Antigenicities and Immunogenicities of Mouse Brain-grown and Formalin-inactivated Dengue Viruses[1)]

Chisato MORI[2)], Keiko BUNDO (MORITA)[3)], Sachiko MATSUO, Akira IGARASHI
Department of Virology, Insitute of Tropical Medicine, Nagasaki Univeksity

Mitsuo TAKAGI, Iwao YOSHIDA, Konosuke FUKAI
Kanonji Institute, Research Foundation for Microbial Diseases of Osaka University

Yoshinobu OKUNO
Department of Preventive Medicine, Research Institute for Microbial Diseases, Osaka University

Abstract: Prototype strains of dengue viruses, type 1, 2, 3 and 4, were grown in brains of suckling mice (SMB) and 10% homogenates were prepared. After low-speed centrifugation, protamine sulfate treatment and formalin inactivation, virions were concentrated and partially purified by activated charcoal treatment and ultracentrifugation, and was inoculated to weaning mice to test their immunogenicities. Antibody titers in the immune sera were measured by indirect ELISA, hemagglutination-inhibition (HI) as well as neutralization (N) tests. Dose-response relationship was observed between the amount of immunogen inoculated and the IgG-ELISA titer of immunized mice. Correlation coefficients (r-value) between the HI and the IgG-ELISA titers of the immune sera were 0.89-0.94 for the homologous type of immunogen and assay anitigen combination, and less r-values (0.76-0.88) were obtained for the heterologous combinations. Serological reactions gave the highest titer for the homologous type of antigen-immunogen combination, however, significant cross-reactions were observed for the heterologous types of dengue antigens especially by the ELISA or the HI test and IgM-ELISA did not give higher type-specificity than the IgG-ELISA. The IgG-ELISA and HI antibody production indicated that the immunogenicity was highest for type 3 (D3) followed by type 1 (D1), and then

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2) On leave of absence from Kanonji Institute, Research Foundation for Microbial Diseases of Osaka University.

3) Present address: Department of Physiology and Biochemistry, University of Medicine and Dentistry of New Jersey, Rutgers Medical School, Piscataway, New Jersey 08854, USA

type 4 (D4), and least for type 2 (D2) antigens. This tendency was also observed when mice were given trivalent, or tetravalent types of combined dengue immunogens and immune sera were measured by the IgG-ELISA. Omission of D2 from the immunogen produced antisera with significantly reduced anti-D2 IgG-ELISA titer, while immunization with D1+D2 or D2+D3 produced antisera with sufficient cross-reactions for all types of dengue antigens in the IgG-ELISA. Thses results indicated the importance to include D2 immunogen in order to produce broadly reactive IgG-ELISA antibodies. On the other hand, results by the HI test on mouse sera obtained with multivalent immunogen showed that ommission of D1 from immunogen resulted in significant reduction in the anti-D1 HI antibody titers, while ommission of D2 or D4 from the immunogen did not affect the resulting anti-D2 or anti-D4 HI antibody titers.

Key werds: ELISA, Dengue viruses, Antigenicities, Immunogenicities

INTRODUCTION

Infections by 4 serotypes of dengue viruses have been prevalent in the Southeast Asia and were considered as a viral disease of the highest medical importance because of large numbers of patients and occurrence of severe dengue hemorrhagic fever (DHF) among affected children (World Health Organization, 1966; Halstead, 1966, 1980). Serodiagnosis on DHF has routinely been performed by the HI test (Clarke & Casals, 1958), however, the result is hard to tell the infecting serotypes of dengue viruses because of high degrees of cross-reactivity. Since ELISA was introduced as a new serological test with various advantages (Engvall & Perlman, 1971), the test has been applied to serodiagnosis on various infectious diseases including viral infections (Voller *et al.*, 1976; Sever & Madden, 1977). In this laboratory, we have been studying the applicability of the ELISA to serodiagnosis and seroepidemiology of Japanese encephalitis (JE) virus infections (Igarashi *et al.*, 1981; Bundo *et al.*, 1981, 1982a, b, 1983a, b, Morita *et al.*, 1982; Fujita *et al.*, 1983; Fukunaga *et al.*, 1983; Chanyasanha *et al.*, 1984a, b) using formalin-inactivated and purified JE vaccine concentrate (Takaku *et al.*, 1968) as assay antigen. Although we have utilized cell culture-grown and purified dengue viruses as assay antigen in the indirect ELISA (Chanyasanha *et al.*, 1984a; May La Linn *et al.*, 1985) or dengue virus-infected cell culture fluid for IgM-capture ELISA on dengue infections (Bundo & Igarashi, 1985; May La Linn *et al.*, 1985), mouse brain-grown dengue viruses have not been tested yet. In this report, we examined their antigenicities regarding the applicability as assay antigen in the indirect ELISA and also their immunogenicities in relation to future development of inactivated immunogen.

