

# 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<sup>1)</sup>, SHINGO INOUE<sup>2)</sup>, TSUYOSHI KURAMOTO<sup>1)</sup>, SHOZABURO ONIZUKA<sup>1)</sup>, MARIKO SAITO<sup>1)</sup>, FUTOSHI HASEBE<sup>2)</sup>, KOUICHI MORITA<sup>2)</sup>, and TSUYOSHI NAGATAKE<sup>1)</sup>

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 of these findings, we propose 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 °C) 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

<sup>1)</sup> Department of Internal Medicine and <sup>2)</sup> 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°C. 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 ( $49 \times 10^3/\mu\text{l}$ ). A small amount of epistaxis was noted on September 9 th and 11th. 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 ( $49.4 \text{ ng}/10^7$  platelets; the normal range is below  $20 \text{ ng}/10^7$  platelets) was found on September 10 th when the peripheral platelet count was the lowest. The levels of fibrin degradation product and fibrinogen were  $2.3 \mu\text{g}/\text{ml}$  and  $256 \text{ mg}/\text{dl}$ , 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  $63 \times 10^3/\mu\text{l}$ ) and on October 23 th, 2001 (convalescent phase; platelet count is  $241 \times 10^3/\mu\text{l}$ ). We also employed platelet samples from three healthy volunteers (platelet count ranges from  $195$  to  $303 \times 10^3/\mu\text{l}$ ). 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  $1.5 \times 10^9$  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  $0.12 \pm 0.07$ . In contrast, an increased activity of anti-dengue virus 2 IgG ( $0.98 \pm 0.06$ ) in the eluate at the acute phase of the presented case was found. A decreased activity of anti-dengue 2 IgG ( $0.19 \pm 0.01$ ) 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

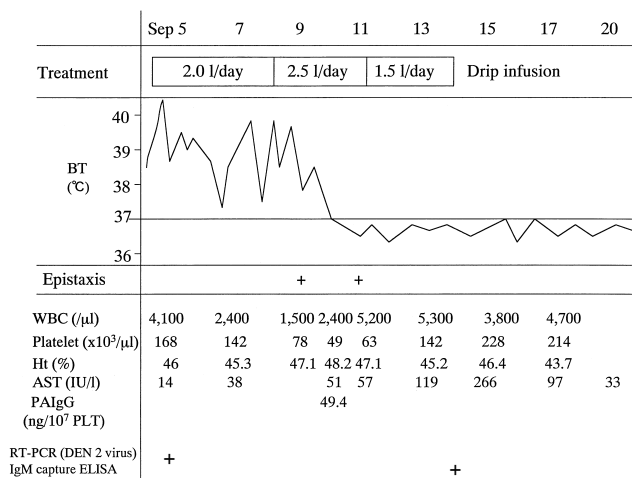


Figure 1. Clinical course of an imported case of dengue fever.

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 defervescence (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

- 1) 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.
- 2) Guzman, M.G., Kouri, G. (2002) Dengue: an update. *Lancet. Infect. Dis.* 2, 33-42.
- 3) Igarashi, A. (1997) Impact of dengue virus infection and its control. *FEMS. Immunol. Med. Microbiol.* 18, 291-300.
- 4) Libraty, D.H., Endy, T.P., Houn, H.H., Green, S., Kalayanaraj, 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.
- 5) Morita, K., Tanaka, M., Igarashi, A. (1991) Rapid identification of dengue virus serotypes by using polymerase chain reaction. *J. Clin. Microbiol.* 29, 2107-2110.

- 6) 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.
- 7) 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.
- 8) Srichaikul, T., Nimmannitya, S. (2000) Hematology in dengue and dengue haemorrhagic fever. *Bailliere's. Clinical. Haematology.* 2000, 13: 261-276.
- 9) 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.
- 10) World Health Organization. (1997) *Dengue haemorrhagic fever: diagnosis, treatment, prevention and control.* 2 nd ed. Geneva.