THE EFFECT OF NONIONIC DETERGENT ON DENGUE AND JAPANESE ENCEPHALITIS VIRUS ANTIGENS IN ANTIGEN DETECTION ELISA AND IgM-CAPTURE ELISA

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Abstract. In order to simplify dengue and Japanese encephalitis (JE) IgM-ELISA, we have been trying to produce antigens as infected C6/36 cell culture fluid. In this study, we examined the effect of nonionic detergents, which were used to inactivate viral infectivity, on dengue and JE antigen titers as well as the results in an IgM-capture ELISA. In the antigen detection ELISA, antigen titers were not significantly reduced after treatment with nonionic detergents (Nonidet P-40 or Triton X-100, at 0.01 to 0.1% final concentration). In contrast, in the IgM-capture ELISA, the color development was significantly reduced when the antigens were pretreated with nonionic detergents. The results suggest that certain epitopes which react with anti-viral IgM antibodies, but not IgG antibodies, have been destroyed by treatment with nonionic detergents. The results indicate that we cannot use nonionic detergents to inactivate the infectivity of assay antigens.

INTRODUCTION

Dengue and JE virus infections have been great medical and public health problems in Southeast Asia (Halstead, 1966, 1980, 1992, 1993; Igarashi, 1994). Most laboratory diagnosis of these viral infections has been carried out by serological tests, classically by the hemagglutination-inhibition test (HI) and recently by the IgM capture ELISA (Burke, 1983; Bundo and Igarashi, 1985; Innis *et al,* 1989). In these tests, assay antigens have been extracted from infected suckling mouse brains by sucrose-acetone and other methods (Clarke and Casals, 1958). These procedures require animal handling and large quantities of organic solvents. In order to simplify the procedures of IgM-ELISA, we have been trying to prepare dengue antigen as infected C6/36 cell culture fluid (Igarashi *et al,* 1995). Since the infected culture fluid contains infective virus, it is recommended that inactivation of the infectivity be carried out without losing antigenicity of the antigen. Our previous experience showed that flaviviruses, including dengue and JE viruses, can easily be disrupted by treatment with nonionic detergents such as Nonidet P-40 (NP40) or Triton X-100 (TX100) (Morita *et al,* 1991).

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The purpose of this study was to evaluate the effect of two nonionic detergents (NP40 and TX100) on the titers of dengue and JE antigen in the infected culture fluid as determined by the antigen-detection ELISA and the efficiency of the IgM-ELISA using such treated antigens.

MATERIALS AND METHODS

Serum sample

A total of 81 serum samples was obtained from patients clinically diagnosed as dengue fever or JE, kindly provided by Dr Masao Yoshida of the Iwate Life Science Institute in collaboration with the Department of Pediatrics, Udayana University, Indonesia.

Cell culture antigen preparation

Virus strains used in this study were dengue virus type 1 (D1) Hawaiian strain, dengue virus type 2 (D2) New Guinea B strain, dengue virus type 3 (D3) H87 strain, dengue virus type 4 (D4) H241 strain, and JE virus JaOArS982 strain. Seed virus specimens were inoculated into C6/36 *Aedes albopictus* cell cultures (Igarashi, 1978). Virus was allowed to be absorbed for two hours and thereafter Eagle's minimal essential medium supplemented with 2% fetal bovine serum and 0.2mM each nones-

sential amino acids was added. Cells were incubated at 28° C for 7 days, infected culture fluid was harvested, and virus antigen titer was determined by the antigen detection ELISA. A part of the infected culture fluid was further incubated overnight at 4°C with NP40 or TX100 at final concentrations of 0.01% and 0.1%.

Antigen detection ELISA

The micro sandwich method of Voller *et al* (1976) with some modifications was used in this study. 96-well ELISA flat bottom plates (Nunc, Denmark) were coated with 100 µl of anti-flavivirus IgG (20 µg/ml in coating buffer of 0.05 M carbonate-bicarbonate buffer, pH 9.6) at room temperature overnight. After washing with PBS-Tween 3 times for 3 minutes each, the plates were blocked with Block Ace (Yukijirushi, Japan) for one hour at room temperature. After washing, test specimens treated with or without NP40 or TX100, standard D2 antigen serially diluted in 2-fold steps, and a negative control (maintenance medium alone) were distributed in duplicate wells. The ELISA plates were incubated for one hour and washed as above. One hundred microliters of HRPO-conjugated anti-flavivirus IgG (1:100 dilution in PBS-Tween) were distributed into each well and incubated as above. Unbound conjugate was washed off as above and plates were incubated with the substrate of *o*-phenylenediamine-2HCl and H_2O_2 in 0.05 M citrate-phosphate buffer, pH 5.0, for one hour in the dark. The color reaction was terminated by adding 1N H_2SO_4. Optical density was measured at 492 nm using an ELISA microplate reader. Antigen titer of test specimen was determined by comparing their ELISA-OD with those of serially diluted standard antigen.

IgM-capture ELISA

The 96-well flat bottomed ELISA plates were coated with antihuman IgM (µ-chain specific) goat IgG (Cappel, PA, USA) diluted 1:200 with coating buffer and incubated at 4° C overnight. Plates were washed with PBS-Tween 3 times for 3 minutes each. Nonspecific binding sites were blocked with Block Ace (Yukijirushi, Japan) for 1 hour at room temperature. Serum samples (1:100 dilution in PBS-Tween) were added and incubated for another hour at room temperature. After another series of PBS-Tween washings, D1, D2 and JE virus antigens with or without NP40 treatment were added and incubated for one hour at room temperature. The plates were subjected to PBS-Tween washing, reaction with HRPO-conjugated anti-flavivirus IgG, another washing, color development with substrate, termination of the reactrion with H_2O_2, and OD reading, as in the case of antigen detection ELISA. The P/N ratio was calculated for each test specimen by dividing its ELISA-OD by ELISA-OD of negative standard serum.

