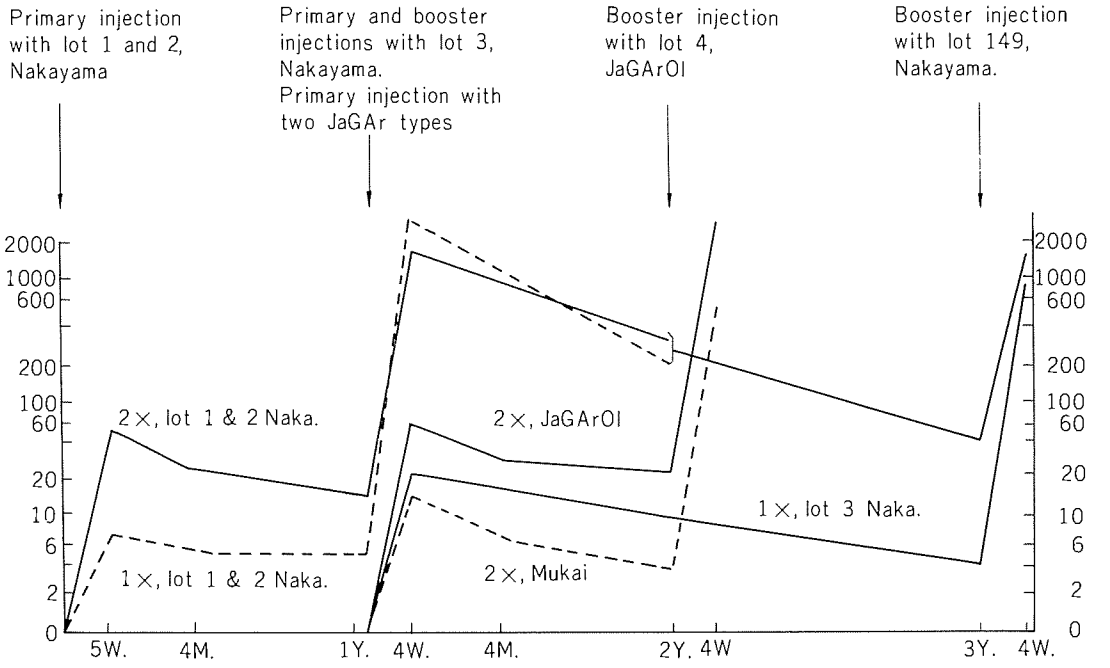


FIGURE 2. *Antibody responses to booster vaccination of groups with varying histories of JE vaccination.*

boosted with Nakayama vaccine, the antibody level before the booster injection did not greatly influence the response. The increased antibody slowly decreased, but all 104 subjects receiving booster injections maintained a detectable antibody titer for 1 year, and 38 of 41(92%) were still seropositive after 2 years. In the group boosted with the JaGAr immunotype vaccine, the antibody level before the booster injection affected the response. The low response of the group with Mukai vaccine was probably due to the low potency of this vaccine.

6. *Antibody response to heterologous viruses to the vaccine*

It is known that almost all of the recently isolated JE viruses are of the JaGAr immunotype (Okuno et al., 1968). The viruses are immunologically distinct from Nakayama-NIH virus, which has usually been used to prepare JE vaccine for human use. Although the two immunotypes share common antigens, the efficacy of Nakayama vaccine in preventing infection of the prevalent immunotype virus required investigation. Sera collected within 4 weeks after a primary or booster vaccination were simultaneously titrated for antibodies against homologous and heterologous viruses to that of the vaccine. A titer of 1:10 was taken as the lowest antibody level effective in preventing JE virus infection by a mosquito bite (Oya, 1967). Results are shown in Table 7. The antibody titer against Nakayama virus increased to the required level after two primary injections of the homologus Nakayama

TABLE 7. *Comparison of antibody responses to booster doses of homologous and heterologous vaccine*

