Title	Association of dengue virus type-specific IgG on platelets is specific for the acute phase in an imported Japanese patient with secondary dengue 2 virus infection
Author(s)	Oishi, Kazunori; Inoue, Shingo; Kuramoto, Tsuyoshi; Onizuka, Shozaburo; Saito, Mariko; Hasebe, Futoshi; Morita, Kouichi; Nagatake, Tsuyoshi
Citation	Vol.31, No.4, 2003, pp.223-225
Issue Date	2003-12-15
URL	http://hdl.handle.net/10069/5657
Right	Copyright(c) 2003 by The Japanese Society of Tropical Medicine

ASSOCIATION OF DENGUE VIRUS TYPE-SPECIFIC IGG ON PLATELETS IS SPECIFIC FOR THE ACUTE PHASE IN AN IMPORTED JAPANESE PATIENT WITH SECONDARY DENGUE 2 VIRUS INFECTION

Kazunori Oishi , Shingo Inoue , Tsuyoshi Kuramoto , Shozaburo Onizuka , Mariko Saito , Futoshi Hasebe , Kouichi Morita , and Tsuyoshi Nagatake
Accepted 24, October, 2003

Abstract: The mechanism of thrombocytopenia in dengue virus infection remains unknown. We report herein an imported case of a 21-year-old male Japanese with dengue fever caused by secondary dengue 2 virus infection. The thrombocytopenia detected around the day of defervescence was associated with an increased level of platelet-associated IgG (PAIgG). The eluate from the platelets during the acute phase of this case contained an increased activity of anti-dengue virus 2 IgG, while the eluate from platelets during the convalescent phase contained a low level of anti-dengue 2 IgG. These findings suggest the transient formation of PAIgG involving anti-dengue 2 virus IgG during the acute phase of secondary dengue 2 virus infection.
Key words: Dengue virus infection, PAIgG, Dengue specific IgG, Thrombocytopenia

INTRODUCTION

The geographical distribution of the dengue viruses, a mosquito-borne human viral pathogen, has expanded greatly and the number of cases has dramatically increased over the past three decades (Igarashi, 1997). Two and a half billion people in more than one hundred countries are currently at risk of infection, with an estimated 50 million infections per year (Guzman and Kouri, 2002). The four serotypes of dengue virus induce a wide spectrum of clinical manifestations, which are frequently associated with thrombocytopenia and hemorrhagic diathesis (Srichaikul and Nimmannitya, 2000). We recently demonstrated a correlation between increased platelet-associated IgG (PAIgG) and thrombocytopenia and the association of anti-dengue virus IgG on platelets from patients during the acute phase of secondary dengue infection. As a result if these findings, we propose and that PAIgG formation involving anti-dengue virus IgG plays a pivotal role in the induction of transient thrombocytopenia (Oishi, 2003). However, the issue of whether the association of anti-dengue virus IgG on the platelets is specific for the acute phase has not been determined. We demonstrate herein the acute phase-specific association of anti-dengue virus IgG on platelets from an imported case of dengue fever.

A 21-year-old Japanese man visited to the Solomon islands between August 19 th and September 2 nd. Immediately after returning to Japan on September 4 th, he was admitted to Nagasaki University hospital on September 5 th, 2001 because of high fever that persisted for 2 days. His medical examination was unremarkable except for the fever (38.4℃) on admission. No bleeding diathesis was noted. A thick film of his blood sample, taken on September 5 th, was negative for the malaria parasite. A plasma sample, obtained on the day of admission, was found to be positive for dengue virus serotype 2, but negative for dengue virus serotypes 1, 3 and 4 by RT-PCR (Morita et al, 1991). IgM capture ELISA was negative on September 5 th, but became positive on September 14 th (Bundo et al, 1985). Parenteral fluid therapy (5% glucose in physiological saline) was

Department of Internal Medicine and Department of Virology, Institute of Tropical Medicine, Nagasaki University
Correspondence to: Kazunori Oishi, M.D.,
1-12-4 Sakamoto, Nagasaki 852-8523, Japan
Tel: +81-95-849-7842, FAX+81-849-7843, e-mail: oishi-k@net.nagasaki-u.ac.jp

started on the day of admission. Acetaminphen (200 mg per dose) was orally given when his body temperature reached in excess of 39℃. The peripheral white blood count decreased on September 7 th and 9 th. More Importantly, his peripheral platelet counts decreased on September 9 th and reached to a minimum (49 x 10 /ml). A small amount of epistaxis was noted on September 9 th and11th. The day of defervescence was September 10 th. This case was diagnosed as dengue fever based on the WHO criteria because the hematocrit increase was less than 20% (WHO, 1997). Further HI tests using serum samples at the acute (September 8 th; 1: 40) and convalescent phase (September 19 th; 1: 5, 120) confirmed the existence of a secondary infection (WHO, 1997).

