

IgG-ELISA Antibody Titers against Japanese Encephalitis (JE) and Dengue Virus Type 1 among Healthy People in JE-endemic Areas in Japan and Thailand

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Abstract: Antibody titers against Japanese encephalitis (JE) and dengue virus type 1 (D1) were measured by enzyme-linked immunosorbent assay (ELISA) among healthy people in JE-endemic areas in Japan (Kumamoto Prefecture) and Thailand (Chiang Mai Province). The IgG-ELISA titers against D1 in Kumamoto distributed in lower range as compared with those against JE, although some people over 50 years of age possessed relatively high IgG-ELISA titers against D1 antigen. On the other hand, adult people in Chiang Mai possessed similar titers against these two antigens as measured by IgG-ELISA.

Key words: ELISA, Japanese encephalitis, Dengue, Healthy people, Thailand, Japan

INTRODUCTION

Japanese encephalitis (JE) is endemic not only in Japan but also in other parts of Asia including Thailand in the Southeast Asia (Miles, 1960) causing many deaths and disabled people, while dengue hemorrhagic fever (DHF) has been the most important viral disease in Southeast Asia resulting in a large numbers of hospitalizations with occasional shock and deaths (Halstead, 1966; 1980; World Health Organization, 1966). Both JE and DHF are caused by mosquito-borne arboviruses belonging to flavivirus genus of family *Togaviridae*. Serodiagnosis and seroepidemiological survey on the diseases have been carried out in most case by the hemagglutination-inhibition (Clarke and Casals, 1958). Recent advance in serology introduced the principle of ELISA (Engvall and Perlman, 1971) also to the field of diagnostic and epidemiological virology as a new technology possessing several advantages (Voller et al., 1976; Sever and Madden, 1977). In this Department, we have been trying to apply the ELISA to measure antibody titers

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against JE in humans (Igarashi et al., 1981; Bundo et al., 1981; 1982b; 1983a; Morita et al., 1982), and animals (Bundo et al., 1982a; 1983b). Recently, the method was extended also to dengue viruses using the specimens obtained from Thailand (Bundo and Igarashi, 1983; Fujita et al., 1983). One of the results in these studies was that both primary dengue and encephalitis cases in Thailand as well as JE case in Japan showed more specific reactions against dengue or JE antigen, respectively, while secondary DHF cases showed high titers both by dengue and JE antigens in the IgG-ELISA. Because dengue virus infection has never been reported in Japan after the World War II, while both JE and dengue viruses have been endemic in Chiang Mai, Thailand (Yamada et al., 1971; Grossman et al., 1973; 1974; Igarashi et al., 1983a; b), it appeared interesting to see whether we can find out any difference between the antibody titers against JE and dengue antigens in JE-epidemic areas in Japan and Thailand as measured by the ELISA.

MATERIALS AND METHODS

Serum specimens: Specimens from 517 individuals in various age groups in Kumamoto Prefecture (JE-endemic area in Japan) were previously described (Bundo et al., 1982b). Sera from 50 healthy adult blood donors were collected during "Virological and epidemiological studies on encephalitis in Chiang Mai Area, Thailand" in July and August, 1982 (Igarashi et al., 1983a). The sera were diluted 1:100 or 1:1000 in PBS-Tween (phosphate buffered saline, pH 7.2, containing 0.05 % Tween 20 and 0.01 % NaN_3).

ELISA: Indirect micro method of Voller et al. (1976) was modified as described (Igarashi et al., 1981; Bundo et al., 1982a). Antigen of JE virus was formalin-inactivated and purified JE vaccine concentrate (Takaku et al., 1968) kindly supplied by the Research Foundation for Microbial Diseases of Osaka University, and was diluted 1:80 in coating buffer before distributing to microplates (100 μl /well). Antigen of D1 virus was prepared from infected culture fluid of *Aedes albopictus*, clone C6/36, cells (Igarashi, 1978), by polyethylene glycol precipitation and ultracentrifugation (Bundo and Igarashi, 1983), and was diluted 1:1600 before coating. Peroxidase-conjugated anti-human IgG (heavy and light chains) goat IgG was obtained from Cappel Laboratories, Pa, USA, and was diluted 1:1000. Titers of test specimens were calculated by a computer system (Morita et al., 1982) comparing the color reaction by each test specimen with those by serial dilution of a standard positive serum with known endpoint titer.