Primary	Nakayama lot 2[a]				JaGArOl lot 2[b]			
Booster	—		Nakayama lot 3		—		JaGArOl lot 4	
Time after last vac.	1 wk		4 wks		4 wks		4 wks	
Antibody	Homo	Hetero	Homo	Hetero	Homo	Hetero	Homo	Hetero
<10	0	14	0	0	2	N.D.	0	0
10	0	20	0	2	9		0	0
40	16	3	0	11	16		0	6
160+	21	0	54	41	16		30	24
Total	37	37	54	54	43		30	30

a Twice 4 weeks apart
b Twice 1 week apart

TABLE 8. *Effect of previous JE vaccination on booster antibody response*

Vaccine		No.	Mean antibody titer			
			Nakayama		JaGArOl	
Primary	Booster		Before	After[a]	Before	After
Nakayama	Nakayama	31	13	984	3.6	173
Nakayama	JaGArOl	32	24	2406	4.6	317
JaGArOl	JaGArOl	31	2.8	191	16	2190
JaGArOl	Nakayama	31	2.6	207	18	2403

a 4 weeks after booster vaccination

vaccine. However, in most subjects a booster injection 1 year later with the same vaccine was needed to increase the antibody to heterologous JaGArOl virus to the level. Similarly, a booster injection of JaGArOl vaccine after a primary vaccination was necessary to increase the antibody against Nakayama virus to the required level.

Next, the anamnestic antibody response induced by booster vaccination was studied. Sixty-three subjects who had received a primary injection with Nakayama vaccine 2 years previously were divided into 2 groups. One group received a booster injection of the homologous Nakayama vaccine, and the other group received the heterologous JaGArOl vaccine. Two other groups of 31 subjects each who had received a primary injection of JaGArOl vaccine 1 year previously received a booster dose of homologous and heterologous vaccines, respectively. Results are shown in Table 8. Groups receiving the same immunotype vaccine for both the primary and booster injections showed greater antibody response to the vaccine after the booster dose, as expected. However, after booster injection with different immunotype vaccines from that of primary injection, the antibody response to the type used in the primary vaccination was always greater than that to the booster type. In other words, the magnitude of the response to the booster vaccination was always greater against the JE virus to which the subjects had first been exposed. This indicates that the doctrine of original antigenic sin known for influenza is also true for JE.

7. *Effects of age on the antibody response*

Forty-six persons of 60 years old or more, living in an asylum were injected with 1 dose of JaOH vaccine lot 4. Paired sera were collected before and 4 weeks after vaccination and their antibody against JaOH virus was titrated by the microtitration technique. Paired sera from high school students who had received a primary injection with 1 dose of JaOH vaccine lot 3 were subjected also to antibody titration in a similar way to compare results from the two age groups. Table 9 shows the results.

Old people are divided into 3 groups based on their histories of living in an endemic area of JE and the immunological status at the time of vaccination. A 2-fold or greater increase in antibody titer was observed in 20% of the high school students, and a 4-fold or greater increase in 4%. Eleven of the 46 older subjects were similar to the students in respect to both the life history and immunological status. Four of the 11(36.3%) responded to the vaccine. The magnitude of antibody response was more marked in subjects with the history of living in an endemic are of JE. Therefore, the capacity of old people to respond to JE vaccine is not necessarily inferior to that of the high school students.

8. *Physicochemical properties of the antibody produced by the vaccine*

Serum samples were collected at intervals from subjects after a primary dose of Nakayama

TABLE 9. *Antibody response of aged persons to primary vaccination with 1 dose of JaOH*

Age	Place of previous residence	Prevaccination antibody	No.	Increase			
				≥×2		≥×4	
60 or older	Hokkaido only	Negative	11	4	36.3%	0	0%
	Hokkaido and Endemic area	Negative	26	9	34.6	1	3.8
		Positive	9	6	66.7	2	22.2
12 and 13	Hokkaido only	Negative	49	10	20.4	2	4.0

vaccine and after a booster dose of the same vaccine. After the primary vaccination neutralizing antibody began to appear within 6 to 12 days, reaching a maximum within 1 month, and then gradually decreasing. The sensitivity of the antibody to 2-ME was tested and the results are shown in Fig. 3. The antibody titer of sera collected in the initial 34 days after vaccination decreased to a quarter or less of the original titer on treatment with 2-ME, however, 3 of the 4 sera collected on or after the 41st day were unaffected by this treatment. Next, three serum samples collected at intervals from the same person were subjected to gel filtration to determine the immunoglobulin class containing the antibody. Results are shown in Fig. 4. The antibody titer of serum on the 14th day was 1:40, and the antibody activity

