8 Gum PA, Kottke-Marchant K, Welsh PA, White J, Topol EJ. A prospective, blinded determination of the natural history of aspirin resistance among stable patients with cardiovascular disease. *J Am Coll Cardiol* 2003; **41**: 961–5.

9 Born GV. Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature* 1962; **194**: 927–9.

10 Ozaki Y, Satoh K, Yatomi Y, Yamamoto T, Shirasawa Y, Kume S. Detection of platelet aggregates with a particle counting method using light scattering. *Anal Biochem* 1994; **218**: 284–94.

11 Sakamoto T, Ogawa H, Kawano H, Hirai N, Miyamoto S, Takazoe K, Soejima H, Kugiyama K, Yoshimura M, Yasue H. Rapid change of platelet aggregability in acute hyperglycemia. Detection by a novel laser-light scattering method. *Thromb Haemost* 2000; **83**: 475–9.

12 Polgar J, Clemetson JM, Kehrel BE, Wiedemann M, Magnenat EM, Wells TN, Clemetson KJ. Platelet activation and signal transduction by convulxin, a C-type lectin from *Crotalus durissus terrificus* (tropical rattlesnake) venom via the p62/GPVI collagen receptor. *J Biol Chem* 1997; **272**: 13576–83.

13 Morton LF, Hargreaves PG, Farndale RW, Young RD, Barnes MJ. Integrin alpha 2 beta 1-independent activation of platelets by simple collagen-like peptides: collagen tertiary and quaternary structures are sufficient alone for alpha 2 beta 1-independent platelet reactivity. *Biochem J* 1995; **306** (Pt 2): 337–44.

14 Granstrom E, Kindahl H. Radioimmunoassay of prostaglandins and thromboxanes. *Adv Prostaglandin Thromboxane Res* 1978; **5**: 119–210.

15 Catella F, Healy D, Lawson JA, FitzGerald GA. 11-Dehydrothromboxane B2: a quantitative index of thromboxane A2 formation in the human circulation. *Proc Natl Acad Sci U S A* 1986; **83**: 5861–5.

16 Kawasaki T, Ozeki Y, Igawa T, Kambayashi J. Increased platelet sensitivity to collagen in individuals resistant to low-dose aspirin. *Stroke* 2000; **31**: 591–5.

17 Weber AA, Zimmermann KC, Meyer-Kirchrath J, Schror K. Cyclooxygenase-2 in human platelets as a possible factor in aspirin resistance. *Lancet* 1999; **353**: 900.

18 Zimmermann N, Wenk A, Kim U, Kienzle P, Weber AA, Gams E, Schror K, Hohlfeld T. Functional and biochemical evaluation of platelet aspirin resistance after coronary artery bypass surgery. *Circulation* 2003; **108**: 542–7.

19 Halushka MK, Walker LP, Halushka PV. Genetic variation in cyclooxygenase 1: effects on response to aspirin. *Clin Pharmacol Ther* 2003; **73**: 122–30.

20 Rocca B, Secchiero P, Ciabattoni G, Ranelletti FO, Catani L, Guidotti L, Melloni E, Maggiano N, Zauli G, Patrono C. Cyclooxygenase-2 expression is induced during human megakaryopoiesis and characterizes newly formed platelets. *Proc Natl Acad Sci USA* 2002; **99**: 7634–9.

21 Cipollone F, Ciabattoni G, Patrignani P, Pasquale M, Di Gregorio D, Bucciarelli T, Davi G, Cuccurullo F, Patrono C. Oxidant stress and aspirin-insensitive thromboxane biosynthesis in severe unstable angina. *Circulation* 2000; **102**: 1007–13.

22 Payne DA, Jones CI, Hayes PD, Webster SE, Ross Naylor A, Goodall AH. Platelet inhibition by aspirin is diminished in patients during carotid surgery: a form of transient aspirin resistance? *Thromb Haemost* 2004; **92**: 89–96.

23 Halushka MK, Halushka PV. Why are some individuals resistant to the cardioprotective effects of aspirin? Could it be thromboxane A2? *Circulation* 2002; **105**: 1620–2.

24 Cipollone F, Toniato E, Martinotti S, Fazia M, Iezzi A, Cuccurullo C, Pini B, Ursi S, Vitullo G, Averna M, Arca M, Montali A, Campagna F, Ucchino S, Spigonardo F, Taddei S, Virdis A, Ciabattoni G, Notarbartolo A, Cuccurullo F, Mezzetti A. A polymorphism in the cyclooxygenase 2 gene as an inherited protective factor against myocardial infarction and stroke. *JAMA* 2004; **291**: 2221–8.

25 Chirkov YY, Chirkova LP, Sage RE, Horowitz JD. Impaired responsiveness of platelets from patients with stable angina pectoris to antiaggregating and cyclicAMP-elevating effects of prostaglandin E1. *J Cardiovasc Pharmacol* 1995; **25**: 961–6.

26 Chirkov YY, Holmes AS, Martelli JD, Horowitz JD. Effect of perindopril on platelet nitric oxide resistance in patients with chronic heart failure secondary to ischemic left ventricular dysfunction. A10. *Am J Cardiol* 2004; **93**: 1438–40.

27 Fukuda K, Ozaki Y, Satoh K, Kume S, Tawata M, Onaya T, Sakurada K, Seto M, Sasaki Y. Phosphorylation of myosin light chain in resting platelets from NIDDM patients is enhanced: correlation with spontaneous aggregation. *Diabetes* 1997; **46**: 488–93.

28 Fontana P, Dupont A, Gandrille S, Bachelot-Loza C, Reny JL, Aiach M, Gaussem P. Adenosine diphosphate-induced platelet aggregation is associated with P2Y12 gene sequence variations in healthy subjects. *Circulation* 2003; **108**: 989–95.

29 Payne DA, Jones CI, Hayes PD, Thompson MM, London NJ, Bell PR, Goodall AH, Naylor AR. Beneficial effects of clopidogrel combined with aspirin in reducing cerebral emboli in patients undergoing carotid endarterectomy. *Circulation* 2004; **109**: 1476–81.

30 Sabatine MS, Cannon CP, Gibson CM, Lopez-Sendon JL, Montalescot G, Theroux P, Claeys MJ, Cools F, Hill KA, Skene AM, McCabe CH, Braunwald E. Addition of clopidogrel to aspirin and fibrinolytic therapy for myocardial infarction with ST-segment elevation. *N Engl J Med* 2005; **352**: 1179–89.

31 Hashimoto Y, Naito C, Kume S, Kato H, Watanabe T, Kawamura M, Teramoto T, Oka H. High concentrations of arachidonic acid induce platelet aggregation and serotonin release independent of prostaglandin endoperoxides and thromboxane A2. *Biochim Biophys Acta* 1985; **841**: 283–91.

© 2006 International Society on Thrombosis and Haemostasis

Severe secondary deficiency of von Willebrand factor–cleaving protease (ADAMTS13) in patients with sepsis-induced disseminated intravascular coagulation: its correlation with development of renal failure

Tomoko Ono, Jun Mimuro, Seiji Madoiwa, Kenji Soejima, Yuji Kashiwakura, Akira Ishiwata, Katsuhiro Takano, Tsukasa Ohmori, and Yoichi Sakata

Deficiency of ADAMTS13 is found in patients with thrombotic thrombocytopenic purpura (TTP), and the genetic defects in the *ADAMTS13* gene or the autoantibody against ADAMTS13 is thought to be responsible for the development of TTP. The clinical correlation and mechanisms of secondary ADAMTS13 deficiency in other disease states were investigated. In addition to TTP, ADAMTS13 levels were severely decreased in patients with sepsis-induced disseminated intravascular coagulation (DIC). The incidence of acute renal failure and serum creatinine levels in patients with ADAMTS13 activity levels lower than 20% (incidence, 41.2%; creatinine, 160 ± 150 μM [1.81 ± 1.70 mg/dL]) (*P* < .05) were significantly higher than they were in patients with ADAMTS13 activity levels higher than 20% (incidence, 15.4%; creatinine, 84 ± 67 μM [0.95 ± 0.76 mg/dL]) (*P* < .01). Additionally, unusually large von Willebrand factor multimers were detected in 26 (51.0%) of 51 patients with ADAMTS13 activity levels lower than 20%. Lower molecular weight forms of ADAMTS13 were found in the plasma of patients with sepsis-induced DIC, suggesting that the deficiency of ADAMTS13 was partially caused by its cleavage by proteases in addition to decreased synthesis in the liver. These data suggested that severe secondary ADAMTS13 deficiency can be associated with sepsis-induced DIC and may contribute to the development of renal failure. (Blood. 2006;107:528-534)

© 2006 by The American Society of Hematology

Introduction

Deficiency of the von Willebrand factor (VWF)–cleaving protease,[1-5] ADAMTS13 (a disintegrin-like metalloprotease with thrombospondin type 1 repeats) is found in most patients with thrombotic thrombocytopenic purpura (TTP), and this deficiency is thought to be responsible for platelet aggregation and microthrombi formation in the circulation, which in turn cause typical thrombotic microangiopathies (TMAs) to develop.[6-9] Deficiency of ADAMTS13 in patients with TTP is caused by genetic defects in the *ADAMTS13* gene (familial TTP, Upshaw-Schulman syndrome) or by autoantibodies against ADAMTS13. Although hemolytic uremic syndrome (HUS) is clinically similar to TTP, the role of ADAMTS13 deficiency in the development of HUS is controversial because reports conflict about whether ADAMTS13 activity remains unchanged[6-8] or decreases.[10-13] It also is possible that secondary deficiency of ADAMTS13 may account for the development of microthrombi formation in disease states other than TTP. To search for the clinical correlation of secondary ADAMTS13 deficiency in disease states, we measured ADAMTS13 activity levels by the standard method[14] and determined antigen levels by our newly developed monoclonal antibody–based enzyme-linked immunosorbent assay (ELISA) for ADAMTS13 in patients with TTP and in patients with sepsis-induced disseminated intravascular coagulation (DIC). We found that severe secondary ADAMTS13 deficiency could occur in patients with sepsis-induced DIC and that it had a clinical correlation with the development of renal failure.

Patients, materials, and methods

Blood samples

All samples were obtained with informed consent from patients according to the Declaration of Helsinki. Blood was drawn from 113 patients (65 men, aged 17-83; 44 women, aged 21-81; idiopathic TTP, 3 patients; Upshaw-Schulman syndrome, 1 patient; sepsis-induced DIC, 109 patients). The diagnosis of TTP was made with note of the presence of typical clinical features (fever, bleeding tendency, neurologic symptoms) laboratory examination results (thrombocytopenia, hemolytic anemia with red blood cell fragmentation, increased levels of LDH, increased levels of serum creatinine), and effectiveness of plasma exchange treatment. Patients with definite infection, such as bacteremia, pneumonia, urinary tract infection, biliary tract infection, or pathogenic *Escherichia coli* O-157 infection, were excluded from the TTP group. The patient with Upshaw-Schulman syndrome had TTP, and plasma transfusion was effective in preventing recurrence.

The diagnosis of DIC was made according to the criteria established in 1988 by the Japanese Ministry of Health and Welfare. Criteria for DIC were

From the Research Division of Cell and Molecular Medicine, Center for Molecular Medicine, Jichi Medical School, Tochigi-ken, Japan; the Research and Development Department, Mitsubishi Kagaku Iatron, Chiba-ken, Japan; and the Chemo-Sero Therapeutic Research Institute, Kumamoto-ken, Japan.

Submitted March 17, 2005; accepted September 1, 2005. Prepublished online as *Blood* First Edition Paper, September 27, 2005; DOI 10.1182/blood-2005-03-1087.