MATERIALS AND METHODS

Dengue viruses: Following prototype strains were used in the study: type 1 (D1), Hawaiian; type 2 (D2), New Guinea B; type 3 (D3), H87; type 4 (D4), H241. Each

strain was inoculated intracerebrally to suckling mice, their brains (SMB) were harvested when they became moribund and stored at −70°C. Preparation of formalin-inactivated and purified virion is as described for JE virus (Takaku *et al.*, 1968). Ten percent homogenate of SMB was prepared in PBS and was clarified by low-speed centrifugation (3,000 $\times g$. 20 min). The supernatant was treated with protamine sulfate (1.8 mg/ml, 4°C, 90 min) and clarified again. The resulting supernatant was inactivated with formalin at final concentration of 0.05% at 4°C for 60 days and clarified again. The inactivated virions were concentrated and partially purified from the supernatant by 1% activated charcoal treatment followed by low-speed centrifugation, membrane filtration (Millipore, type HA), and ultracentrifugation (59,000$\times g$, 6 hours). The pelleted virion was homogenized with PBS containing 0.02% gelatin (Gel-PBS) and subjected to the second ultracentrifugation (59,000$\times g$, 6 hours). The pellet was homogenized with Gel-PBS, passed through the membrane filter, and diluted with Medium 199 containing 0.02% gelatin and M/150 phosphate buffer, pH 7.2 (Gel-PB-199), to final 1/4 volume of the starting 10% homogenate of SMB, and stored at 4°C.

Immunization of mice: Groups of 4-weeks old weaning mice were intraperitoneally inoculated with 0.5 ml of inactivated and partially purified virion diluted in 4-fold steps in Gel-PBS, using 26 mice for each dilution. After 7 days, the mice were given the second immunization with the same route and dose, and were bled 1 week later. Sera from 13 mice each from a group of 26 immunized with the same dilution of antigen were combined to make 2 different pools as serum A and serum B before tested in the serology. The immunization experiment was repeated with 4 different lots of inactivated antigen for each type of dengue virus.

ELISA: Indirect micromethod (Voller *et al.*, 1976) was followed with modifications as described (Igarashi *et al.*, 1981; Bundo *et al.*, 1982a). Plastic U-shaped 96-well plate (Greiner Laborteknik, West Germany) was coated with formalin-inactivated and partially purified dengue virion appropriately diluted in coating buffer and incubated at 4°C overnight. After washing with PBS-Tween, the plate was reacted with test sera diluted 1:100 or 1:1000 in PBS-Tween. Standard serum of predetermined endpoint titer was serially diluted in 2-fold steps starting from 1:100 up to 1:12800, and run in parallel with the test specimens on the same plate. The plate was incubated at 37°C for 1 hour followed by washing with PBS-Tween. Then, the plate was reacted with peroxidase-conjugated anti-mouse IgG (or IgM) goat IgG from Cappel Laboratories, USA, diluted 1:1000 (or 1:200) in PBS-Tween. The plate was incubated at 37°C for 1 hour followed by washing with PBS-Tween. Peroxidase reaction was performed with *o*-phenylenediamine and H_2O_2 in citrate-phosphate buffer, pH 5.0, at room temperature for 1 hour in a dark box. The reaction was stopped by adding H_2SO_4 and optical density of the colored product was measured at 490 nm in a Micro ELISA Autoreader (Dynatech, USA) with reference wavelength of 630 nm. Titers of test specimens were estimated by comparing their optical density with those by serial dilution of the standard serum using a computer system

(Morita *et al.*, 1982).

HI test: The method of Clarke and Casals (1958) was followed with modification to use microtiter system. The antigens were prepared by sucrose-acetone extraction from infected SMB.

N test: Micro N test combined with immunoperoxidase method (Okuno *et al.*, 1977) was used (Okuno *et al.*, 1978).

Statistical method: Methods described by Snedecor (1952) were followed.