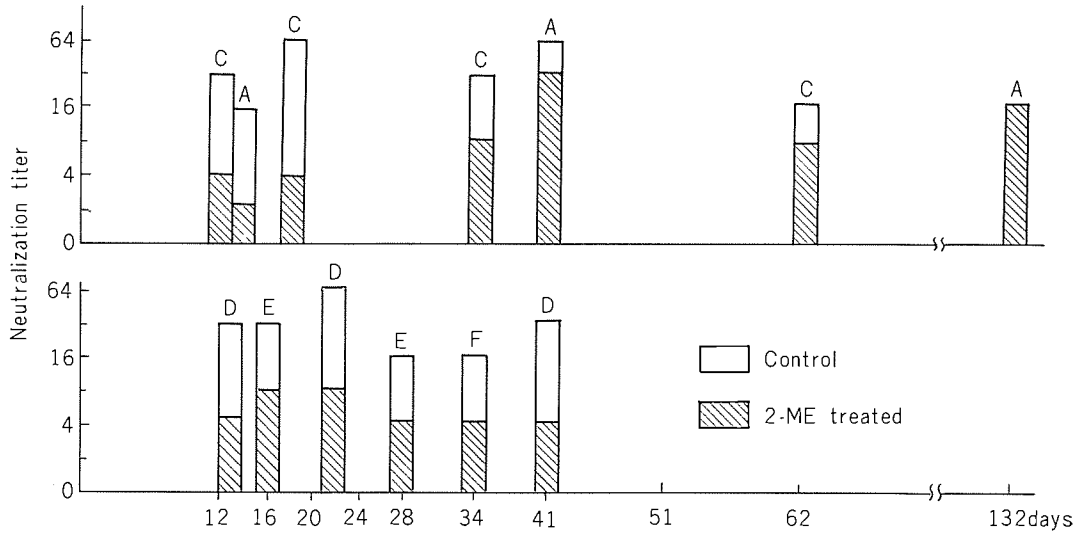


FIGURE 3. *Susceptibility to 2-ME of antibody produced by a single injection of Nakayama vaccine.*

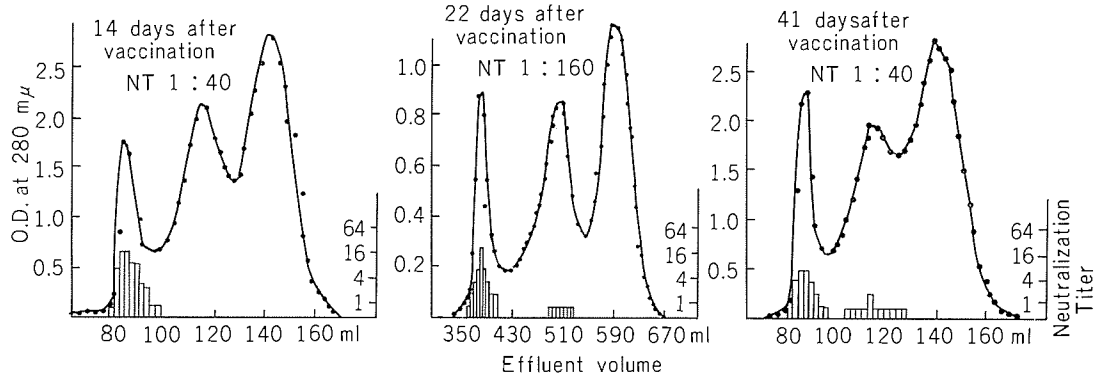


FIGURE 4. *Gel filtration of serum antibody produced by primary and booster injections of Nakayama vaccine.*