Supported by Grants-in-Aid for Scientific Research from the Japanese Ministry of Education and Science; Health and Labor Science Research Grants for Research from the Japanese Ministry of Health, Labor, and Welfare; and Grants for "High-Tech Center Research" Projects for Private Universities: matching fund subsidy from MEXT (Japanese Ministry of Education, Culture, Sports, Science, and Technology), 2002-2006.

Reprints: Jun Mimuro, Research Division of Cell and Molecular Medicine, Center for Molecular Medicine, Jichi Medical School, 3311-1 Yakushiji, Tochigi-ken 329-0498, Japan; e-mail: mimuro-j@jichi.ac.jp.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

© 2006 by The American Society of Hematology

reported previously.[15] Briefly, the presence of underlying disease—such as infection and malignancies—specific clinical conditions (bleeding symptoms, organ dysfunction), and results of laboratory examinations (platelet counts, prothrombin time, fibrinogen, fibrin degradation products) were quantified based on score. If the score was 7 or more, the diagnosis of DIC was made. In patients with hematologic malignancy, scores on the bleeding symptom and platelet counts were excluded, and the diagnosis of DIC was made if the total score was 4 or more.

The diagnosis of sepsis was made according to the guidelines of the Society of Critical Care Medicine Consensus Conference Committee.[16] Briefly, patients had to meet at least 3 of the 4 criteria for systemic inflammatory response and had to have a known infection or a suspected infection, as evidenced by one or more of the following: bacteremia, pathologic microorganisms or white blood cells in a normally sterile body fluid such as urine or joint fluid; purulent sputum; radiographic evidence of pneumonia; clinical signs associated with high risk for infection (eg, cholangitis, peritonitis) or increased levels of endotoxin, β-D-glucan, or *Candida* antigen.

Thirty-nine patients with DIC were shown to have bacteremia, as evidenced by their blood cultures. Twelve patients, whose bacteremia was not evidenced by blood culture, had increased levels of endotoxin, β-D-glucan, or *Candida* antigen. Twenty-eight patients who were negative for bacteria in blood culture or who did not have increased levels of endotoxin, β-D-glucan, or *Candida* antigen, had pneumonia as evidenced by radiography, 11 patients had urinary tract infection, 4 patients had wound infection during postoperative periods, 1 patient had biliary tract infection, 1 patient had bacterial arthritis, 1 patient had bacterial osteomyelitis, and 12 patients had suspected respiratory infection with the presence of pathogenic microorganisms, such as methicillin-resistant *Staphylococcus aureus* in sputum cultures.

Citrated platelet-poor plasma samples were prepared and stored at −80°C until use. Blood was also drawn from 12 healthy volunteers (7 men, aged 25-53; 5 women, aged 25-48) for the preparation of normal pooled plasma. Laboratory analyses of patients' blood were performed by the standard methods using automated analyzers. Complete blood cell counts, serum creatinine (normal range, 35-97 μM [0.4-1.1 mg/dL]), serum bilirubin (normal range, 3-21 μM [0.2-1.2 mg/dL]), aspartate aminotransferase (AST; normal range, 8-35 IU/L), alanine aminotransferase (ALT; normal range, 5-40 IU/L), serum albumin (normal range, 39-51 g/L [3.9-5.1 g/dL]), and C-reactive protein (CRP; normal range, less than 5 mg/L [0.5 mg/dL]) were measured in this study.

Determination of ADAMTS13 antigen and activity levels

The human *ADAMTS13* cDNA used in this study was described previously.[5] Human ADAMTS13 was expressed in human embryo kidney 293 cells stably transfected with pCAG-ADAMTS13 Neo and was purified. Murine monoclonal antibodies (mAbs) to human ADAMTS13 were generated by the standard method[17] after immunization of BALB/c mice with recombinant human ADAMTS13. Two mAbs, WH10 and WH2-22-1A, were selected for ELISA, which was shown to bind to the third TSP-1 motif and to the disintegrin domain of ADAMTS13 by the binding study to recombinant ADAMTS13 mutants, respectively.[5,14,18] WH10 (2 μg/mL) was used for microtiter plate coating (Maxi Sorp plate; Nalge Nunc International, Rochester, NY). After blocking with 1% casein, plasma samples from healthy subjects and patients were diluted in phosphate-buffered saline, pH 7.2/0.1% casein, and then incubated in WH10-coated plates. ADAMTS13 bound to the microtiter plates was detected by peroxidase-conjugated WH2-22-1A. Purified recombinant ADAMTS13 was used as the standard to determine ADAMTS13 antigen levels in normal plasma. The ADAMTS13 level in each patient's plasma was expressed as the percentage of that in normal pooled plasma. ADAMTS13 activity levels in plasma were measured according to the previously described method.[14] Briefly, 10 μL plasma was mixed with purified VWF (1 μg) in 100 μL reaction buffer (5 mM Tris [pH 8.0]/1.5 M urea/10 mM $BaCl_2$/0.4 mM Pefabloc SC [Roche Diagnostics, Mannheim, Germany]) at 37 °C for 24 hours. Reaction was terminated by the addition of 10 μL of 500 mM EDTA, pH 8.0.[14] Portions of samples were subjected to 1.4% sodium dodecyl sulfate–agarose gel electrophoresis to determine the extent of VWF degradation. After electrophoresis, proteins were transferred to polyvinylidene fluoride (PVDF) membranes, and VWF multimers were detected by peroxidase-labeled rabbit anti–human VWF antibodies (Dako, Glostrup, Denmark).[12-14]

Quantification of molecular markers of DIC

Plasma levels of fibrin degradation products (FDPs) were quantified with commercial kits (Roche Diagnostics, Tokyo, Japan) used for laboratory examinations. Given that the quantification of free thrombin concentration in plasma is technically difficult, we used ELISA (Sysmex, Kobe, Japan) to quantify plasma levels of thrombin/antithrombin III (TAT) complexes. Similarly, plasma levels of plasmin/α2 plasmin inhibitor complexes (PICs) were measured using ELISA with commercial kits (Sysmex) used for laboratory examinations. Plasma plasminogen activator inhibitor 1 (PAI-1) levels were quantified by the latex photometric immunoassay by using a commercial kit (Mitsubishi Kagaku Iatron, Tokyo, Japan), as described previously.[19] The granulocyte elastase digests of cross-linked fibrin (granulocyte elastase–dependent fibrin degradation products [E-XDPs]) were measured by the automated latex photometric immunoassay using IF-123 monoclonal antibody, which is specific for the fibrin fragment D species generated by granulocyte-elastase digestion.[20] Monoclonal antibody IF-123–bound latex particles (Mitsubishi Kagaku Iatron) were used for the assay. A 2.4-μL aliquot of sample plasma was mixed with 32 μL latex reagents in 250 μL Tris-buffered saline, and then absorbance changes were analyzed with an automated analyzer for latex photometric immunoassay (model LPIA-NV7; Mitsubishi Kagaku Iatron). The standard E-XDP was purified according to the method of Kohno et al.[20] The normal range of plasma E-XDP levels is less than 3 U/mL.

Effect of granulocyte elastase on ADAMTS13

Recombinant ADAMTS13 (250 nM) was incubated in 20 μL Tris-buffered saline, pH 7.4, in the absence or presence of granulocyte elastase (Elastin Products, Owensville, MO) at 5 nM and 50 nM. Aliquots (5 μL each) were harvested after incubation at 37°C for 5, 15, and 30 minutes. The reaction of each aliquot was terminated by addition of the sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer containing 2% SDS. Samples were then analyzed by SDS-PAGE followed by Western blotting with anti-ADAMTS13 monoclonal antibody WH2-22-1A and peroxidase-labeled anti–mouse IgG.

Detection of ADAMTS13 molecular forms in plasma

Western blot analysis of ADAMTS13 in plasma by mAb WH2-22-1A was performed after immunoprecipitation with anti-ADAMTS13 polyclonal antibody immobilized to protein G-Sepharose.

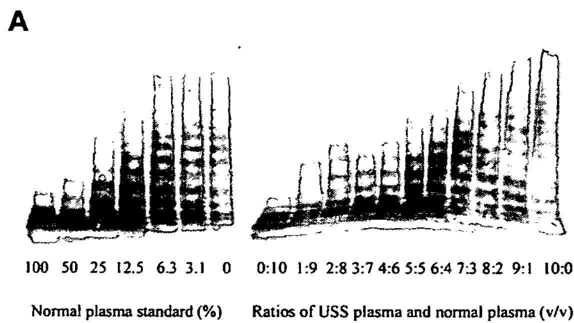
Analysis of VWF multimers in patient plasma

VWF multimers in patient plasma were analyzed by SDS-agarose gel electrophoresis according to the method described previously.[12-14]

Results

ELISA for ADAMTS13

We generated mAbs against recombinant human ADAMTS13 and used them to develop an mAb-based ADAMTS13 ELISA. To determine the specificity of this assay, plasma obtained from a patient with Upshaw-Schulman syndrome was mixed with normal plasma at various ratios, and the ADAMTS13 activity and antigen levels were measured. As shown in Figure 1, ADAMTS13 activity and ADAMTS13 antigen levels in the plasma of the patient with Upshaw-Schulman syndrome were less than 1%, and the ADAMTS13 antigen level in the patient plasma increased linearly in parallel with the ADAMTS13 activity in the presence of increasing amounts of normal plasma. The correlation coefficient



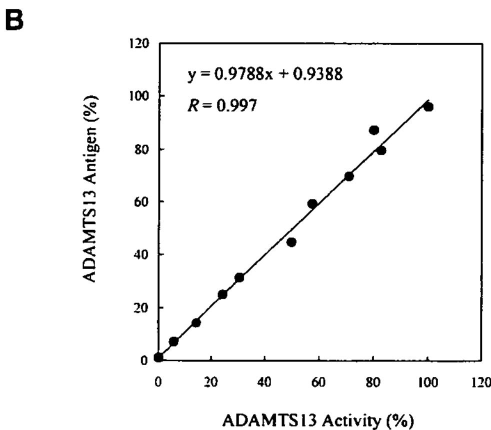


Figure 1. Analysis of ADAMTS13 activity and antigen levels in plasma of patients with Upshaw-Schulman syndrome. ADAMTS13 activity and antigen levels were determined in the plasma of a patient with Upshaw-Schulman syndrome (USS) mixed with normal pooled plasma at various ratios. (A) Result of ADAMTS13 activities in the plasma of the USS patient mixed with normal plasma at various ratios (0:10-10:0). (B) Correlation of ADAMTS13 activity and antigen levels in these samples.

between ADAMTS13 antigen and ADAMTS13 activity was 0.997. The ADAMTS13 level in normal pooled plasma was 1.57 μg/mL when recombinant human ADAMTS13 was used as the standard. The calibration curve was linear ($r = 0.999$), and the ELISA could distinguish absorbance changes of ADAMTS13 at 0.3% of the normal plasma level from ADAMTS13-depleted plasma. Interassay variability in samples containing 50% and 100% of ADAMTS13 were 7.9% and 5.2%, respectively.