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

RESULTS

IgG-ELISA titers in Kumamoto: Tables 1 and 2 show the number of people in various age groups according to their IgG-ELISA titers against JE and D1 antigens, respectively. The data were summarized into Fig. 1 to compare the geometrical mean titers (GMT) and antibody positive rate (titer ≥ 400) in each age group as measured by JE and D1 antigens. Significant difference was observed between the GMTs measured by these 2 antigens among people of 5 to 49 years old. Also antibody positive rate by JE antigen was significantly higher than that by D1 antigen in groups over 5 years old. Both GMT

Table 1. Age distribution of IgG-ELISA titers against D1 antigen among healthy people in Kumamoto Prefecture, Japan

Age group	IgG-ELISA titer						Total	% positives	GMT	Average with SD (in log)
	0 { 99	100 { 199	200 { 399	400 { 799	800 { 1599	1600 {				
0-4	32	18	8	4			62	6.45	98.4	1.99 \pm 0.35
5-9	13	11	6				30	0	100.2	2.00 \pm 0.29
10-14	8	7	3				18	0	100.9	2.00 \pm 0.31
15-19	22	11	8		1		42	2.38	93.0	1.97 \pm 0.32
20-29	25	10	2	1			38	2.63	73.2	1.86 \pm 0.27
30-39	43	20	7	2			72	2.78	81.4	1.91 \pm 0.28
40-49	50	23	9	2			84	2.38	82.8	1.91 \pm 0.29
50-59	34	32	8	1	1	2	78	5.13	106.3	2.03 \pm 0.38
60-	35	25	18	6	5	2	91	14.29	139.4	2.14 \pm 0.45
Total	262	157	69	16	7	4	515	5.24		

Table 2. Age distribution of IgG-ELISA titers against JE antigen among healthy people in Kumamoto Prefecture, Japan

Age group	IgG-ELISA titer									Total	% positives	GMT
	0 { 99	100 { 199	200 { 399	400 { 799	800 { 1599	1600 { 3199	3200 { 6399	6400 { 12799	12800 {			
0-4	36	17	1	2		2	2	2		62	12.9	129
5-9				6	5	12	3	3	1	30	100	1943
10-14			1	5	6	6				18	94.4	1109
15-19		1	1	7	15	9	8	1		42	95.2	1450
20-29	1	3	5	7	12	5	6			39	76.9	876
30-39	9	4	11	22	18	3	5			72	66.6	521
40-49	6	3	12	24	24	5	6	3	1	84	75.0	738
50-59	6	5	12	23	16	8	6	2		78	70.5	664
60-	7	3	8	17	19	14	19	5		92	80.4	1098
Total	65	36	51	113	115	64	55	16	2	517	70.5	