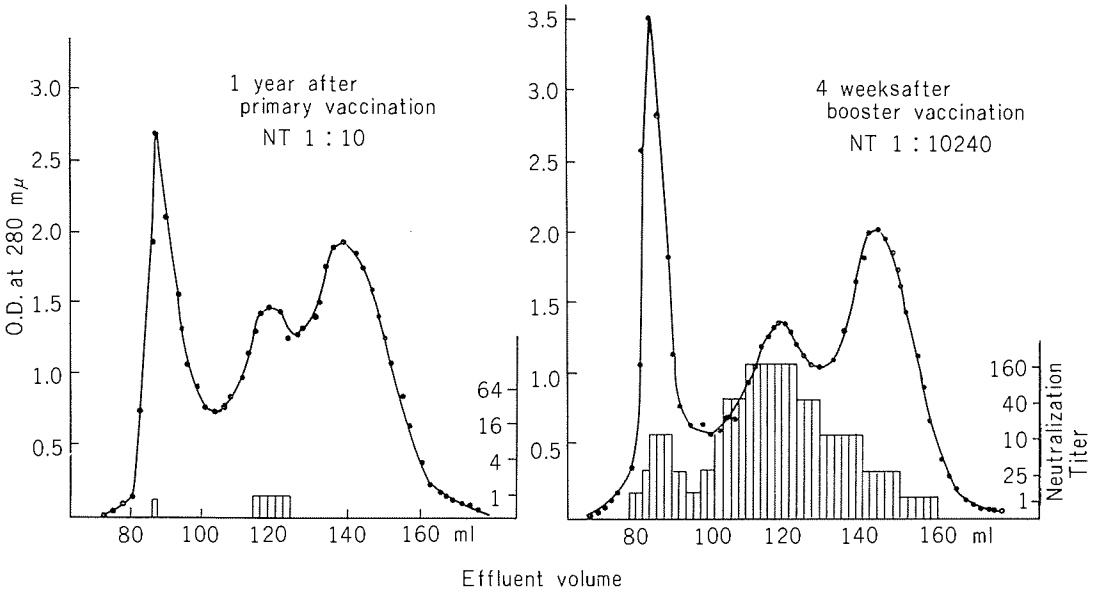


FIGURE 4. Continued

of the serum was located entirely in the 19S macroglobulin fractions. On the 22nd day the activity was still predominantly in the 19S fractions, but there was some activity in the 7S also. The activity in the 7S fractions subsequently increased and on the 41st day fair activity was found in both 19S and 7S. Paired sera collected from another subject before and 4 weeks after a booster vaccination were also examined. Before the booster dose the antibody titer was 1 : 10, and the activity was almost exclusively in the 7S fractions. The booster

TABLE 10. *Side reactions 24 and 48 hours after the primary injection with 1 dose of vaccines*

Vaccine	Nakayama lot 149[a]		JaGArOl lot 2[a]		Mukai[b]	
Number of subject	52		118		130	
Time after vaccination	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
Redness						
0	87%	91%	28%	66%	81%	86%
<50 mm	7	7	24	18	17	13
50+	6	2	48	16	2	1
Spontaneous pain	7	0	13	3	1	1
Tenderness	44	23	100	77	50	13
Itching	6	0	2	9	8	4
Headache	4	2	21	4	10	4
Lassitude	23	6	30	8	16	8
Fever	0	0	7	0	6	2

a Mouse brain type *b* Tissue culture type

vaccination increased the titer to 1: 10,240 after 4 weeks, and this increase was largely due to increase in the activity in the 7S fractions.

9. *Side reactions following vaccination*

High school students who had received a primary injection with a single dose of vaccines differing in the immunotype and the method of preparation were examined for side reactions occurring 24 and 48 hr after vaccination. Results are shown in Table 10. Among the three vaccines tested, JaGArOl vaccine was the greatest in the frequencies of occurrence in both local and systemic side reactions. The frequencies were approximately the same between the groups injected with Nakayama and Mukai vaccines, and the reactions were greatly reduced 48 hr later.

Side reactions were examined more carefully in 14 medical students injected primarily with a single dose of JaGArOl lot 4 vaccine of the mouse brain type. Redness of 50 mm diameter or more developed at the site of injection in 9 students (64.2%). It developed within 24 hr after vaccination and lasted for 1 or 2 days. The maximum size of the redness was 140×140 mm. Three of the 14 students felt lassitude or feverish on the day of vaccination. The maximum body temperture of the subjects with fever was 37.6 C. These symptoms, however, disappeared within 2 days after vaccination. None of the high school or medical students developed longlasting sequelae.

DISCUSSION

JE has been studied intensively for years, but its ecology is still largely unknown. Therefore, the only possible way of preventing the virus infection is immunization. Recently, large-scale production of highly purified potent JE vaccine has been achieved, so this was possible. The effect of JE vaccine has been evaluated by investigating the morbidity from the disease (Tigertt et al., 1956). Recently, a similar but more extensive investigation was made in Formosa (Wei et al., 1966). The vaccine employed was found to be 81% effective.

The antibody response to JE vaccine is also an useful measure of the efficacy of the vaccine. This method is easier than a survey of morbidity, but when it is employed in an area where JE is endemic, it is difficult to evaluate results because natural JE virus infection may modify the response (Matsumoto et al., 1956; Ezaki et al., 1966). In this respect, the present study has been conducted under ideal conditions.