ADAMTS13 levels in disease states

ADAMTS13 antigen and activity levels in the plasma of patients with sepsis-induced DIC or TTP were studied (Figure 2). The correlation coefficient of ADAMTS13 antigen and ADAMTS13 activity was 0.80. As shown in Figure 2A, discrepancies between ADAMTS13 antigen levels and activity levels were observed in many samples. These discrepancies mainly were caused by the decreased level of specific ADAMTS13 activity compared with the ADAMTS13 antigen level. Some samples had higher specific activity of ADAMTS13. To explore the possibility that decreased levels of the ADAMTS13-specific activity correlated with disease states, Western blot analysis of ADAMTS13 molecular forms in patient plasma was performed. Low molecular–weight ADAMTS13 species were observed in DIC patient plasma by Western blotting (Figure 2B), indicating that proteolytic cleavage of ADAMTS13 could occur in this disease state. The recent report showed that ADAMTS13 could be digested in vitro by proteases such as thrombin and plasmin.[21] Because thrombin and plasmin can be generated in DICs, we tested the correlation between ADAMTS13 levels and molecular markers of coagulation and fibrinolysis. There was no correlation of ADAMTS13 activity, antigen, or specific activity level with levels of fibrinogen, FDP, TAT, PIC, PAI-1, or platelet counts (Table 1). We could only find a negative correlation between activity levels and antigen levels of ADAMTS13 and plasma levels of granulocyte elastase digests of fibrin (E-XDP) (Table 1; Figure 3A-B). Based on these results, we studied the effects of granulocyte elastase on ADAMTS13 in vitro. In accordance with previous reports, recombinant ADAMTS13 was determined to migrate at approximately 190 kDa by SDS-PAGE, followed by Western blotting.[14,21] As shown in Figure 3C, recombinant ADAMTS13 migrating at approximately 190 kDa was converted to the 120-kDa and 100-kDA fragments and finally to the approximately 40-kDa fragment on incubation with granulocyte

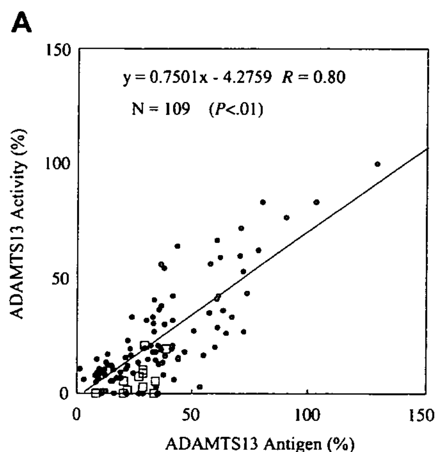
Table 1. Correlation between the ADAMTS13 levels and molecular markers of DIC in patients with sepsis-induced DIC

	ADAMTS13*		
	Activity	Antigen	Activity-antigen ratio
Fibrinogen	−0.347	−0.244	−0.219
FDP	0.354	0.242	0.274
TAT	0.246	0.379	0.367
PIC	0.370	0.357	0.327
PAI-1	−0.230	−0.300	−0.006
Platelet	0.260	0.245	0.239
E-XDP	−0.399†	−0.404†	−0.229

n = 109 patients.

*Values are *rs* determined by Spearman rank correlation test.

†Statistically significant ($P < .01$).



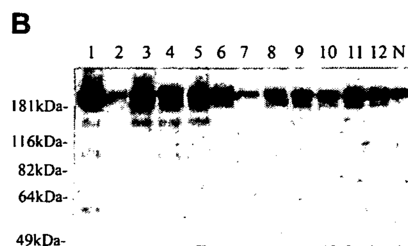


Figure 2. Analysis of ADAMTS13 activity, antigen, and molecular forms in plasma of patients with sepsis-induced DIC. (A) ADAMTS13 activity and antigen levels in the plasma of patients with sepsis-induced DIC were determined as described in "Patients, materials and methods." Samples (□) were subjected to immunoprecipitation followed by Western blotting to investigate the cleavage of ADAMTS13, as described in "Patients, materials, and methods." (B) Typical Western blot of degraded ADAMTS13 found in the patients' plasma indicated in panel A (□) is shown. Western blotting of ADAMTS13 antigen in normal pooled plasma (N) is shown as the control. ADAMTS13 molecules in normal plasma migrated at approximately 190 kDa.

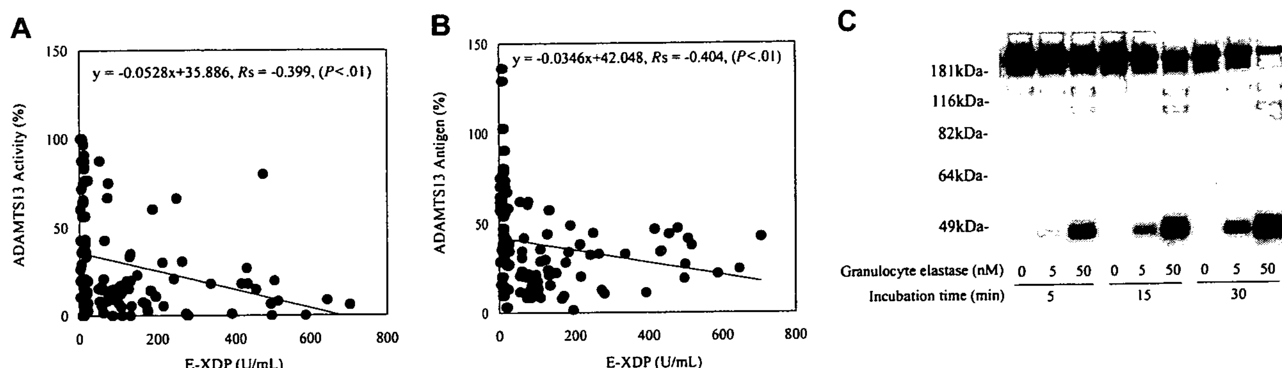


Figure 3. Correlation between the ADAMTS13 levels and the granulocyte elastase digests of cross-linked fibrin (E-XDP) levels in plasma of patients with sepsis-induced DIC and the effect of granulocyte elastase on ADAMTS13 in vitro. Correlations between the activity levels of ADAMTS13 and the plasma levels of granulocyte-elastase digests of fibrin (E-XDP) (A) and between the antigen levels of ADAMTS13 and the plasma levels of granulocyte-elastase digests of fibrin (E-XDP) (B) in patients with sepsis-induced DIC are shown. Values were analyzed by Spearman correlation coefficient by rank test. Recombinant ADAMTS13 was incubated with granulocyte elastase at 5 nM or 50 nM, and degradation of ADAMTS13 by granulocyte elastase was studied after the indicated time and analyzed as described in "Patients, materials, and methods" (C).

elastase in a dose-dependent and a time-dependent manner in vitro. A variety of lower molecular–weight ADAMTS13 fragments were detected in DIC patient plasma by Western blot (Figure 2B). According to the previous report, the ADAMTS13 fragments migrating approximately 150 to 170 kDa could be generated by thrombin.[21] ADAMTS13 fragments migrating approximately 120 kDa and 100 kDa in patient plasma might correspond to the granulocyte elastase digests of ADAMTS13. However, the 120-kDa ADAMTS13 fragment and the 100-kDa ADAMTS13 fragment could be generated by thrombin and plasmin, respectively.[21] It also is possible that thrombin-cleaved ADAMTS13 or plasmin-cleaved ADAMTS13 could be digested by granulocyte elastase or vice versa. These data may suggest that granulocyte elastase, together with other proteases (thrombin and plasmin plays a role in ADAMTS13 cleavage under certain pathologic conditions), may partially account for the decrease of the ADAMTS13-specific activity observed in DIC patients.

ADAMTS13 deficiency in disease states

ADAMTS13 antigen and activity levels in patient groups and in healthy subjects are shown in Figure 4. The plasma ADAMTS13 antigen and activity levels in untreated patients with TTP (no plasma exchange treatment, no fresh frozen plasma transfusion) were 13.5% ± 7.1% (range, 5.1%-19.6%) and 6.3% ± 5.7% (range, 0%-12.5%), respectively (idiopathic TTP 3, Upshaw-Schulman syndrome 1). Decreased levels of ADAMTS13 antigen and activity were observed in patients with sepsis-induced DIC compared with healthy subjects ($P < .01$) in this study, and severe decreases of ADAMTS13 activity and antigen levels were observed in patients with sepsis-induced DIC. Of the 109 patients with sepsis-induced DIC, decreases in ADAMTS13 activity levels (less than 5%) were found in 17 (15.6%) patients; clinical features and laboratory data of these patients are summarized in Table 2. Consciousness disturbance, thrombocytopenia, decreased hemoglobin levels, and increased LDH levels were commonly found in these patients. Clinical features were indistinguishable from those of patients with TTP, though patients with sepsis-induced DIC had evidence of the infection. Given that the highest ADAMTS13 activity level in patients with TTP without plasma exchange or blood transfusion was 12.5%, patients with sepsis-induced DIC were divided into 2 groups. One included patients with decreased ADAMTS13 activity levels (less than 20%; n = 51), and the other included patients with ADAMTS13 activity levels greater than 20% (n = 52). Patients with chronic renal failure before infection were excluded from this analysis. Patients were in severe condition; 25 (49.0%) of 51 patients in the former group and 35 (67.3%) of 52 patients of the latter group received transfusions of fresh frozen plasma, platelet concentrates, or both within 5 days of the determination of ADAMTS13 levels. This might have affected the activity and antigen levels of ADAMTS13.

Correlation between secondary ADAMTS13 deficiency and organ failure

Analyses of clinical and laboratory data showed that the patients with severe ADAMTS13 deficiency (ADAMTS13 activity less than 20%) had elevated serum creatinine levels (Figure 5) that were

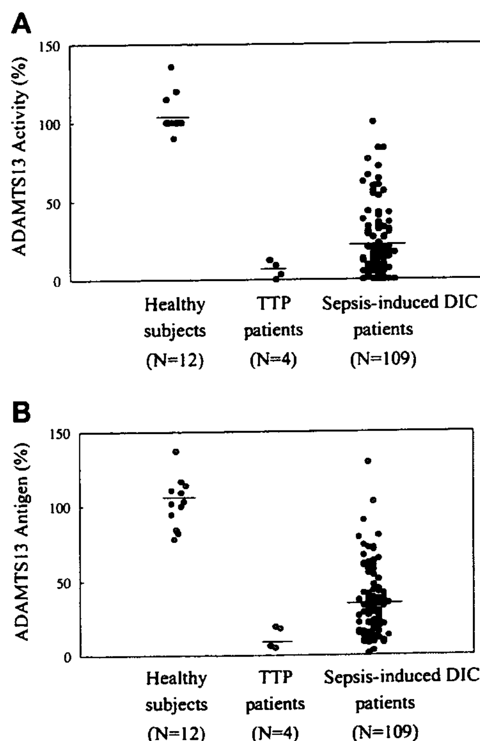


Figure 4. Plasma ADAMTS13 levels in patients and healthy subjects. ADAMTS13 activity levels (A) and antigen levels (B) of healthy subjects, patients with TTP (idiopathic TTP, 3; Upshaw-Schulman syndrome, 1) before plasma exchange treatment, and patients with sepsis-induced DIC (n = 109) are shown. Differences in the mean values (horizontal lines) between the healthy subject group and patient groups were statistically significant (nonrepeated measures ANOVA and Dunnett test; $P < .01$).

Table 2. Clinical profiles of patients with sepsis-induced DIC whose ADAMTS13 activity levels were lower than 5%

Characteristic	Value
Age, y	56.9 ± 21.3
Consciousness disturbance, no. (%)	8 (47.1)
Blood transfusion, no. (%)	11 (64.7)
ADAMTS13 antigen, %	25.5 ± 13.6
Creatinine, mg/dL	1.88 ± 2.06
Albumin, g/dL	2.2 ± 0.5
WBC count, cells/μL	11 200 ± 7 500
RBC count, × 10⁴/μL	260 ± 86
Hemoglobin, g/dL	8.3 ± 2.0
Platelet count, × 10⁴/μL	6.7 ± 5.3
LDH, IU/L	2481.3 ± 4107.8
CRP, mg/dL	18.11 ± 13.41

n = 17 patients.

Values for all categories except consciousness disturbance and blood transfusion are mean ± SD.

To convert creatinine from milligrams per deciliter to micromoles per liter, multiply milligrams per deciliter by 88.4.

To convert albumin from grams per deciliter to grams per liter, multiply grams per deciliter by 10.