RESULTS

In this study, we examined the effect of nonionic detergent treatment on dengue and JE antigen titers as determined by the antigen detection ELISA as well as the results in the IgM capture ELISA. The effect of TX100 and NP40 on the titer as determined by antigen capture ELISA is shown in Tables 1 and 2. Incubation of D1 antigen with TX100 and NP40 slightly increased the antigen titer. No effect was seen when D2 antigen was treated with either TX100 or NP40. However there was a slight decrease in antigen ELISA titer when D3 was treated with NP40 and a further significant decrease when treated with TX100. An increase in antigen ELISA titer was obtained when D4 was treated with TX100 or NP40. On the other hand, there was no significant change in the ELISA titer when JE virus antigen was treated with 0.01% TX100 or NP40. As a whole, NP40 or TX100 treatment did not significantly reduce the dengue and JE antigen titers as determined by the sandwich ELISA.

The effect of NP40 on the efficiency of positive case detection in the IgM-capture ELISA is shown in Table 3. By incubation of viral antigens (D1, D2 and JE) with 0.1% NP40, the P/N ration of the IgM-capture ELISA was substantially decreased. The percentage of the specimens showing decreased P/N ration was 76% for D1, 100% for D2, and 75% for JE antigens, respectively. Figs 1, 2 and 3 showed comparison of P/N ratio of test sera using D1, D2 and JE antigen with or without treatment by NP40 at final concentration of 0.1%. Most of the samples which were scored positive (P/N ratio more than 2) by nontreated antigen were scored as negative when detergent treated antigen was used.

Table 1

Effect of TX100 on the ELISA titer of viral antigen.

Antigen	ELISA titer of antigens		
	Without TX100 treatment	With TX100 treatment	
		0.01%	0.1%
D1	9	26	20
D2	35	37	35
D3	15	8	4
D4	1.5	5.5	9
JE	30	37	26

Table 2

Effect of NP40 on the ELISA titer of viral antigens.

Antigen	ELISA titer of antigens		
	Without NP40 treatment	With NP40 treatment	
		0.01%	0.1%
D1	9	14	14
D2	35	37	35
D3	15	16	10
D4	1.5	4.5	8
JE	30	33	20

Table 3

Change of the P/N ratio of the test serum specimens in the IgM-ELISA by NP40 treatment of viral antigens.

Antigen	No. of specimens			% decrease P/N ratio
	Total	Decreased P/N ratio	Increased P/N ratio	
D1	21	16	5	76%
D2	40	40	0	100%
JE	20	15	5	75%

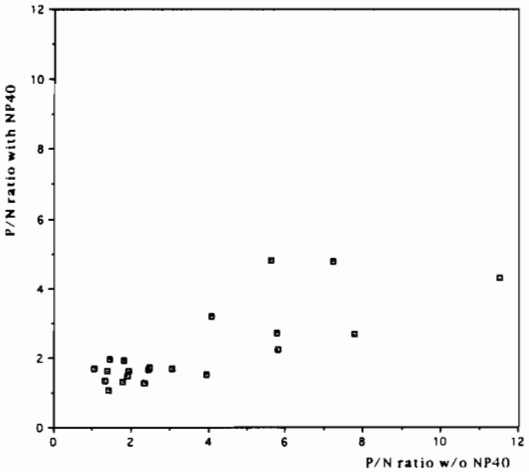

Fig 1–Comparison of P/N ratio of test sera in the IgM ELISA using D1 antigen with or without NP40 treatment.

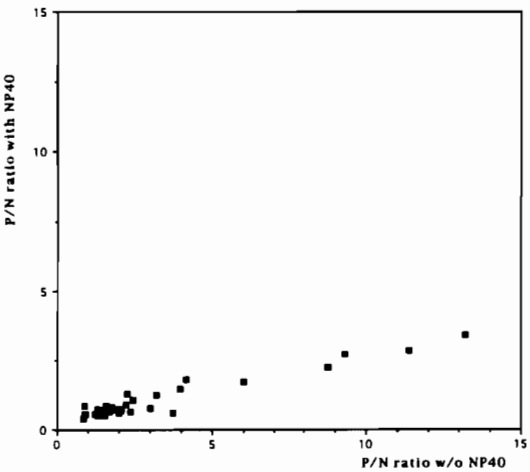

Fig 2–Comparison of P/N ratio of test sera in the IgM ELISA using D2 antigen with or without NP40 treatment.

DISCUSSION

The results shown above suggest that certain epitopes which react with anti-dengue or anti-JE IgM antibodies, but not IgG antibodies, have been destroyed by the treatment with nonionic detergents. The results also indicated that we cannot use nonionic detergent in order to inactivate infectivity of assay antigens.

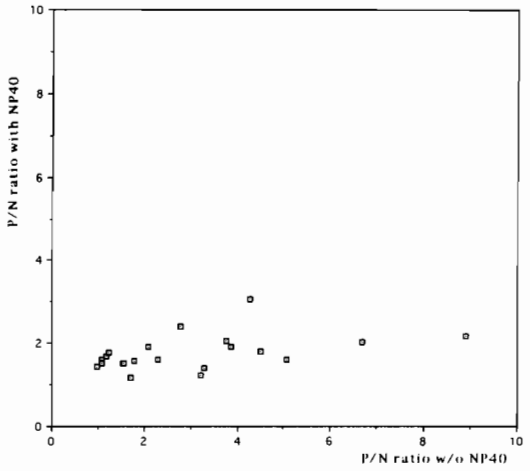

Fig 3–Comparison of P/N ratio of test sera in the IgM ELISA using JE antigen with or without NP40 treatment.

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