There are various methods available for testing the potency of JE vaccine (Darwish and Hammon, 1966b; Oya, 1966; Kurata et al., 1964; Lee et al., 1967). However, the potency is useful only when it was correlated with the ability of vaccine to produce antibody in man. This was found true with Nakayama vaccine, but not always true with JaGAr immunotype vaccines. Two primary injections with the latter vaccines greatly enhanced the antibody response, however, the magnitude of the response was not always correlated with the potency of the vaccines. There is evidence that the immunological characters of JE virus are altered by serial passage in animals or cell cultures (Watanabe et al., 1954; Fujie et al., 1962; Okuno et al., 1965). Ozaki and Kumagai (1969) suggested that the alteration might be due to a change in the avidity of JE virus for antibody. Recently, a certain JE virus of the JaGAr immunotype was found to differ from Nakayama-NIH virus both in the physicochemical properties and the lipid metabolism of the infected mouse brain (Nishimura et al., 1968). These factors are possibly involved in causing such a peculiar relationship between the potency and the ability to produce antibody in man of JaGAr immunotype vaccines.

Mukai vaccine is a cell culture vaccine which was first tested in man in Japan. Thus, it was unexpected that the response to this vaccine should be poor. This vaccine lot showed an unsual increase in pH value on treatment with sodium bisulfate to neutralize residual formaldehyde (personal communications). Over-neutralization may have reduced its potency.

The antibody response to JaOH vaccine, another one of cell culture vaccines, was as expected. It was prepared by propagating the monkey kidney (MK) cell-adapted JaOH virus in MK cell cultures. Unfortunately, antibody response to this vaccine was measured on CEF cell cultures in the present study. A greater response could probably have been obtained if the antibody titration was made on MK cell cultures. At any rate, the results indicate that large-scale production of a potent JaGAr immunotype vaccine completely free of mouse brain substances is possible.

A booster injection of JE vaccine is known to have a considerable effect (Warren et al., 1948; Kitaoka, 1965; Darwish et al., 1967). This was confirmed in our study. A booster injection had a marked effect, irrespective of the previous history of JE vaccination, the antibody level before the booster injection and the immunotype of vaccine injected. The response was marked even after a primary injection of vaccine with low potency such as Mukai. A booster vaccination was also needed to increase the antibody to the level required to prevent natural infection of heterologous JE virus.

There are many reports that previous exposure to or previous vaccination against group B arboviruses other than JE enhanced the antibody response to JE vaccine injected later, and vice versa (Hammon et al., 1956; Imam and Hammon, 1957; Darwish et al., 1967). Nakayama and JaGArOl viruses are immunologically distinct from each other, but the difference is much less than that between JE and other group B arboviruses. The anamnestic responses induced by booster injections with JE vaccines homologous and heterologous to that injected primarily, however, clearly distinguished between viruses of the two immunotypes.

The capacity of older people to respond serologically to JE vaccine is controversial (Ishii, 1967; Kunita et al., 1968; Otsuka et al., 1970). This problem is of particular importance since both the morbidity and mortality from JE increase with age in man (Miura, 1967). We did not test very many old people, but the percentage of those showing 2-fold or more increase in antibody titer was rather higher in the aged than in the high school students. The potency of the vaccine lot which was given to the aged was slightly lower than that to the students, as seen in Table 4; nevertheless, the antibody response of the aged was greater than that of the students.

Bellanti et al(1965) reported that guinea pig responded serologically to experimental JE virus infection in a similar manner to that on immunization of animals with nonreplicating antigens; that is, the antibody began to appear initially in the 19S macroglobulin fractions followed by appearance in the 7S globulin fractions as early as 14 days after infection. Results on patients with JE were different in that the 19S antibody persisted for 30 days or more after the onset of the disease (Ogata et al., 1967; Ishii et al., 1968). Our results on JE vaccination agree with those on patients.

Side reactions following injection of the improved JE vaccine have been investigated extensively (Okinaka et al., 1967). Most persons subjected to the investigation were, however, those living all their life in an endemic area of JE. The previous exposure to the virus or previous JE vaccination may affect the occurrence or severity of the side reactions. We investigated the reactions on junior high school and medical students without the history of living in the endemic area and also without the history of the vaccination. Among the three vaccines tested, both local and systemic side reactions occurred with the highest frequency and severity in the group receiving JaGArOl vaccine. Mukai and Nakayama vaccines were the same to JaGArOl vaccine in respect to the immunotype and the method of preparation, respectively. Side reactions in the groups with the two vaccines were, however, very mild. The high frequency of the reactions following injection with JaGArOl vaccine may not be due to impurities in the vaccine since the amount of TCA-precipitable nitrogen

did not differ greatly from those in the two other vaccines. There were considerable differences in the potencies between the three vaccines tested, but it is still unknown whether this is the only factor responsible for the side reactions.

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