To convert WBC count from cells per microliter to $\times 10^9$ cells per liter, divide cells per microliter by 1000.

To convert RBC count from $\times 10^4$ cells per microliter to $\times 10^{12}$ cells per liter, divide $\times 10^4$ cells per microliter by 100.

To convert hemoglobin from grams per deciliter to grams per liter, multiply grams per deciliter by 10.

To convert platelet count from $\times 10^4$ platelets per microliter to $\times 10^9$ per liter, multiply $\times 10^4$ platelets per microliter by 10.

To convert CRP from milligrams per deciliter to milligrams per liter, multiply milligrams per deciliter by 10.

significantly higher than those in patients with ADAMTS13 levels higher than 20% (Table 3). The incidence of renal injuries in patients with severe ADAMTS13 deficiency (ADAMTS13 activity less than 20%) was significantly higher than in patients with ADAMTS13 activity levels higher than 20% (Table 3). However, there were no differences in the incidence of liver dysfunction or serum levels of bilirubin, AST, and ALT among these groups (Table 3), suggesting that severe ADAMTS13 deficiency in these patients may be linked to the development of renal injuries. There was a significant difference in serum albumin levels between both groups, suggesting that the decrease of ADAMTS13 activity and antigen levels in patients was at least partially caused by reduced synthesis in the liver.

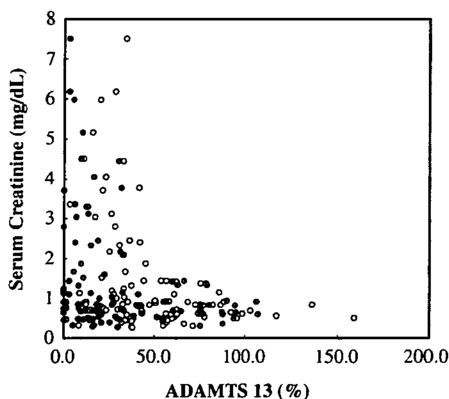


Figure 5. Correlation between the plasma ADAMTS13 levels and the serum creatinine levels. Correlation between serum creatinine levels and ADAMTS13 activity (●) levels or antigen (○) levels in patients with sepsis-induced DIC is shown (n = 103). Patients with a history of chronic renal failure were excluded from the study.

Table 3. Correlation between ADAMTS13 levels and organ injury in patients with sepsis-induced DIC

	ADAMTS13 activity less than 20%, n = 51	ADAMTS13 activity greater than 20%, n = 52	P
Creatinine, mg/dL	1.81 ± 1.70	0.95 ± 0.76	< .01*
AST, IU/L	106 ± 128	182 ± 290	NS
ALT, IU/L	72 ± 109	122 ± 160	NS
Bilirubin, mg/dL	2.70 ± 3.13	2.20 ± 2.53	NS
Albumin, g/dL	2.3 ± 0.4	2.9 ± 0.7	< .05*
CRP, mg/dL	13.50 ± 10.51	6.90 ± 8.61	< .01*
Organ injury, no. (%)			
Renal injury	21 (41.2)	8 (15.4)	< .05†
Liver injury	40 (78.4)	38 (73.1)	NS

Values for all categories except organ injury are mean ± SD. Renal injury: serum creatinine greater than 1.2 mg/dL.

Liver injury: elevation of bilirubin (> 2.0 mg/dL), AST (> 40 IU/L), or ALT (> 40 IU/L).

To convert creatinine from milligrams per deciliter to micromoles per liter, multiply milligrams per deciliter by 88.4.

To convert bilirubin from milligrams per deciliter to micromoles per liter, multiply milligrams per deciliter by 17.1.

To convert albumin from grams per deciliter to grams per liter, multiply grams per deciliter by 10.

To convert CRP from milligrams per deciliter to milligrams per liter, multiply milligrams per deciliter by 10.

NS indicates not significant.

*Statistically significant (Welch *t* test).

†Statistically significant (Fisher exact probability test).

Analysis of VWF multimers in patients with severe secondary ADAMTS13 deficiency

Additionally, unusually large VWF multimers were detected in the plasma of patients with severe secondary ADAMTS13 deficiency (ADAMTS13 activity less than 20%), as shown in Figure 6. Serum creatinine levels in patients in whom unusually large VWF multimers and severe ADAMTS13 deficiency were detected were significantly higher than in patients in whom the unusually large VWF multimers were absent (Table 4). There was no significant difference in ADAMTS13 activity (Table 4) and ADAMTS13-specific activity (activity-antigen ratio) between these patient groups (not shown).

There was a significant difference in CRP levels between the ADAMTS13 activity less than 20% group and the ADMATS13 activity greater than 20% group, but their platelet counts were not significantly different (not shown), indicating that the decrease in ADAMTS13 may be related to inflammatory responses. These results are consistent with the data showing a negative correlation between the activity and antigen levels of ADAMTS13 and the plasma levels of granulocyte elastase digests of fibrin (E-XDP).

Discussion

ADAMTS13 has been shown to play an important role in VWF processing.[1-14,22,23] As shown previously, ADAMTS13 may cleave the unusually large multimers of VWF on the endothelial cell surface, preventing entrance of such unusually large multimers into the circulation.[8,24] Without this processing of VWF multimers, the unusually large multimers of VWF secreted from endothelial cells would enter the circulation and initiate platelet thrombus formation, which in turn would cause the development of TMA.[8,24] Patients with primary ADAMTS13 deficiency caused by defects in the *ADAMTS13* gene or with autoantibodies against ADAMTS13 have been shown to develop TTP, suggesting the important physiologic role of ADAMTS13-catalyzed cleavage of these

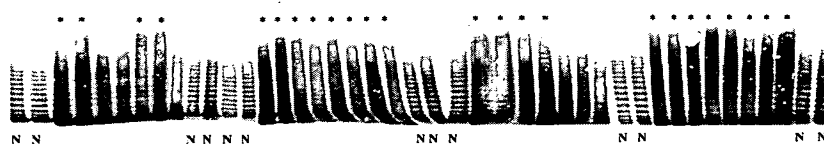


Figure 6. Analysis of VWF multimers of patients with sepsis-induced DIC. VWF multimers in the plasma of patients with sepsis-induced DIC with ADAMTS13 activity levels lower than 20% were analyzed by SDS-agarose gel electrophoresis, as described in "Patients, materials, and methods." VWF multimer patterns of patients and healthy subjects (N) were analyzed simultaneously. *Representative unusually large multimers of VWF found in the plasma of patients with ADAMTS13 activity levels lower than 20%.

unusually large VWF multimers in humans. TTP is a fatal thrombotic microangiopathic disease if patients are not treated appropriately, but the incidence of TTP is low.[8,22] While searching for the role of ADAMTS13 in common thromboembolic diseases, we found severe secondary ADAMTS13 deficiency in patients with sepsis-induced DIC and showed its clinical correlation to the development of renal failure in this study.

DIC is associated with a variety of disease states such as sepsis, advanced malignancy, severe tissue damage, and pregnancy-related complications. Sepsis may be the most common pathogenic disease that leads to the development of DIC, and the endotoxemia and high cytokine levels in the circulation are thought to induce tissue factor expression that in turn initiates fibrin thrombus formation in the circulation. Microthrombi formed in the circulation cause ischemia of and damage to a variety of organs. Lines of evidence have suggested that proteases released from white blood cells may also be involved in the development of organ injuries. This study showed that patients with sepsis-induced DIC frequently exhibited decreased antigen and activity levels of ADAMTS13 and that severe ADAMTS13 deficiency was found in these patients at high incidence. Many patients in this study had undergone transfusion with ADAMTS13-containing blood products, such as fresh frozen plasma and platelet concentrates, soon before blood sample collection for the determination of ADAMTS13 levels, suggesting that the levels of ADAMTS13 in the plasma samples of these patients might not reflect the severity of ADAMTS13 deficiency before blood transfusion. Thus, severe secondary ADAMTS13 deficiency in sepsis-induced DIC might be more common. Clinical manifestations and laboratory data of these patients with sepsis and secondary severe ADAMTS13 deficiency were nearly indistinguishable from those of patients with TTP, though the former had evidence of infection (Table 2), indicating that there exists a subset of patients who have secondary severe ADAMTS13 deficiency caused by sepsis and in whom the disease course is clinically similar to that of TTP. In addition, they might also have the same ADAMTS13 deficiency pathophysiology for the development of TMA seen in patients with idiopathic TTP.

Organ failure might be caused by tissue factor–dependent fibrin thrombus formation and platelet aggregation because of severe

Table 4. Correlation between presence of unusually large multimers of VWF and serum creatinine levels of patients with sepsis-induced DIC and ADAMTS13 activity levels lower than 20%

	Presence, n = 26	Absence, n = 25	P
Creatinine, mg/dL	2.39 ± 2.24	1.34 ± 1.35	< .05*
ADAMTS13 activity, %	6.6 ± 6.8	8.9 ± 6.0	NS

Values are mean ± SD.

To convert creatinine from milligrams per deciliter to micromoles per liter, multiply milligrams per deciliter by 88.4.

Presence indicates unusually large VWF multimers present in the plasma of patients; absence, unusually large VWF multimers absent in the plasma of patients. NS, not significant.

*Statistically significant (Welch *t* test).

ADAMTS13 deficiency in the patients with sepsis-induced DIC with ADAMTS13 activity levels lower than 20%. This notion was supported by the correlation between severe secondary ADAMTS13 deficiency and renal failure in patients with sepsis-induced DIC with ADAMTS13 activity levels lower than 20%. We could not find any significant difference in the ADAMTS13-specific activity levels between these 2 groups (not shown). One possibility is that small molecular forms of ADAMTS13 could be lost in urine because of renal injuries. However, we could not determine whether this was the case because no urine samples were available for study.

In a previous report by Reife et al,[25] patients with TMA who did not have DIC were analyzed for the correlation between ADAMTS13 activity levels and serum creatinine levels without distinguishing TTP from HUS. They found that creatinine levels in patients with severely decreased ADAMTS13 activity levels were significantly lower than those in patients without severely decreased ADAMTS13 activity levels. These data are contrary to our findings that patients with severe ADAMTS13 deficiency (ADAMTS13 activity less than 20%) had significantly higher serum creatinine levels than did patients with the ADAMTS13 activity levels higher than 20%. Given that patients with HUS were not distinguished from patients with TTP in the report by Reife et al,[25] it is possible that the patients without severe ADAMTS13 deficiency in that study included patients with HUS. We studied patients with sepsis-induced DIC, and this difference in patient groups explains the opposing findings. There was no apparent difference between the platelet counts of patients with ADAMTS13 activity levels less than 20% and those of patients with ADAMTS13 activity levels greater than 20%. The combination of underlying DIC and platelet transfusion in these patients may account for the data.

The presence of the unusually large multimers of VWF in the plasma of patients with severe secondary ADAMTS13 deficiency and its correlation with serum creatinine levels supports the notion that severe secondary ADAMTS13 deficiency may correlate with the development of renal failure in sepsis-induced DIC. There was no significant correlation between the unusually large multimers of VWF and ADAMTS13 activity levels, possibly because of technical difficulties in determining the unusually large VWF multimers and the differences in endothelial cell damage among these patients.