RESUTS

Dose-response of Immunogen and ELISA Titer in Sera of Immunized Mice

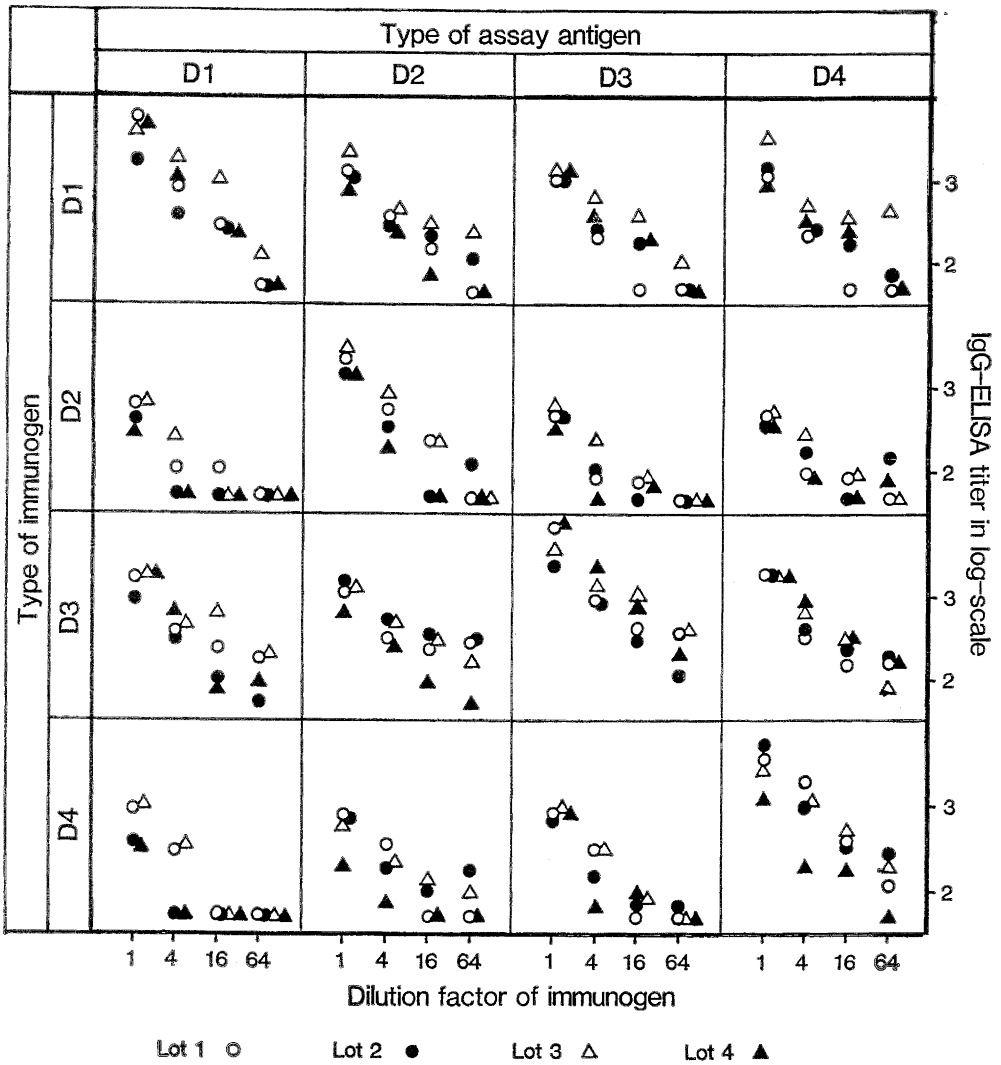

Fig. 1. IgG-ELISA titers in sera from mice immunized with varying concentrations of formalin-inactivated dengue virions in 4 different lots of preparations.

In Fig. 1 is shown IgG-ELISA titer in mouse sera immunized with formalin-inactivated dengue virions prepared from infected SMB and serially diluted in 4-fold steps. Preparation of the immunogen, immunization schedule, bleeding, and pooling of the immune sera were performed as described in the Materials and Methods. The IgG-ELISA titer was determined for serum A and serum B using each of the 4 different types of formalin-inactivated dengue virions as assay antigens, and the average titer of serum A and B is shown in the Figure.

The result shows dose-response relationships between the concentration of the immunogen and the IgG-ELISA titer of the immunized mice. The antibody production appears to be quite reproducible for 4 lots of the immunogens, especially when the titer was assayed by the homologous antigen. All the 4 lots of D3 and 3 out of the 4 lots

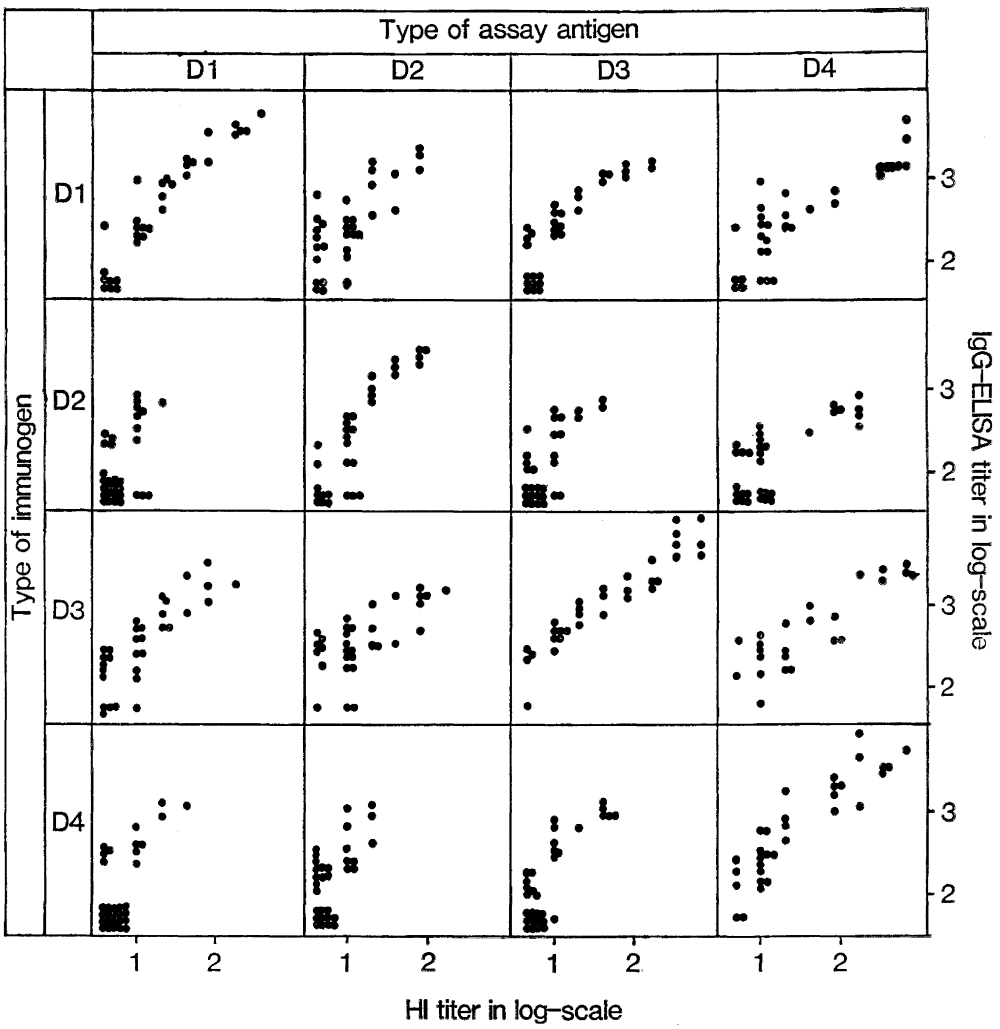

Fig. 2. Correlation between IgG-ELISA and HI titers in sera from mice immunized with formalin-inactivated dengue virions.