An increased level of PAIgG (49.4 ng/ 10 platelets; the normal range is below 20 ng/ 10 platelets) was found on September 10 th when the peripheral platelet count was the lowest. The levels of fibrin degradation product and fibrinogen were 2.3 μg/ml and 256 mg/dl, respectively. The prothrombin time ratio (divided by the normal value) was 1.14. Consequently, the total disseminated intravascular coagulation (DIC) score was determined to be 5, which is suggestive of a low probability of DIC (Nishiyama et al, 2000). Our recent paper demonstrated that PAIgG contains anti-dengue virus IgG during the acute phase of secondary dengue virus infection. We thus compared the levels of anti-dengue virus serotype 2 IgG in between the eluted sample from platelets on September 12 (acute phase; platelet count is 63 x 10 /μl) and on October 23 th, 2001 (convalescent phase; platelet count is 241 x 10 /μl). We also employed platelet samples from three healthy volunteers (platelet count ranges from 195 to 303 x 10 /μl). The elution of IgG from platelet samples was carried out according to previously described procedures and the eluate was dialyzed against phosphate buffered saline (PBS) and concentrated to a final volume of 1ml with 1.5 x 10 platelets (Oishi et al, 2003). The eluates (1:5 diluted in PBS) were used in an indirect ELISA as previously described (Oishi et al, 2003). The optical density (OD at 405 nm; the values are the mean of three determinations) was measured. The OD at 405 nm of the anti-dengue 2 IgG in the eluates from the three healthy volunteers was 0.12 ± 0.07. In contrast, an increased activity of anti-dengue virus 2 IgG (OD at 405 nm; 0.98 ± 0.06) in the eluate at the acute phase of the presented case was found. A decreased activity of anti-dengue 2 IgG (OD at 405 nm; 0.19 ± 0.01) was also found in the eluate at the convalescent phase of this patient.

The imported case of dengue fever presented in this study was a secondary infection and was due to a dengue 2 virus infection. A transient increase in anti-dengue virus IgG on the surface of platelets was found to be associated

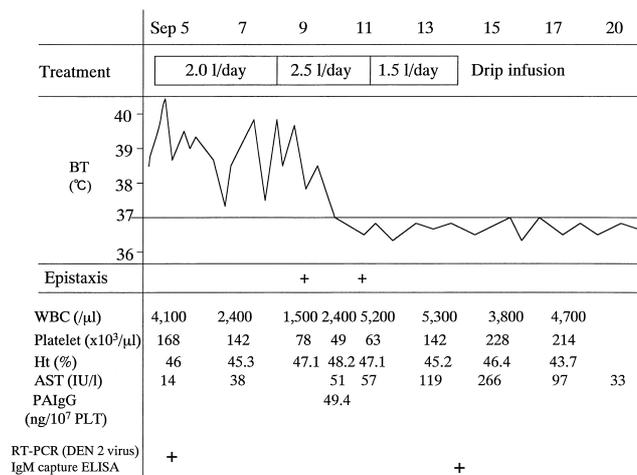|	Sep 5	7	9	11	13	15	17	20
Treatment	2.0 l/day		2.5 l/day	1.5 l/day	Drip infusion			
BT (℃)								
Epistaxis			+	+				
WBC (/μl)	4,100	2,400	1,500	2,400 5,200	5,300	3,800	4,700	
Platelet (x10³/μl)	168	142	78	49 63	142	228	214	
Ht (%)	46	45.3	47.1	48.2 47.1	45.2	46.4	43.7	
AST (IU/l)	14	38		51 57	119	266	97	33
PAIgG (ng/10⁷ PLT)			49.4					
RT-PCR (DEN 2 virus) IgM capture ELISA								

Figure 1. Clinical course of an imported case of dengue fever.

with an increased level of PAIgG during the acute phase of this case. These data support our hypothesis that the transient formation of PAIgG could be one of the important factors inducing thrombocytopenia during the acute phase of secondary infection. Furthermore, an *in vitro* study has reported that the dengue 2 virus binds to platelets in the presence of a virus specific antibody (Wang et al, 1995). The authors suggest that the Fc receptor is not involved in the antibody-dependent binding of the dengue 2 virus to platelets. The fact that plasma viremia emerges, peaks during the early phase and then disappears around the time of defervesence (Libraty et al, 2002), indicates the immune complexes could be formed on the platelet via the direct binding of a virus to the platelets in the present case of dengue fever.

REFERENCES

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

Guzman, M.G., Kouri, G. (2002) Dengue: an update. Lancet. Infect. Dis. 2, 33-42.

Igarashi, A. (1997) Impact of dengue virus infection and its control. FEMS. Immunol. Med. Microbiol. 18, 291-300.

Libraty, D.H., Endy, T.P., Houng, H.H., Green, S., Kalayanarooj, S., Suntayakorn, S., Chansiriwongs, W., Vaughn, D.W., Nisalak, A., Ennis, F.A., Rothman, A.L. (2002) Differing influences of virus burden and immune activation on disease severity in secondary dengue-3 virus infection. J. Infect. Dis. 185, 1213-1221.

Morita, K., Tanaka, M., Igarashi, A. (1991) Rapid identification of dengue virus serotypes by using polymerase chain reaction. J. Clin. Microbiol. 29, 2107-2110.

Nishiyama, T., Matsukawa, T., Hanaoka. K. Is protease inhibitor a choice for the treatment of pre- or mild disseminated intravascular coagulation? (2000) Crit. Care. Med. 28, 1419-422.

Oishi, K., Inoue, S., Cinco, M.T.D.D., Dimaano, E.M., Alera, M.T.P., Afron, J.A.R., Abanes, F., Cruz, D.J.M., Matias, R.R., Matsuura, H., Natividad, F.F., Nagatake, T. (2003) Correlation between increased platelet-associated IgG and thrombocytopenia in secondary dengue virus infections. J. Med. Virol. 71, 259-264.

Srichaikul, T., Nimmannitya, S. (2000) Hematology in dengue and dengue haemorrhagic fever. Bailliere's. Clinical. Haematology. 2000, 13: 261-276.

Wang, S., He, R., Patarapotikul, J,. Innis, B.L., Anderson, R. (1995) Antibody-enhanced binding of dengue-2 virus to human platelet. Virology. 213, 254-257.

World Health Organization. (1997) Dengue haemorrhagic fever: diagnosis, treatment, prevention and control. 2 nd ed. Geneva.