Decreased specific activity of ADAMTS13, presumably caused by its cleavage by proteases, was a mechanism for severe secondary ADAMTS13 deficiency in patients with sepsis-induced DIC. Various proteases have been shown to degrade ADAMTS13 in vitro.[21] Thrombin and plasmin are generated in DIC, and these enzymes may cleave ADAMTS13, resulting in the inactivation of ADAMTS13. Our data suggest that granulocyte elastase may be one of the proteases that cleave ADAMTS13, together with thrombin and plasmin, under in vivo pathologic conditions. In this regard, the case report by Galbusera et al[26] of chronically relapsing

TTP—which showed that α1-antitrypsin (the physiologic granulocyte elastase inhibitor) therapy was effective at preventing the appearance of unusually large VWF multimers in the circulation but not at preventing TTP relapse—was interesting and suggested the link between granulocyte elastase and cleavage of ADAMTS13. Correlation between ADAMTS13 activity and antigen levels and E-XDP levels, not only in patients with TTP but also in patients with pathogenic *E coli* infection–related HUS, would be a further study to investigate the role of granulocyte elastase in TMA development. Specific inhibitors of these proteases are present at high concentrations in blood, indicating that cleavage of ADAMTS13 by these proteases may depend on the kinetic balance between ADAMTS13, the proteases, and their inhibitors. Thus, cleavage of ADAMTS13 by these proteases may not proceed completely in vivo. It is possible that other proteases could also digest ADAMTS13 in the disease state. This possibility should be investigated in a future study.

Because serum albumin levels decreased in most patients, liver injuries associated with the underlying disease might be an additional mechanism for decreasing ADAMTS13 antigen levels given that this enzyme is synthesized in the liver. Mutations or polymorphisms of the *ADAMTS13* gene are another possible cause of a decrease or an increase of ADAMTS13-specific activity. These possibilities should also be explored in future studies.

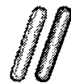
In conclusion, the precise analysis of ADAMTS13 antigen and activity levels in disease states offers insight into the roles of ADAMTS13 in thromboembolic diseases. Severe ADAMTS13 deficiency takes place secondarily in disease states such as sepsis-induced DIC, and it may not be specific for idiopathic TTP and may not have a solo diagnostic value for idiopathic TTP. Although the mechanisms of severe ADAMTS13 deficiency in sepsis are different from those of idiopathic TTP, the clinical features of patients with sepsis-induced DIC and severe ADAMTS13 deficiency are similar to those of patients with idiopathic TTP. Sepsis may have the same pathophysiology of severe ADAMTS13 deficiency for TMA development as idiopathic TTP, raising the possibility of novel supportive therapies for patients with sepsis and severe ADAMTS13 deficiency, such as ADAMTS13 supplementation, α1-antitrypsin administration, and use of synthetic granulocyte elastase inhibitors. Given that severe secondary ADAMTS13 deficiency might correlate with the development of organ injury in patients with sepsis-induced DIC, determining the ADAMTS13 levels of patients in severe condition at the time of hospital admission would provide better understanding of the extent of disease. Current analyses of ADAMTS13 levels in disease states are retrospective; thus, prospective study is needed for the timely execution of ADAMTS13 supplementation for patients not only with TTP but also with secondary ADAMTS13 deficiency.

References

1. Gerritsen HE, Robles R, Lämmle B, Furlan M. Partial amino acid sequence of purified von Willebrand factor-cleaving protease. Blood. 2001;98:1654-1661.
2. Fujikawa K, Suzuki H, McMullen B, Chung D. Purification of human von Willebrand factor-cleaving protease and its identification as a new member of metalloprotease family. Blood. 2001;98:1662-1666.
3. Levy GG, Nicholas WC, Lian EC, et al. Mutation in a member of ADAMTS gene family cause thrombotic thrombocytopenic purpura. Nature. 2001;413:488-494.
4. Zheng X, Chung D, Takayama TK, Majerus EM, Sadler JE, Fujikawa K. Structure of von Willebrand factor-cleaving protease (ADAMTS-13), a metalloprotease involved in thrombotic thrombocytopenic purpura. J Biol Chem. 2001;276:41059-41063.
5. Soejima K, Mimura N, Hirashima M, et al. A novel human metalloprotease synthesized in the liver and secreted into the blood: possibly, the von Willebrand factor-cleaving protease? J Biochem (Tokyo). 2001;130:475-480.
6. Moake JL Thrombotic microangiopathies. N Engl J Med. 2002;347:589-600.
7. Furlan M, Robles R, Lamie B. Partial purification and characterization of a protease from human plasma cleaving von Willebrand factor to fragments produced by in vivo proteolysis. Blood. 1996;87:4223-4234.
8. Furlan M, Robles R, Galbusera M, et al. Von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and the hemolytic uremic syndrome. N Engl J Med. 1998;339:1578-1584.
9. Tsai HM, Lian EC. Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. N Engl J Med. 1998;339:1585-1594.
10. Loof AH, van Vliet HH, Kappers-Klunne MC. Low activity of von Willebrand factor-cleaving protease is not restricted to patients suffering from thrombotic thrombocytopenic purpura. Br J Haematol. 2001;112:1087-1088.
11. Veyradier A, Brivet F, Wolf M, et al. Total deficiency of specific von Willebrand factor-cleaving protease and recovery following plasma therapy in one patient with hemolytic-uremic syndrome. Hematol J. 2001;2:352-354.
12. Remuzzi G, Galbusera M, Noris M, et al. von Willebrand factor cleaving protease (ADAMTS-13) is deficient in recurrent and familial thrombotic thrombocytopenic purpura and hemolytic uremic syndrome. Blood. 2002;100:778-785.
13. Studt JD, Bohm M, Budde U, Girma JP, Varadi K, Lämmle B. Measurement of von Willebrand factor-cleaving protease (ADAMTS-13) activity in plasma: a multicenter comparison of different assay. J Thromb Haemost. 2003;1:1882-1887
14. Kokame K, Matsumoto M, Soejima K, et al. Mutations and common polymorphisms in ADAMTS13 gene responsible for von Willebrand factor-cleaving protease activity. Proc Natl Acad Sci U S A. 2002;99:11902-11907.
15. Gando S, Kameue T, Morimoto Y, Matsuda N, Hayakawa M, Kemmotsu O. Tissue factor production not balanced by tissue factor pathway inhibitor in sepsis promotes poor prognosis. Crit Care Med. 2002;30:1729-1734
16. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: Definitions for sepsis and multiple organ failure and guidelines for the use of innovative therapies in sepsis. Crit Care Med. 1992;20:864-874.
17. Köhler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature. 1975;256:495-497.
18. Soejima K, Matsumoto M, Kokame K, et al. ADAMTS-13 cysteine-rich/spacer domains are functionally essential for von Willebrand factor cleavage. Blood. 2003;102:3232-3237.
19. Ono T, Sogabe M, Ogura M, Furusaki F. Automated latex photometric immunoassay for total plasminogen activator inhibitor-1 in plasma. Clin Chem. 2003;49:987-989.
20. Kohno I, Inuzuka K, Itoh Y, et al. A monoclonal antibody specific to the granulocyte-derived elastase-fragment D species of human fibrinogen and fibrin: its application to the measurement of granulocyte-derived elastase digests in plasma. Blood. 2000;95:1721-1728.
21. Crawley JT, Lam JK, Rance JB, Mollica LR, O'Donnell JS, Lane DA. Proteolytic inactivation of ADAMTS13 by thrombin and plasmin. Blood. 2005;105:1085-1093.
22. Bianchi V, Robles R, Alberio L, Furlan M, Lammle B. von Willebrand factor cleaving protease (ADAMTS-13): in thrombocytopenic disorders: a severely deficient activity is specific for thrombotic thrombocytopenic purpura. Blood. 2002;100:710-713.
23. Mannucci PM, Canciani MT, Forza I, Lussana F, Lattuada A, Rossi E. Change in health and disease of the metalloprotease that cleaves von Willebrand factor. Blood. 2001;98:2730-2735.
24. Dong J, Moake JL, Nolasco L, et al. ADAMTS-13 rapidly cleaves newly secreted ultralarge von Willebrand factor multimers on the endothelial surface under flowing conditions. Blood. 2002;100:4033-4039.
25. Raife T, Atkinson B, Montgomery R, Vesely S, Friedman K. Severe deficiency of VWF-cleaving protease (ADAMTS13) activity defines a distinct population of thrombotic microangiopathy patients. Transfusion. 2004;44:146-150.
26. Galbusera M, Ruggenenti P, Noris M, et al. α1-Antitrypsin therapy in a case of thrombotic thrombocytopenic purpura. Lancet. 1995;345:224-225.

平成19年度　研究成果の刊行物・別冊

消化器外疾患に対する *Helicobacter pylori* 除菌療法の適応


藤村欣吾*

世界中の多くのヒトが感染している *Helicobacter pylori*（*H. pylori*）は胃を中心とした病変との関連に加えて，最近では多くの全身性の疾患との関連が検討されている．このなかでエビデンスレベルはⅢないしⅣであるが除菌により病態が改善する疾患が注目されている．とくに原因不明の鉄欠乏性貧血で経口鉄剤不応性の鉄欠乏性貧血症例，さらには特発性（免疫性）血小板減少性紫斑病（ITP）においては除菌成功によって貧血の改善や血小板増加反応が認められた報告が多くなされ，除菌適用が検討されている．*H. pylori* によるこれらの疾患の発症には，*H. pylori* による胃分泌機能，胃粘膜組織への影響あるいは抗原分子相同性を介した局所，全身性免疫反応への影響が考えられている．しかし一部の症例しかこれらの病態が発症しない点に関しては，個人のヒト白血球抗原（HLA）の問題や *H. pylori* の遺伝子の多様性によるらしい．

はじめに

最近，単離 *Helicobacter pylori*（*H. pylori*）菌株の遺伝的変異と人種地理学との関係を調査した結果，*H. pylori* の遺伝的多様性の分布にみられるパターンとヒトの遺伝的多様性の分布にみられるパターンとが類似していることが報告された．このなかで人類がアフリカから離れ，ほかの大陸へ移動をはじめた約 58 万年前にはすでに *H. pylori* に感染していることが推測され，人類と *H. pylori* は同じ時間経過で東アフリカからほうぼうへ拡散したらしいと述べられている[1]．このように人類と歴史的に深くかかわってきたと思われる *H. pylori* は 1983 年に発見されたグラム陰性桿菌で，上部消化管疾患の発症機序や治療概念に大きな変化をもたらした．すなわち胃粘膜局所に持続感染し，好中球をはじめとした炎症細胞浸潤をきたし萎縮性胃炎，胃潰瘍の主たる原因として考えられ，さらには胃癌や胃のリンパ腫（MALT リンパ腫）の発症

key words
経口鉄剤不応性鉄欠乏性貧血
特発性（免疫性）血小板減少性紫斑病（ITP）
自己免疫胃炎
分子相同性
除菌療法

* FUJIMURA Kingo/広島国際大学薬学部病態薬物治療学講座，広島赤十字原爆病院内科

との関連が示されている．1994 年には疫学的研究から type I （definite）の癌原因子として認定されている[2]．

最近では *H. pylori* に全人類の半数以上が感染していることから，上部消化管疾患以外にも全身性疾患との関連が注目を集めている．

1．*H. pylori* 感染と消化器以外の疾患の概要

たとえば甲状腺炎やシェーグレン症候群，関節リウマチ，膜性腎症など自己免疫疾患との関連をはじめとして血液疾患では悪性貧血，自己免疫性好中球減少症，シェーンライン・ヘノッホ紫斑病や *H. pylori* 除菌により特発性（免疫性）血小板減少性紫斑病（ITP）における血小板減少の改善や経口鉄剤治療抵抗性の鉄欠乏性貧血の改善，monoclonal gammopathy of undetermined significance (MGUS) の一部に単クローン性 γ グロブリン血症が改善する例が報告され，さらには一般の動脈硬化症や冠動脈疾患，慢性蕁麻疹との関連も示唆されている[3]．しかし，なかには疑問視されている疾患もあり，さらにどのような機序でこれらの疾患が生じるのか明らかではない．