of D4 immunogens at 1:64 dilution still could elicit detectable levels of IgG-ELISA titers, in contrast to D1 and D2 immunogens, which produced barely detectable antibodies by only 1 of the 4 lots of immunogens at 1:64 dilution. In the case of D2 immunogen, only 2 of the 4 lots at 1:16 dilution could produce detectable antibodies in contrast to other types which produced detectable antibodies at 1:16 dilution of all the 4 lots. The titers assayed by heterologous types of antigen were generally lower than the titer assayed by the homologous type of the antigen.

Correlation between the HI and the IgG-ELISA Titer of Immune Sera

Each of the pooled serum A and B was assayed by the HI test using each of the 4 types of dengue antigen. The HI titers were compared with those by the IgG-ELISA showing linear relationships with homologous immunogen and assay antigen combination (Fig. 2). For heterologous combinations, the linear relationship was less marked, probably because of the lower titer of both HI and IgG-ELISA. The results are numerically expressed by the correlation coefficients (r-values) in Table 1, with higher r-value (0.887-0.936) for homologous compared with heterologous combinations (0.654-0.886). Among the heterologous combinations of immunogen and assay antigen, D1-D3, D3-D4, D4-D3, D1-D4, and D3-D1 gave relatively high r-values, showing closer relationships among D1, D3, and D4 viruses. Whereas, D2-D1 and D3-D2 combinations gave lower r-values. The results appear to indicate that D2 antigen is rather remote from other types of dengue viruses.

Table 1. Correlation coefficient between IgG-ELISA and HI titers of mouse sera immunized with and assayed by 4 different types of formalin-inactivated dengue virions.

		Type of assay antigen			
		D1	D2	D3	D4
Immunogen	D1	**0.935**	0.760	0.886	0.852
	D2	0.654	**0.899**	0.792	0.759
	D3	0.815	0.667	**0.936**	0.883
	D4	0.760	0.791	0.863	**0.887**

Cross-reactions between Different Serotypes of Dengue Antigens

The pooled serum A and B from mice immunized with undiluted lot 1 immunogen were tested by IgG-ELISA, IgM-ELISA, HI, and N tests using 4 different serotypes of inactivated assay antigens or infective virus. The titers for serum A and serum B were averaged and normalized to show the titer for the homologous immunogen-assay antigen combination as 100 (Fig. 3). The results indicated that the N test is most type-specific among 4 serological tests and that significant cross-reactivity were observed for other tests. Especially, high cross-reactivity was obtained by the HI using type 4 assay antigen. The

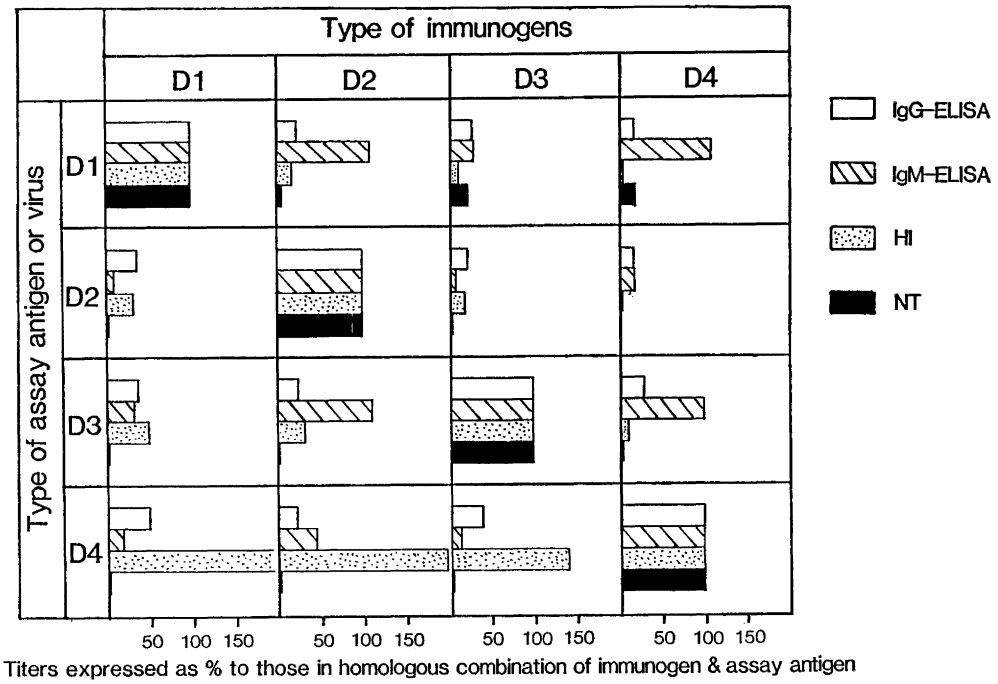

Fig. 3. Cross-reactions of anti-dengue mouse sera assayed by IgG-ELISA, IgM-ELISA, HI, and N tests.

result of IgM-ELISA is not always more type-specific than the IgG-ELISA, especially immunogen and assay antigen combination of D2-D1, D2-D3, D4-D1, and D4-D3 giving high cross-reactivity.