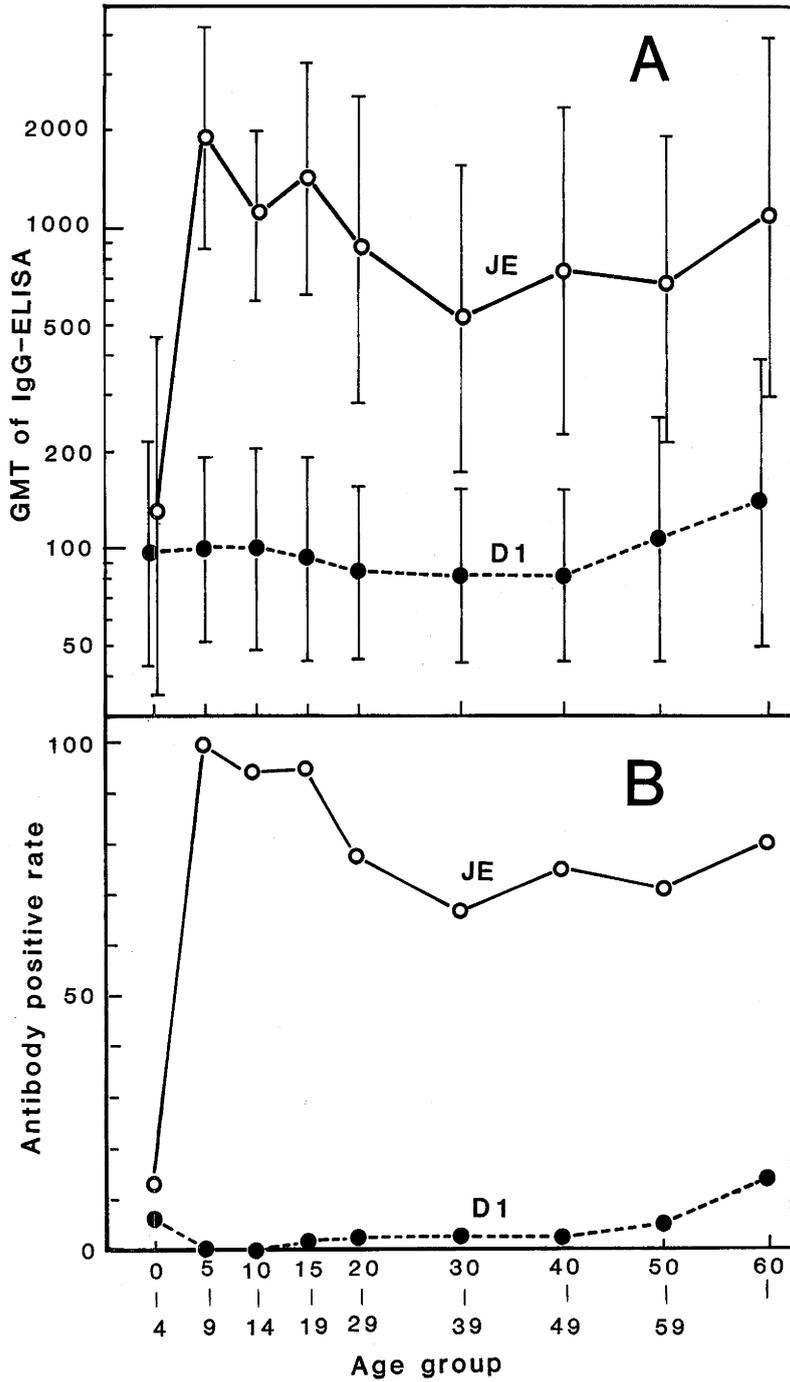


Fig. 1. Age distribution of the GMT of IgG-ELISA (A), and antibody positive rate (B), among healthy people in Kumamoto Prefecture, Japan, as measured by JE (○—○) and D1 (●.....●) antigens. Vertical bars in (A) represent standard deviations.

and antibody positive rate tended to increase slightly in groups over 50 year old. Individuals were divided into groups according to their titers by JE and D1 antigens as shown in Table 3 in order to show the correlation between these 2 titers. Majority of the people show higher titers against JE than D1 antigen, although several specimens possessed

Table 3. Correlation between the IgG-ELISA titer against JE and that against D1 antigen in healthy people in Kumamoto, Japan

IgG-ELISA titer against D1 antigen	IgG-ELISA titer against JE antigen									
	0	100	200	400	800	1600	3200	6400	12800	25600
	99	199	399	799	1599	3199	6399	12799	25599	
0-99	41	19	34	74	65	24	7			
100-199	17	12	13	30	36	27	20	2		
200-399	4	4	3	6	8	8	23	11	1	1
400-799	2	1	1	1	5	1	4	1		
800-1599	1			1	2	2	1			
1600-				1		1		2		