推測されている機序としては，①*H. pylori* の直接作用，②サイトカインの遊離を伴う炎症反応の活性化による，③*H. pylori* 菌体と宿主のあいだでの抗原の相同性などがある．ここでは除菌療法の効果が顕著に認められ，除菌療法が適応であると検討されている特発性血小板減少性紫斑病〔idiopathic (immune) thrombocytopenic purpura：ITP〕と鉄欠乏性貧血を中心に話を進める．

2．*H. pylori* 感染と血液疾患

1）経口鉄剤治療不応性鉄欠乏性貧血

A．疫学

血液疾患のなかでは最も早く *H. pylori* との関連が報告され，1991 年，1993 年にそれぞれベルギー，イタリア，フランスから 1 例ずつ症例報告されたのがはじまりと思われる．いずれの症例も小児ないし青春期で経口鉄剤に不応の鉄欠乏性貧血症例で *H. pylori* 陽性であり，これらの症例に除菌療法をおこなったところ鉄剤の補充がなくともヘモグロビン値が上昇した報告である．

以来各地で原因不明の鉄欠乏性貧血についての臨床疫学的研究がなされ，*H. pylori* 感染と貯蔵鉄減少とのあいだには関連性があるとの報告が多い．すなわち *H. pylori* 感染者では血清フェリチン値が 17%減少している報告や，デンマークやアラスカ，オーストラリア，米国からの報告でも *H. pylori* 陽性例ではフェリチン値の減少リスクが陰性例にくらべ高く，実際血清フェリチン値が低い[4)5)]．

韓国の報告では，青春期の貧血を対象とした場合，鉄欠乏性貧血や，低フェリチン，鉄欠乏状態では対照群にくらべ有意に *H. pylori* 感染率が高い．

一方では鉄欠乏性貧血と非貧血症例のあいだでは *H. pylori* 感染の頻度には差がないとする報告やフェリチン値と *H. pylori* 感染のあいだには関連を認めないとする否定的報告もわずかではあるが存在する[6]．

しかし *H. pylori* 感染症例と鉄欠乏状態との関連性を示唆する報告が多く，臨床的に経口鉄剤不応の症例で *H. pylori* 陽性であれば除菌を勧めている報告もある．

B．鉄欠乏状態や経口鉄剤不応の機序について

いくつかの可能性が示されているが確定的なものはない．しかしこれらの可能性はいずれも理論的であり，複合的に作用して発症するものと考えられる．

①*H. pylori* 感染後の胃炎による微量の持続的出血：慢性のびらん性胃炎が引き起こされることによるとの考えがある．

②*H. pylori* 感染後の胃炎によって胃酸分泌低下，アスコルビン酸分泌減少により鉄の吸収障害が生じる．

一般に食事中の鉄は 3 価であり，これは胃酸，アスコルビン酸の作用によって 2 価の鉄となってはじめて吸収される．したがって胃酸分泌低下，アスコルビン酸分泌減少がつづくと鉄吸収が障害され鉄欠乏状態が引き起こされることになる．実際原因不明の鉄欠乏性貧血症例について検討した報告では，*H. pylori* 陽性例では陰性例にくらべ有意に胃酸 pH は高く，胃液中のアスコルビン酸濃度も低い．また *H. pylori* 陽性であっても鉄欠乏性貧血のない症例では鉄欠乏性貧血例にくらべ胃酸 pH は低く，アスコルビン酸濃度も高く正常と差がない．したがって *H. pylori* 感染による *H. pylori* 胃炎は胃酸分泌，アスコルビン酸分泌に影響し鉄吸収を妨げると考えられる[7]．

③細菌による鉄の取り込みと利用

多くの細菌と同様に *H. pylori* も増殖因子として鉄は必要で，鉄の取り込みと貯蔵にかかわる蛋白を発現しているといわれている[4]．

以上が代表的機序であるが，この他にも鉄欠乏状態を引き起こす機序が報告されており，これらが複合して作用しているものと考えられる．

2）自己免疫胃炎と鉄欠乏性貧血，ビタミン B_{12} 低下症との関係

原因不明の *H. pylori* 陽性鉄欠乏性貧血においては無酸症である症例があること，胃体部にも萎縮性胃炎のあるいわゆる pangastritis の所見を示す症例のあることから，胃の所見と貧血の関係について研究がなされている[8)9)]．

すなわちいわゆる自己免疫胃炎（高ガストリン血症，高い抗壁細胞抗体価を伴う）160 例について赤血球のサイズを検討した．その結果鉄欠乏性貧血（小球性貧血）は 83 例，正球性赤血球は 48 例（このうち半数 24 例は鉄欠乏を示していた），大球性赤血球 29 例（このうち鉄欠乏は 3 例）で，小球性の症例の年齢は大球性よりも平均 21 歳若く，ほとんどが女性であった．20 歳以下の若年者から 60 歳以上の高年齢者にいくにしたがい赤血球サイズは大きくなり，血清フェリチン値，ガストリン値も上昇するのに対し，血清コバラミン値は減少する傾向を示した．また *H. pylori* 感染率は 20 歳以下 87.5%であったが加齢とともに徐々に減少し，60 歳以上では 12.5%であった．以上の結果から，*H. pylori* 感染が引き金になって自己免疫過程により胃炎が発症し，年月を重ねるにしたがって胃粘膜萎縮が拡大し，鉄吸収障害からビタミン B_{12} 吸収障害となり大球性貧血が生じてくると考えられる（図❶）．大球性ではほぼ全例が（100%）血清ビタミン B_{12} 値が低下し悪性貧血と考えられ，正球性では 54%に，小球性では 27%にビタミン B_{12} が低下し悪性貧血が疑われた．また高度の粘膜萎縮になると *H. pylori* 自体の発育，増殖は影響を受け感染頻度が低下すると推測している．事実悪性貧血（大球性貧血）においては抗 *H. pylori* 抗体が 1 年あたり 6%の頻度で陰性化

20 歳 → 60 歳		
赤血球サイズ		
microcytic	normocytic	macrocytic
Hb（g/dl）		
9.6±1.8	10.1±2.1	11.5±2.5
ガストリン値		
↑	↑↑	↑↑↑
血清フェリチン値		
↓↓↓	↓↓	↓
血清 B_{12} 値		
正常	↓	↓↓↓
H. pylori 陽性率（%）		
87.5	47.1	12.5
萎縮性胃炎（%）		
41	54	69
慢性胃炎（%）		
56	38	15

図❶ 貧血と胃機能の関係

（Hershko C *et al* 2006[8] より引用）

しているとの報告がある．

これら一連の過程はレトロスペクティブな研究の結果から組み立てられたものであり今後プロスペクティブな研究によって実証される必要があるが，*H. pylori* の関与として以下の推測がおこなわれている．

H. pylori 感染は局所に IL-β や TNFα を誘導し胃壁細胞や腸管クロム親和性細胞（enterochromaffine cell：ECL）の機能を抑制し，酸分泌を抑制するとともに壁細胞のアポトーシスを誘導し低酸を伴う粘膜萎縮を生じる．さらには *H. pylori* によるアスコルビン酸の分泌抑制により，酸分泌低下とあいまって食餌中の鉄の吸収障害が起こり，小球性傾向になる．また *H. pylori* 陽性症例においては壁細胞と *H. pylori* とのあいだの抗原分子相同性により，たとえば H^+/K^+-ATPase 蛋白に対する抗体の出現頻度が高く，*H. pylori* 感染が胃粘膜に自己免疫病変を引き起こすと考えられている．一方，これらの所見は除菌療法によってガストリン値の低下，胃酸分泌の改善，アスコルビン酸の分泌回復，鉄剤治療の奏功，さらには症例によっては萎縮病変の消失などがみられ，胃の分泌機能，粘膜病変へ *H. pylori* 感染が影響していることが裏付けられている．

しかし除菌をおこなっても胃粘膜抗原に対する抗体が消失したり，力価が低下する症例は少なく，また必ずしも除菌によって改善しない症例もあり，*H. pylori* 感染は自己免疫胃粘膜萎縮の引き金として作用し，自己免疫過程ができあがってしまうともはや *H. pylori* の存在は必ずしも必要ないことを示している．このことは加齢に伴って自己免疫性萎縮性病変の拡大による胃酸，内因子分泌の低下，内因子抗体の出現に伴いビタミン B_{12} の吸収低下をきたし，終末像としての悪性貧血に至るものと推測される．

3）*H. pylori* 感染と特発性（免疫性）血小板減少性紫斑病（ITP）

特発性血小板減少性紫斑病（ITP）は後天性の血小板減少症で，皮膚や粘膜の紫斑を主体とする出血症状を主徴とし，発症に血小板膜に対する抗体が関与する自己免疫疾患のひとつである．慢性に経過するいわゆる慢性型は成人に多く，とくに女性が男性の 2.5 倍多い．わが国における最近の調査では年齢分布は男女とも 20 歳ごろから増加しはじめ，51～70 歳にピークを認めている．

年間発生率は人口 10 万人あたり 1.25 人で発症機序の解明，診断・治療法の確立を目指して厚生労働省の難病指定を受けている疾患である．治療として副腎皮質ステロイド薬を中心とした免疫抑制療法や血小板破壊場所ならびに抗体産生の中心的役割を果たす脾臓の除去（摘脾療法）が定着している．これらによる治療成績はおおまかには約 40%の完全寛解（CR），いわゆる治癒といってよい症例と，日常生活には何ら支障がないが軽度の血小板数減少が持続する部分寛解（PR）と考えられる症例が 40%，血小板減少に対する何らかの治療介入が必要な，いわゆる難治症例が約 20%である．

最近本疾患のなかで *H. pylori* 陽性症例に対して除菌療法により約 40～60%の症例が血小板増加効果を示し，*H. pylori* が一部の ITP 症例の発症に関係していることが示唆されている．

A．ITP における *H. pylori* 感染頻度

慢性 ITP における *H. pylori* 感染率も一般人口と同様に加齢とともに上昇し，イタリア，わが国からの報告では，一般人口の感染率と同様に中高年症例の 70～90%と先進国でも頻度が高く，年齢をマッチさせた一般人口とのあいだでは感染率に差はない．したがって ITP 症例に *H. pylori* に感染率が高いわけではなく，*H. pylori* 感染者の一部に ITP が発症したものと考えられる．また *H. pylori* 陽性 ITP 症例に胃を中心とした消化器症状や消化器病変が多いわけではない．

小児に多い感染を契機とする急性の ITP については *H. pylori* 感染との関連は有意ではなく，急性 ITP の発症に *H. pylori* 感染はかかわっていないとの報告がある．

B．*H. pylori* 感染 ITP における除菌療法による血小板増加について

1998 年の Gasbarrini ら[10]の報告以来，除菌療法による血小板増加効果を検討した報告が多くなされている．その一覧を表❶に示すが除菌による血小板増加効果は国によって有効率に差があり，スペイン，英国，米国からの報告では除菌による血小板増加効果はほとんど認められないか増加反応を示す症例が少ない．スペインの報告では 13%が PR となっているにすぎない[11)~13)]．一方，イタリアやわが国からの報告では一部を除き約 40%以上に血小板の増加反応が認められている（33～100%）[14)~17)]．

このように除菌後の血小板増加効果についておおまかに二極化した報告になっているのが現状である．この原因は不明であるが，①対象者の免疫学的背景に差がある，②地域的に感染した *H. pylori* 株によって発現している抗原性の程度が異なる〔Cag, Vac がコードする蛋白抗原，Lewis（Le）抗原など〕などが推測されている．

このうち感染菌株についてわが国の報告では血小板減少を伴わない単なる胃・十二指腸潰瘍症例と同様で，*cagA*，*vacA*，*iceA*，IL-8 の発現なども他の *H. pylori* 感染消化器疾患と差はないとされている．したがってわが国では血小板減少を引き起こす特有の菌株や菌の性状は見当たらない[18)]．