Immunization with Multivalent Antigen Mixtures

Equal volumes of 2 different serotypes of undiluted dengue immunogens were mixed and used to immunize mice by the same schedule as described in the Materials and Methods. Sera from immunized mice were pooled and tested for their antibody titers by the IgG-ELISA and HI test using each of the 4 types of dengue antigens. The result in Fig. 4 shows that IgG-ELISA titer assayed by the antigen which was not included in the immunogen was generally lower than the titers assayed by the antigens included in the immunogen. Especially, reduced level of anti-D2 titer in sera obtained by D1+D3, or D1+D4, or D3+D4 immunogens were remarkable, while D1+D2, or D2+D3 immunogen gave significant levels of anti-D3, or anti-D1 titer, respectively. When D1 or D3 was included in the immunogen, serum IgG-ELISA titer by D1 or D3 tended to be high. This relationship between the antibody titer by the assay antigen and presence of the homologous type in the immunogen was not marked by the HI test, showing the highest titer by D4 antigen and the lowest titer by D1 antigen, irrespective of their presence in the immunogen.

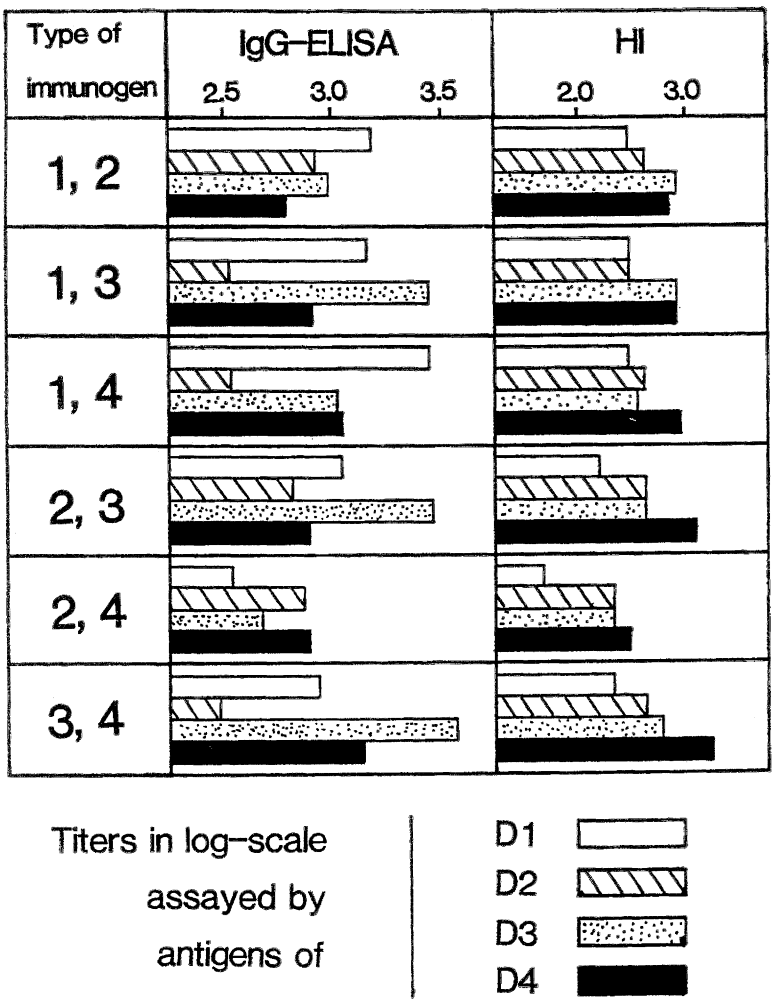

Fig. 4. IgG-ELISA and HI antibody titers in mouse sera immunized with mixtures of 2 different types of dengue immunogen.

Fig. 5 shows similar results obtained with tri-or tetravalent mixture of immunogens. Equal volumes of 3 or 4 different serotypes of undiluted immunogens were mixed and used to immunize mice according to the schedule described in the Materials and Methods, and immune sera were pooled before tested by the IgG-ELISA and HI test. Here again, the IgG-ELISA titer assayed by the antigen which was not included in the immunogen was generally lower than the titers assayed by the assay antigen included in the immunogen. Again the reduced level of anti-D2 titer of D1+D3+D4 immunized serum was remarkable, and the tetravalent immune serum showed that anti-D2 titer was the least among the titers assayed by 4 types of dengue antigens. In contrast, relatively high anti-D3 titer or anti-D4 titer was obtained in D1+D2+D4, or D1+D2+D3 immunized serum. Again this relationship between the high antibody titer and the presence of the homologous type in