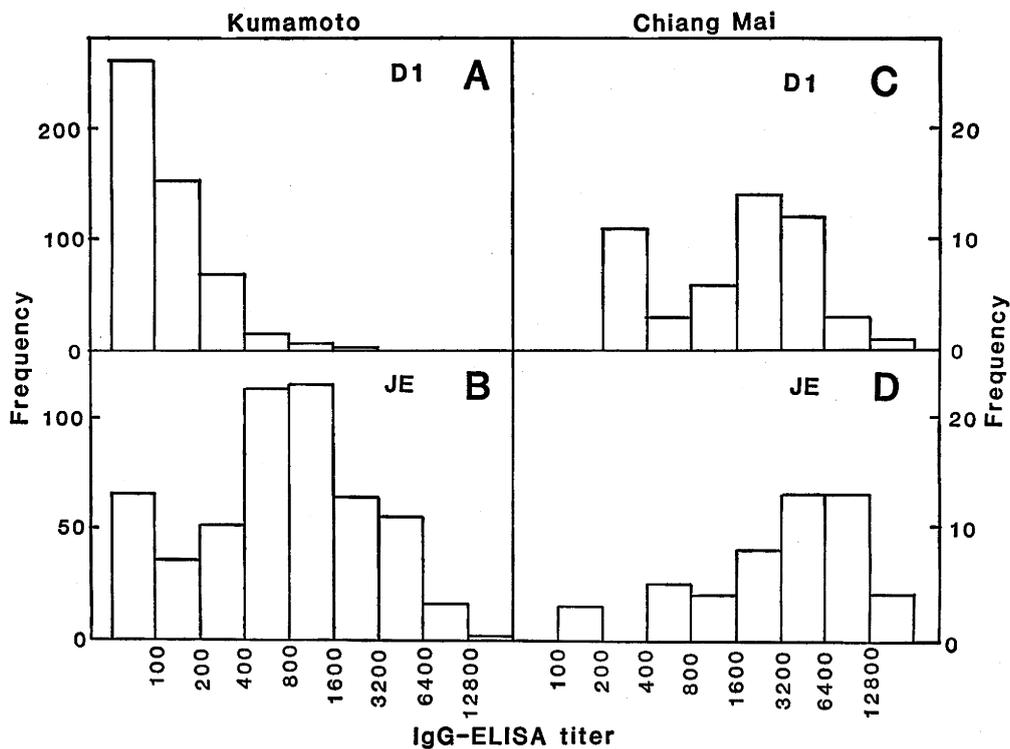


Fig. 2. Distribution of IgG-ELISA titers among healthy people in Kumamoto Prefecture, Japan (A, B), and in Chiang Mai, Thailand (C, D), as measured by D1 (A, C), and JE (B, D) antigens.

DISCUSSION

The data show that healthy adults in Kumamoto possess more or less specific IgG-ELISA antibodies against JE compared with D1 antigen. The tendency that some old people possessed relatively high IgG-ELISA titers against D1 antigen might reflect their past exposure to dengue viruses because dengue epidemics were recorded in Japan during World War II, almost 40 years ago (Taniguchi, 1943; Kimura and Hotta, 1943). This possibility should be verified by the neutralization test. On the other hand, people in Chiang Mai appear to have been exposed both to JE and dengue viruses, as shown by field studies (Fukunaga et al., 1983 a; b). It may be interesting to see the IgG-ELISA titers in people who have been exposed only to dengue viruses, preferably only to 1 type of the virus, in order to see whether their antibody titers are more or less specific to that type of dengue antigen.

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日本とタイ国の日本脳炎流行地における健康住民の日本脳炎ウイルスとデングウイルス1型に対する IgG-ELISA 抗体価

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日本脳炎流行地である日本の熊本県とタイ国のチェンマイにおける健康住民の日本脳炎ウイルス (JE) とデングウイルス1型 (D1) に対する抗体価を免疫酵素測定法 (ELISA) により測定した。熊本の検体の D1 に対する IgG-ELISA 抗体価は JE に対する価に比べて低値側に分布し、わずかに50才以上の検体が D1 に対し高い値を示したにすぎなかった。一方チェンマイの成人は JE と D1 両抗原に対しほぼ同程度の IgG-ELISA 抗体価を保有していた。

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