C．わが国のレトロスペクティブ共同研究による *H. pylori* 陽性 ITP に対する除菌療法の血小板増加反応について[17)]

厚生労働省研究班において 2002 年 7 月～2003 年 12

表❶ 成人慢性 ITP 症例における *H. pylori* 陽性率と除菌による血小板増加効果（2006 年 5 月）

報告者	報告年	症例数	*H. pylori* 感染率 (%)	除菌成功症例数	血小板増加反応例 (%)	平均観察期間（月）
Gasbarrini *et al*（伊）	1998	18	11（61）	8	8（100）	4
Emilina *et al*（伊）	2001	30	13（43）	12	6（50）	8.3
Jarque *et al*（スペイン）	2001	56	40（71）	23	3（13）	24
Kohda *et al*（日）	2002	48	27（56）	19	12（63）	14.8
Hino *et al*（日）	2003	30	21（70）	18	10（56）	15
Hashino *et al*（日）	2003	22	14（64）	13	5（39）	15
Ando K *et al*（日）	2003	61	50（82）	27	16（59）	11
Michel *et al*（米）	2004	76	16（21）	14	0	11.5
Takahashi *et al*（日）	2004	20	15（75）	13	7（53.8）	4
Fujimura *et al*（日）	2004	435	300（69）	155	88（57）	12<
Ando T *et al*（日）	2004	20	17（85）	17	15（85）	24
Sato *et al*（日）	2004	53	39（74）	27	15（55.6）	6
Inaba *et al*（日）	2005	35	25（71）	25	11（44）	6
Veneri D *et al*（伊）	2005	43	43	41	20（49）	31.2
Stasi R *et al*（英，伊）	2005	137	64（47）	52	17（33）	12<
Suzuki *et al*（日）	2005	36	25（69）	23	10（44）	6
Suvajdzic N *et al*（英）	2006	54	39（72）	23	6（26.1）	18
計		1,174	759（64.7）	510	248（48.8）	13.1<

月の 18 ヵ月間に血液専門 11 施設の協力のもとにレトロスペクティブ共同研究をおこなった結果は以下のとおりである．

a．*H. pylori* 陽性 ITP の臨床病態

登録された慢性 ITP435 例のうち 300 例（69%）が *H. pylori* 陽性で，*H. pylori* 陽性 ITP の頻度は加齢とともに上昇し，わが国における一般人口にみられる年齢別感染率と類似している（図❷）．

H. pylori 陽性 ITP 症例群と *H. pylori* 陰性 ITP 症例群との臨床的背景については（表❷），性差，ITP 罹病期間，出血症状を中心とする臨床症状において両群間で差は認められない．ただし年齢は *H. pylori* 陽性群が陰性群にくらべ有意に高い．また *H. pylori* 陽性 ITP では有意に初診時血小板数は激減している症例が少なく，骨髄巨核球数は増加している症例が多く，ITP の病態としては重症例は少ない傾向がある．*H. pylori* 感染による胃腸障害の頻度や程度は非感染 ITP と同等で，*H. pylori* 陽性 ITP は消化器症状の面から明らかにすることはできない．

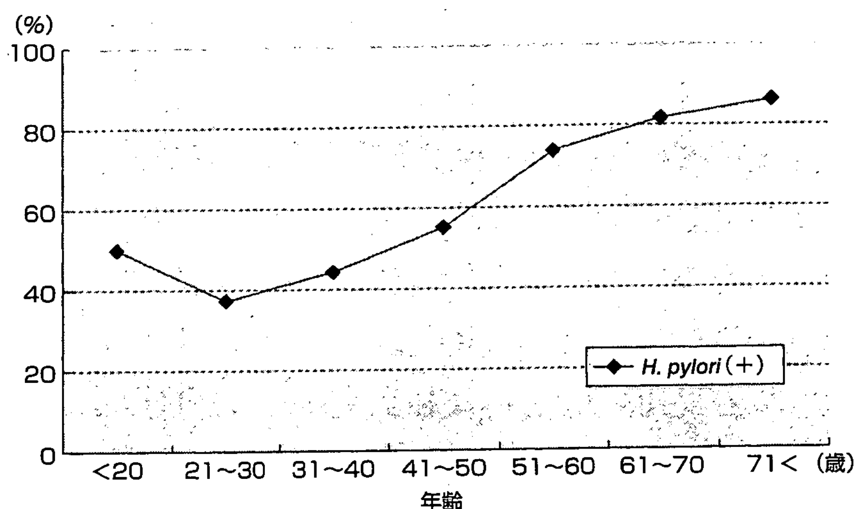
b．除菌による血小板増加効果（表❷）

H. pylori 陽性 ITP 300 例のうち 228 例に除菌療法がおこなわれ，除菌効果判定が可能であった 207 例中 161 例 78%に除菌が成功している．用いられた除菌療法はほとんどが通常のアモキシシリン，クラリスロマイシン，プロトンポンプ阻害薬（PPI）の 3 剤，7 日間併用療法である．

除菌後 12 ヵ月以上経過観察された 155 例（除菌成功群 122 例，除菌不成功群 33 例）の長期予後は，除菌成功群のうち 79 例は血小板数増加を維持し，このうち 28 例は除菌後無治療で血小板数 15 万以上となり CR の症例である（23%）．残り 51 例の多くは血小板数 5 万以上となりほとんどが無治療観察（PR）となっている（42%）．43 例は除菌に成功したものの血小板数の増加が認められない症例で（35%），除菌後の血小板数は除菌前値と変わりなく軽微な増減にとどまっている（表❷）．すなわち除菌成功群では 65%の症例が 12 ヵ月以上のあいだ何らかの血小板増加を維持したことになる．

この頻度は除菌不成功群（9/34，27%）にくらべ有意に高く，*H. pylori* の除去が血小板増加に密接にかかわっていることが確認された．

さらに *H. pylori* 除菌療法が *H. pylori* 陽性 ITP に特異



図❷ ITP 症例における年齢別 *H. pylori* 陽性率

(Fujimura K *et al*, 2005[17] より引用)

表❷ 除菌後 12 ヵ月を経た時点での血小板増加反応
(Retrospective analysis in Japan 2003)

除菌直前の血小板数 (×10⁴/μl)	CR	PR	NR	計
除菌成功群 (n=122)				
<1	0	2 (67)	1 (33)	3
1~3	8 (20)	23 (58)	9 (23)	40
3~5	8 (21)	16 (42)	14 (37)	38
5~10	12 (29)	10 (24)	19 (46)	41
計	28 (23)	51 (42)	43 (35)	122
除菌不成功群 (n=33)				
<1	0	0	2 (100)	2
1~3	1 (11)	1 (11)	7 (78)	9
3~5	1 (7)	3 (21)	10 (71)	14
5~10	3 (38)	0	5 (63)	8
計	5 (15)	4 (12)	24 (73)	33

CR：血小板数>15 万　PR：血小板数　3~15 万
NR：除菌前値血小板数 1 万以下の場合：除菌後 3 倍以上増加しない．
　　除菌前値血小板数 1~3 万の場合：除菌後血小板数 5 万以上とならない．
　　除菌前血小板数 3~10 万の場合：除菌後血小板数 3 万以上増加しない．

(Fujimura K *et al*, 2005[17] より引用)

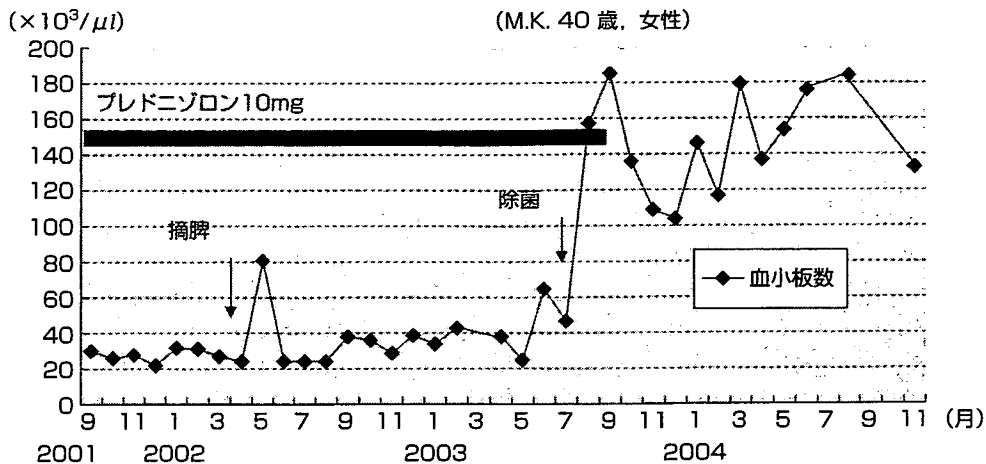
的な現象で，かつ血小板増加反応に直接的に関係していることは以下の報告からも支持されている．

①全身性エリテマトーデス（SLE）に伴う血小板減少においては *H. pylori* 陽性であっても，除菌による血小板増加反応は認められない[15]．

②*H. pylori* 陰性 ITP 症例に無作為的に除菌を試みた報告では血小板増加反応を示した症例は認められず，除菌療法による非特異的血小板増加作用ではない[13]．

③*H. pylori* 陽性 ITP 症例を無作為的に除菌療法をおこなう群（13 例）とおこなわない群（12 例）の 2 群に分け血小板増加反応を検討した報告では除菌群のみに血小板増加が認められる[18]などである．

以上より，*H. pylori* 陽性 ITP のなかには除菌治療と臨床的に密接な関係を示す症例が多いことからこのよう



図❸ プレドニゾロン，摘脾無効　除菌有効症例　2001 年 1 月診断

な ITP 症例を *H. pylori* 関連 ITP と呼称する根拠としている.

c. 除菌による血小板増加反応の特徴

わが国での臨床研究から性差，年齢，除菌直前の血小板数，除菌直前の ITP 治療の有無，治療の内容などいわゆる ITP の臨床病態や過去の治療経過は除菌による血小板増加効果に影響しない結果を得た．すなわちステロイド療法や摘脾に対して不応性の症例に対しても除菌療法により血小板増加が認められる点は大きな利点である[15)17)] (図❸)．しかし極度に血小板数が少ない 1 万以下の症例では血小板増加反応が認められても軽度である傾向がある．ITP としての罹病期間が短い群 (6.52±4.67 年) が長い群 (9.85±7.77 年) にくらべ有意に除菌成功後の血小板増加を示し，罹病期間が血小板増加反応に影響している傾向がある[19)].

以上，除菌後の血小板増加効果は従来の ITP に対する副腎皮質ステロイド療法や摘脾療法を凌駕し，これらの治療よりもはるかに副作用も少なく，除菌後 1ヵ月には効果が出はじめ，再発がみられない特徴がある．このことから現在では *H. pylori* 陽性 ITP に対する除菌療法の保険適用を申請中である.

D. *H. pylori* 感染による血小板減少機序

前述の経口鉄剤不応性鉄欠乏性貧血のように，*H. pylori* 感染部位である胃を中心とした病変ないしその病変に誘導される疾患については，*H. pylori* 感染による発症機序は理解可能であるが，直接的にも間接的にも関連が見出せない ITP の発症機序については種々の臨床的研究がなされてきた．通常 *H. pylori* 陽性症例においては，*H. pylori* に対する特異的な全身性の免疫反応は末梢血リンパ球の検索からは認められず，また臓器非特異的，特異的自己抗体 (抗核抗体，抗平滑筋抗体，抗ミクロソーム抗体など) の出現頻度はコントロールと差がないと報告されている[20)]．しかし最近小児においては *H. pylori* 陽性群においては臨床症状とは関係なく陰性群にくらべ，抗胃壁細胞抗体，抗ミクロソーム抗体の出現頻度が有意に高く，小児における *H. pylori* 菌感染は自己免疫的胃炎や他の自己免疫疾患の誘引となると報告され[21)]，局所の *H. pylori* 感染から全身的免疫反応への進展の可能性を示唆している.