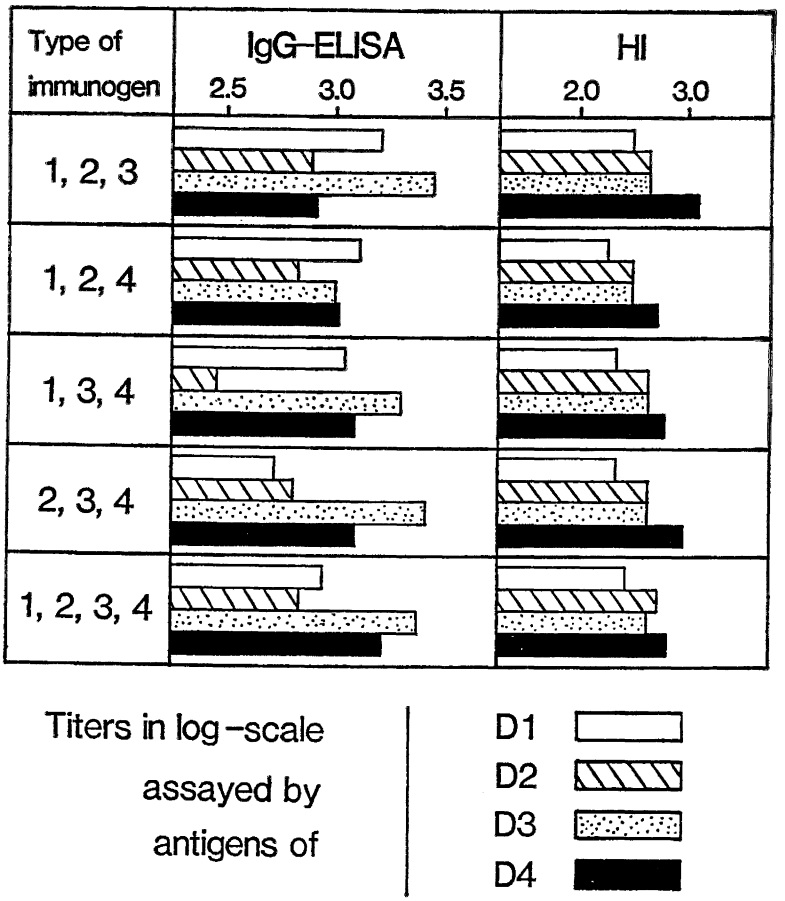

Fig. 5. IgG-ELISA and HI antibody titers in mouse sera immunized with mixtures of 3 or 4 different types of dengue immunogen.

the immunogen was not marked by the HI test, showing the highest titer by D4 and the lowest titer by D1 antigen irrespective of the presence or absence of these types in the immunogen.

Relationship between the presence or the absence of a given type of dengue virion in the immunogen and the IgG-ELISA or HI titer assayed by the respective antigen was numerically shown in Table 2. For monovalent immunogen, D2 gave the lowest titer even by the homologous assay antigen in the IgG-ELISA and HI test, while D3, followed by D1, gave the highest titer. High immunogenicity of D3 (and D1) was also indicated by the high ratio of antibody titers in sera obtained by the presence to that obtained by the absence of the respective type in divalent or trivalent immunogen. Low immunogenicity of D2 is again remarkable in divalent or trivaent immunogen since its absence showed remarkable reduction in anti-D2 titer of immune sera.

Tabe 2. IgG-ELISA and HI titers im mouse sera immunized with monovalent, divalent, and trivalent immunogens and assayed by 4 different types of formalin-inactivated dengue virions.

Type of immunogen		Assay antigen	IgG-ELISA			HI		
			Assay antigen in immunogen		Ratio of +/−	Assay antigen in immunogen		Ratio of +/−
			+	−		+	−	
	Divalent	D 1	1905	692	2.75	316	126	2.51
		D 2	692	339	2.04	355	398	0.89
		D 3	3162	794	3.98	631	423	1.49
		D 4	1122	759	1.48	800	962	0.83
	Trivalent	D 1	1349	525	2.57	238	224	1.06
		D 2	692	288	2.40	398	447	0.89
		D 3	2493	1000	2.49	447	316	1.41
		D 4	1175	832	1.41	671	1259	0.54
	Monovalent	D 1	3350			457		
		D 2	1972			226		
		D 3	5129			543		
		D 4	1841			320		

DISCUSSION

Our data show that the SMB-grown and formalin-inactivated dengue antigens could successfully be used as assay antigen in the indirect ELISA. This is especially useful for D 3 and D 4, which could not give higher yield in cell culture system compared with D 1 and D 2. We have compared the reactivity of SMB-grown and formalin-inactivated dengue virion with SMB-grown live dengue virion, and also with type 1 and 2 dengue virions prepared from infected *Aedes albopictus* clone C6/36 cells (Igarashi, 1978) with or without formalin-inactivation. Their reactivities were similar except that live D 2 virion from C 6 / 36 cells gave higher IgG-ELISA titer by anti-D 1 and anti-D 3 sera, compared with SMB-grown formalin-inactivated virions (data not shown). The specificity of the IgG-ELISA appears to be higher than that by the HI although it is lower than the N test. Unfortunately, the type-specificity will not sufficient to tell the infecting serotype of dengue virus by the serology on patient's sera. The indirect IgM-ELISA was not so type-specific compared with IgG-ELISA, although IgM-antibody assay was reported to be more specific than conventional assay on total immunoglobulins to differentiate different flavivirus infections (Westaway, 1968a, b: Westaway *et al.*, 1975).

