Short Communication

**HEMAGGLUTINATION-INHIBITING IMMUNOGLOBULIN A
ANTIBODY IN THE SERUM OF PATIENTS
WITH DENGUE HEMORRHAGIC FEVER**

SAKAE INOUE, SHIGEO MATSUNO, REISAKU KONO,
NADHIRAT SANGKAWIBHA* and PRASERT THONGCHAROEN**

*Central Virus Diagnostic Laboratory, National Institute of Health, Musashimurayama,
Tokyo 190-12, *Virus Research Institute, Department of Medical Sciences,
Ministry of Public Health, and **Department of Microbiology, Faculty of
Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand*

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SUMMARY: In convalescent-phase sera of patients with dengue hemorrhagic fever, hemagglutination-inhibiting (HI) antibody of immunoglobulin (Ig) A class, in both monomeric and oligomeric forms, was detected. It was found to be less type-specific than IgM HI, and as broadly cross-reacting as IgG HI antibodies.

It has been well documented that hemagglutination-inhibiting (HI) antibody of immunoglobulin (Ig) M class, produced after primary and secondary infections of animals with flaviviruses (group B arboviruses), is relatively specific for the infecting virus, whereas IgG HI antibody is broadly cross-reactive with other members of this group (Westaway, 1968; Edelman et al., 1973; Westaway, Della-Porta and Readman, 1974; Westaway, Shew and Della-Porta, 1975). IgM HI antibody produced after dengue type 2 infection in persons previously immunized against yellow fever was also found more type-specific than the IgG antibody, thus its detection has a diagnostic value (Scott, McCown and Russell, 1972). However, there has been no report on the IgA HI antibody in cases of flavivirus infections. We investigated whether the sera of patients with dengue hemorrhagic fever (DHF), which is one of the most important viral diseases in Southeast Asia, contain IgA HI antibody, and, if so, how its specificity is in comparison with the IgG and IgM antibodies. We have already reported that, in human rubella virus infection, oligomeric IgA antibody appears in the serum for a limited duration (Inouye, Kono and Takeuchi, 1978). The same method as used in the rubella study was employed in this investigation.

Convalescent-phase sera were collected in Bangkok, Thailand, from DHF patients with serologic evidence of secondary dengue infection. Five serum

Fig. 1. Sedimentation profile of HI activity in the serum after treatment with protein A-Sepharose of a convalescing DHF patient. One-tenth milliliter of serum was passed through a small column of protein A-Sepharose. The eluate was concentrated to 0.4 ml with a single hollow fiber and then centrifuged in a 12.5 to 37% (w/v) sucrose at 35,000 rpm for 16 hr at 4 C in a Spinco SW50.1 rotor. Sedimentation velocity markers were rabbit hemolysin (19S, 7S) and catalase (11S). Each fraction was tested for HI activity against dengue type 1 (D1) (.....), D2 (—), D4 (.....) and Japanese encephalitis (JE) virus (—). The HI test was carried out according to Clarke and Casals (1953) in a micromethod.

specimens were treated with protein A-Sepharose (Pharmacia) for removal of IgG, then centrifuged in sucrose density gradients. After fractionation of the gradients, each fraction was tested for its HI activity against four flaviviruses, dengue (D) types 1, 2 and 4, and Japanese encephalitis. The sedimentation profile of the HI activity of one serum is shown in Fig. 1B. The 19S IgM fractions possessed HI only against D1 and D2, while in the 10 to 7S fractions HI activity was detectable against all the four viruses used. The HI activity in the top fractions was due to serum lipoproteins. The HI activity of the broad peak at the middle position was confirmed to be due to IgA antibody by use of immunoglobulin class-specific immunosorbents (Table I). As for the other four serum specimens, IgM antibody was detected against D1 and D2 in one,

TABLE I

Removal of HI activity by immunoglobulin class-specific immunosorbents

Antibody source		Anti-D2 HI titers after absorption with		
Treatment of serum	Fractions	PBS	Anti-IgA*	Anti-IgG*
none	7 S**	8	8	0***
Protein A	7 S	8	0	8
Protein A	10 S	8	0	8

* Immunosorbents were prepared as described previously (Inouye, Kono and Takeuchi, 1978)

** Appropriately diluted

*** No HI activity with undiluted samples.

and against D1, D2 and D4 in another serum. However, IgA antibody was detected against all the viruses used in all the samples. It was reported that little or no IgM antibody is detectable after secondary dengue infection (Russell, Udomsakdi and Halstead, 1967). Our study showed that the IgM antibody response, if it occurs, is not always monospecific within the dengue subgroup, and that the IgA antibody is more cross-reacting than the IgM. When the 10S fraction shown in Fig. 1B was treated with 0.1 M 2-mercaptoethanol, the HI activity was maintained but sedimented to the 7S position (data not shown), whereas the 19S HI activity was abolished after the treatment. This suggests that the 10S HI activity consisted of IgA oligomers, and that the affinity of the IgA antibody to the antigens is higher, thus the specificity is lower than those of the IgM antibody (Eisen, 1973). Implications of the presence of both monomeric and oligomeric IgA antibody in the serum of DHF patients concerning the pathogenesis of the disease are not now known.

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