Mouse immunization experiments showed closer relationship between D 1 and D 3 in contrast to relatively remote antigenicities of D 2 from other types of dengue vi-

ruses. Henchal *et al.*, (1982) demonstrated closer relationship between D 1 and D 3 compared with other types by D 1 -D 3 subcomplex specific monoclones. IgG-ELISA antibody levels of mouse immune sera against each type of dengue assay angen indicated that D 2 should be present in the immunogen in order to produce broadly reactive antisera, while, D 3 and D 1 might be omitted. Different results on the same immune sera were observed by the HI test, indicating the importance to include D 1 and irrelevance of D 4 or D 2 in the immunogen. These difference appear to indicate that the antigenic sites or epitopes reacting in the IgG-ELISA are different from those in the HI, and also that the reactivity of the epitopes is not always similar to their immunogenicities.

Because of the technical difficulty, the N test was not performed on the immune sera obtained by multivalent immunogens. Therefore, present results could not directly evaluate the importance or irrelevance of each immunogen in order to produce inactivated dengue vaccine which could cover all the types of infective dengue viruses. However, these asessments should be performed by considering that humans are almost exclusively sensitive vertebrate hosts to dengue viruses while mice are rather insensitive ones.

REFERENCES

1) Bundo, K., & Igarashi, A. (1985): Antibody-capture ELISA for detection of immunoglobulin M antibodies in sera from Japanese encephalitis and dengue hemorrhagic fever patients. J. Virol. Methods, 11, 15−22.

2) Bundo, K., Matsuo, S., & Igarashi, A. (1981): Enzyme-linked immunosorbent assay (ELISA) on Japanese encephalitis virus. II. Antibody levels in the patient sera. Trop. Med., 23, 135−148.

3) Bundo, K., Morita, K., & Igarashi, A. (1982a): Enzyme-linked immunosorbent assay (ELISA) on Japanese encephalitis virus. III. Assay on antibody titers in swine sera. Trop. Med., 24, 87−102.

4) Bundo, K., Igarashi, A., Morita, K., Hayashi, K., Yamada, A., Goto, I., Douke, S., Sakai, S., Katsuki, K., Watanabe, K., Ishii, K. (1982b): Enzyme-linked immunosorbent assay (ELISA) on Japanese encephalitis virus. V. Antibody levels among inhabitants in endemic and nonendemic areas. Trop. Med., 24, 139−150.

5) Bundo, K., Igarashi, A., Morita, K., Hayashi, K., Urasawa, S., & Ura, M. (1983a): Enzyme-linked immunosorbent assay on Japanese encephalitis virus. VI. Antibody response in human vaccinees. Trop. Med., 25, 25−35.

6) Bundo, K., Morita, K., Igarashi, A., Yamashita, K., Aizawa, M., & Miura, N. (1983b): Antibodies against Japanese encephalitis virus in bovine sera in Nagasaki, 1981. Trop. Med., 25, 73−82.

7) Chanyasanha, C., Bundo, K., & Igarashi, A. (1984a): IgG-ELISA antibody titers against Japanese encephalitis (JE) and dengue virus type 1 among healthy people in JE-endemic areas in Japan and Thailand. Trop. Med., 26, 1−9.

8) Chanyasanha, C., Bundo, K., Igarashi, A., Supawadee, J., Pholprasert, B., & Chiowanich,

P. (1984b); Serodiagnosis by the ELISA on encephalitis in Chiang Mai, Thailand, 1983. Trop. Med., 26, 147−156.

9) Clarke, D. H., & Casals, J. (1958): Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. Amer. J. Trop. Med. Hyg., 7, 561−573.

10) Engvall, E., & Perlman, P. (1971): Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. Immunochemistry, 8, 871−874.

11) Fujita, N., Igarashi, A., Bundo, K., Ogata, T., Supawadee, J., Peerakome, S., Leechanachai, P., Panasampol, K., Chanyasanha, C., & Chatiyanonda, K. (1983): Virological and epidemiological studies on encephalitis in Chiang Mai Area, Thailand, in the year of 1982. IV. Serological examination on hospitalized patients. Trop. Med., 25, 155−164.

12) Fukunaga, T., Igarashi, A., Ogata, T., Fujita, N., Charoensook, O., Jatanasen, S., Chanyasanha, C., Chatiyanonda, K., Peerakome, S., Supawadee, J., & Panasampol, K. (1983): Virological and epidemiological studies on encephalitis in Chiang Mai Area, Thailand in the year of 1982. V. Seroepidemiological survey on humans. Trop. Med., 25, 169−181.

13) Halstead, S. B. (1966): Mosquito-borne haemorrhagic fevers of South and Southeast Asia. Bull. WHO., 35, 3−15.

14) Halstead, S. B. (1980): Dengue haemorrhagic fever- a pubic health problem and a field for research. Bull. WHO., 58, 1−21.

15) Henchal, E. A., Gentry, M. K., McCown, J. M., & Brandt, W. E. (1982): Dengue virus specific and flavivirus group determinants identified with monoclonal antibodies by inirect immunofluorescence. Amer. J. Trop. Med. Hyg., 31, 830−836.

16) Igarashi, A. (1978): Isolation of a Singh's *Aedes albopictus* cell clone sensitive to dengue and chikungunya viruses. J. Gen. Virol., 40, 531−544.

17) Igarash', A., Bundo, K., Matsuo, S., Makino, Y., & Lin, W-j. (1981): Enzyme-linked immunosorbent assay (ELISA) on Japanese encephalitis virus. I. Basic condition of the assay on human immunolgobulin. Trop. Med., 23, 49−59.

18) May La Linn, Bundo, K., Igarashi, A., Soe Thein, & Mi Mi Khin (1985): Dengue and Japanese encephalitis antibody levels in sera from dengue haemorrhagic fever patients in Rangoon, Burma. Trop. Med., 27, 23−36.

19) Morita, K., Bundo, K., & Igarashi, A. (1982): Enzyme-linked immunosorbent assay (ELISA) on Japanese encephalitis virus. IV. A computer system to calculate ELISA endpoint titer from ELISA-OD at a single dilution of test sera. Trop. Med., 24, 131−137.

20) Okuno, Y., Sasao, F., Fukunaga, T., & Fukai, K. (1977): An application of PAP (peroxidase-anti-peroxidase) staining technique for the rapid titration of dengue virus type 4 infectivity. Biken J., 20, 29−33.

21) Okuno, Y., Igarashi, A., & Fukai, K. (1978): Neutralization tests for dengue and Japanese encephalitis viruses by the focus reduction method using peroxidase-anti-peroxidase staining. Biken J., 21, 137−147.

22) Sever, J. L., & Madden, D. L. (1977): Enzyme-linked immunosorbent assay (ELISA) for infectious agent. J. Infect. Dis., 136 (Suppl.), S257−S340.

23) Snedecor, G. W. (1952): Statistical Methods Applied to Experiments in Agriculture and Biology. The Iowa State College Press.

24) Takaku, K., Yamashita, T., Osanai, T., Yoshida, I., Kato, M., Goda, H., Takagi, M.,

Hirota, T., Amano, T., Fukai, K., Kunita, N., Inoue, K., Igarashi, A., & Ito, T. (1968): Japanese encephalitis purified vaccine. Biken J., 11, 25−39.

25) Voller, A., Bidwell, O., & Bartlet, A. (1976): Microplate enzyme immunoassay for the immunodiagnosis of viral infections. pp506−512. *In* N. R. Rose & N. Friedman (ed.). Manual of Clinical Immunology, American Society of Microbiology, Washington, D. C.

26) Westaway, E. G. (1968a): Antibody response in rabbits to the group B arbovirus Kunjin: Serologic activity of the fractionated immunoglobulins in homologous and heterologous reactions. J. Immunol., 100: 569−580.

27) Westaway, E. G. (1968b): Greater specificity of 19S than 7S antibodies in haemagglutinaion-inhibition tests with closely related group B arboviruses. Nature, 219, 78−79.

28) Westaway, E. G., Shew, M., & Della-Porta, A. J. (1975): Reactions of purified hemagglutinating antigen of flaviviruses with 19S and 7S antibodies. Infect. Immun., 11, 630−634.

29) World Health Organization (1966): Mosquito-borne hemorrhagic fever of Southeast Asia and the Western Pacific. Bull. WHO., 35, 17−33.

マウス脳増殖フォルマリン不活化デングウイルスの抗原性と免疫原性

森　千里，森田（分藤）桂子，松尾幸子，五十嵐　章（長崎大学熱帯医学研究所ウイルス学部門）高木光生，吉田　巌，深井孝之助（阪大微生物病研究会）奥野良信（大阪大学微生物病研究所）

デングウイルス1，2，3，4型原株を乳呑マウス脳で増殖させた後10%乳剤を作成し，低速遠心の上清を硫酸プロタミン処理し，フォルマリンで不活化した．その低速遠心上清から活性炭処理と超遠心でウイルス粒子を精製し，乳離れした幼若マウスに接種して免疫原性を検討した．免疫血清の抗体価は間接 ELISA，血球凝集抑制（HI）反応，及び中和試験で測定した．免疫原の濃度と免疫マウス血清の IgG-ELISA 抗体価の間には用量反応関係が認められた．同型の免疫原と測定抗原に対しては免疫血清の HI 価と IgG-ELISA 価の相関係数は 0.88-0.94 と高かったが異型の免疫原と測定抗原の組み合わせでは 0.76-0.88 とやや低い値を示した．種々の血清反応における抗体価も同型の免疫原と測定抗原の組み合わせで最も高かったが異型の組み合わせの場合も可なりの交差反応が ELISA と HI で認められ，IgM-ELISA が IgG-ELISA よりも特異性が高いとは云えなかった．IgG-ELISA 抗体の産生状況から，用いたデングウイルスの中で3型が最も免疫原性が高く，次いで1型，4型であり，2型は最も低いと考えられた．この傾向は異なる型のデングウイルスを混合した2価及び3価の免疫原を用いた場合にも認められた．2型ウイルスを含まない免疫原で得られた抗血清は2型抗原に対する抗体価が有意に低かったが，1型と2型又は2型と3型の混合物で免疫すると凡ての型の測定抗原に対して十分高い IgG-ELISA 抗体価が得られた．従って4つの型のデングウイルスに広く反応する IgG-ELISA 抗体を産生するには免疫原に2型ウイルスを含める必要がある．一方，HI 試験では免疫原に1型ウイルスを含まないと得られた抗血清の1型に対する抗体価が低く，2型又は4型を含まなかっても2型又は4型に対する抗体価は大差がなかった．

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