除菌により血小板が増加した ITP 症例では Th1/Th2 リンパ球の比が除菌後上昇することは，*H. pylori* 感染が全身的な免疫反応の不均衡を引き起していることを裏づけ興味深い．また最近 *H. pylori* 陽性 ITP 症例においては末梢血において特異的な T 細胞のクローナルな増殖が認められ，除菌効果に伴ってクローンが消失すると報告され，*H. pylori* に対する全身的な免疫反応が血小板減少と関連していることが示されている[22)]．局所感染から血小板減少を引き起こす機序として以下のような説が提示されている.

a．Lewis 抗体の関与

H. pylori 感染症例においては高い Lewis 抗体価を示す例がある．この抗 Lewis 抗体が認識するエピトープを有する組織にこの抗体が結合し自己免疫疾患を起こす (molecular mimicry)．たとえば胃壁細胞の H^+/K^+-ATPase の一部は Lewis 抗原エピトープを有し抗 Lewis 抗体が壁細胞障害を生じ胃炎を引き起こす説もある．この抗 Lewis 抗体が血小板に非特異的に吸着され血小板減少を引き起こすと考えられる[23]．

b．分子相同性

従来から微生物由来のリポポリサッカライドや DNA などはアジュバントとしてはたらき，無関係の抗原に対して容易に免疫反応を起こす結果，自己抗原に対しても異種抗原と同じように T 細胞の反応を引き起こしやすいといわれている．また一方，抗原分子相同性 (molecular mimicry) により微生物由来の抗原ペプチドと自己抗原ペプチドのあいだに交差性があれば自己抗体として認識され自己免疫疾患が発症すると推測される．*H. pylori* 陽性 ITP 症例の血小板から誘出した抗体は *H. pylori* の CagA 抗原と反応することが免疫ブロッティング法で明らかにされた[16]．さらに除菌により血小板数が回復すると CagA 抗原と反応する抗体は誘出液中には認められなくなるが，除菌による血小板数の増加が認められない症例においては抗体の消失は認められない．さらに *H. pylori* 陰性 ITP 症例の血小板誘出液も CagA を認識することから，*H. pylori* 陰性 ITP の血小板に結合している自己血小板抗体は CagA にも血小板抗原にも反応すると理解される．すなわち CagA 抗原と血小板膜抗原 (この場合 GP Ⅱb/Ⅲa と考えられている) のあいだの分子相同性が推測されている．また抗 CagA IgG 抗体価が高い症例には除菌による血小板増加反応を示す症例が有意に多いとの報告は，CagA 抗原が ITP 発症の免疫反応にかかわっていることを別の角度から示唆したものである[18]．

いずれにしても CagA 抗原とこれに反応する抗体が血小板減少の発症に関連している報告が集積されつつあるのが現状である．

c．vWF (von Willebrand factor)，抗 *H. pylori* 抗体による血小板活性化反応[24]

ある *H. pylori* 株は vWF，抗 *H. pylori* 抗体の存在下で血小板凝集反応を引き起こすことが報告された．この血小板活性化が局所の炎症反応，潰瘍形成に，また心血管傷害に関係し，さらには血小板活性化による慢性的血小板消費による血小板減少が引き起こされるとする報告もある．

これら諸説のなかでは分子相同性を示唆する機序が理解しやすい．*H. pylori* 菌に対する免疫反応の発現については *H. pylori* 菌の菌体自体が免疫反応にかかわる必要はなく，むしろ *H. pylori* 菌が産生する CagA 抗原のように胃粘膜細胞に侵入し細胞の増殖，分化に影響を与え，炎症反応を引き起こし細胞が破壊される．その結果抗原認識細胞をはじめとする免疫担当細胞と CagA 抗原が接触し，免疫反応が引き起こされる可能性がある．また感染から ITP 発症までに時間が経過する点については自己抗原認識のための T 細胞のプライミングに時間が必要で，いわゆる抗原エピトープの拡大に要する時間と考えれば理解可能である．今後はこれらの仮定を証明することが必要である．

E．*H. pylori* 感染によって ITP を生じる症例の特徴

H. pylori 感染者は非常に多いにもかかわらず ITP を発症する症例はそのごく一部で，多くは ITP を発症しない．これについては，たとえば CagA と交差反応する血小板抗原を認識する免疫反応の個体差が関係していると考えられ，ヒト白血球抗原 (HLA) 系を検索した報告がある．それによると *H. pylori* 陽性 ITP では HLA-DRB1*11，14，と HLA-DQB1*03 が，*H. pylori* 陰性 ITP にくらべ有意に高く，HLA-DRB1*03 が有意に低い結果が得られている．このような HLA 系を有するヒトが *H. pylori* 感染により *H. pylori* 関連 ITP を発症する可能性が高くなることが示唆される[19]．しかし HLA 系の頻度は人種によって差があり普遍化には問題があり，今後それぞれの人種間で検討し結論を出す必要がある．

おわりに

以上 *H. pylori* との関連性が多く検討されている代表

的消化器外疾患について述べた．この他にも冒頭に述べたようにシェーグレン症候群，リウマチ様関節炎，I型糖尿病，ギラン-バレー症候群，慢性蕁麻疹など免疫異常が推測されている疾患の発症に *H. pylori* がかかわっている報告がある．その多くは *H. pylori* の抗体保有頻度が高い，また *H. pylori* 感染によって誘導される抗熱ショック蛋白 65（Hsp65）抗体の頻度が高い，一部には除菌により症状が軽快するなどの臨床研究からその関連を指摘している．しかしこれらの報告に対しては反証もあり現時点では積極的に除菌を支持するほどの説得力に乏しくここでは割愛した．

一方，ここに取り上げた経口鉄剤不応性鉄欠乏性貧血や ITP に関しては除菌による治療的意義を認める報告が多く，またこれらの疾患には除菌を認めるべきとのコメントもあり，今後 *H. pylori* 感染と消化器外疾患の関連を研究するうえでのモデル疾患と考えられる．しかしながら，これらの消化器外疾患は *H. pylori* 感染者のごく一部でありほとんどの感染者はこれらの病変を引き起こさない．このことが通常の感染症とは趣を異にし，関連性が認知されにくい点である．この理由は不明であるが，関連性を問題にされている疾患は免疫機序が考えられている疾患であることから，*H. pylori* に対する反応の個体差がその原因のひとつと考えられる．これに対しては ITP において HLA の違いが感染後の ITP の発症に差があることが今後有力な根拠となるであろう．また *H. pylori* の遺伝子に多様性がありこれは人種によって異なることが報告され，このことは除菌による治療効果が国によって異なる理由の説明となるかもしれない．いずれにしても多くの人類が *H. pylori* に感染している現状では，今後は消化管病変のみならず全身的な病変とのかかわりあいをプロスペクティブに検討すべきであるとともに，HLA の検索や *H. pylori* の遺伝的変異と疾患，人種との関連性を検索すべきであろう．

謝辞

わが国でのプロスペクティブ研究は厚生労働省　難治性疾患克服事業　血液凝固異常症に関する調査研究班班長 池田康夫教授（慶應義塾大学）のもと，大阪大学　倉田義之先生，慶應義塾大学 桑名正隆先生，ならびに北海道大学，旭川赤十字病院，昭和大学藤が丘病院，東京都立駒込病院，東京女子医科大学，東京医科大学，関西医科大学，広島大学，広島赤十字原爆病院の各血液内科の先生方の協力を得ておこなわれたもので，改めて深謝いたします．

文　献

1) Linz B, Balloux F, Moodley Y *et al*：An African origin for the intimate association between humans and *Helicobacter pylori*. *Nature* **445**：915-918, 2007
2) Suerbaum S, Michetti P：*Helicobacter pylori* infection. *N Engl J Med* **347**：1175-1186, 2002
3) Gasbarrini A, Carloni E, Gasbarrini G *et al*：*Helicobacter pylori* and extragastric diseases-Other *Helicobacters*. *Helicobacter* **8**：68-76, 2003
4) DuBois S, Kearney DJ：Iron-deficiency anemia and *Helicobacter pylori* infection：A review of the evidence. *Am J Gastroenterol* **100**：453-459, 2005
5) Cardenas VM, Mulla ZD, Ortiz M *et al*：Iron deficiency and *Helicobacter pylori* infection in the United States. *Am J Epidemiol* **163**：127-134, 2006
6) Gessner BD, Baggett HC, Muth PT *et al*：A controlled, household-randomized, open-label trial of the effect that treatment of *Helicobacter pylori* infection has on iron deficiency in children in rural Alaska. *J Infect Dis* **193**：537-546, 2006
7) Annibale B, Capurso G, Lahner E *et al*：Concomitant alternations in intragastric pH and ascorbic acid concentration in patients with *Helicobacter pylori* gastritis and associated iron deficiency anaemia. *Gut* **52**：496-501, 2003
8) Hershko C, Ronson A, Souroujon M *et al*：Variable hematologic presentation of autoimmune gastritis：age-related progression from Iron deficiency to cobalamin depletion. *Blood* **107**：1673-1679, 2006
9) Hershko C, Hoffbrand AV, Keret D *et al*：Role of autoimmune gastritis, *Helicobacter pylori* and celiac disease in refractory or unexplained Iron deficiency anemia. *Haematologica* **90**：585-595, 2005
10) Gasbarrini A, Franceschi F, Tartaglione R *et al*：Regression of autoimmune thrombocytopenia after eradication of *Helicobacter pylori*. *Lancet* **352**：878-878, 1998
11) Jarque I, Andreu R, Llopis I *et al*：Absence of platelet response after eradication of *Helicobacter pylori* infection in patients with chronic idiopathic thrombocytopenic purpura. *Brit J Haematol* **115**：1002-1003, 2001

12) Michel M, Khellaf M, Desforges L *et al* : Autoimmune thrombocytopenic purpura and *Helicobacter pylori* infection. *Arch Intern Med* **162** : 1033-1036, 2002

13) Michel M, Cooper N, Christelle J *et al* : Does *Helicobacter pylori* initiate or perpetuate immune thrombocytopenic purpura? *Blood* **103** : 890-896, 2004

14) Emillia G, Longo G, Luppi M *et al* : *Helicobacter pylori* eradication can induce platelet recovery in idiopathic thrombocytopenic purpura. *Blood* **97** : 812-814, 2001

15) Kohda K, Kuga T, Kogawa K *et al* : Effect of *Helicobacter pylori* eradication on platelet recovery in Japanese patients with chronic idiopathic thrombocytopenic purpura and secondary autoimmune thrombocytopenic purpura. *Brit J Haematol* **118** : 584-588, 2002

16) Takahashi T, Yujiri T, Shinohara K *et al* : Molecular mimicry by *Helicobacter pylori* CagA protein may be involved in the pathogenesis of *H. pylori*-associated chronic idiopathic thrombocytopenic purpura. *Brit J Haematol* **124** : 91-96, 2004

17) Fujimura K, Kuwana M, Kurata Y *et al* : Is eradication therapy useful as the first line of treatment in *Helicobacter pylori*-positive idiopathic thrombocytopenic purpura? Analysis of 207 eradicated chronic ITP cases in Japan. *Int J Hematol* **81** : 162-168, 2005

18) Suzuki T, Matsushima M, Masui A *et al* : Effect of *Helicobacter pylori* eradication in patients with chronic idiopathic thrombocytopenic purpura-a randomized controlled trial. *Am J Gastroenterol* **100** : 1265-1270, 2005

19) Stasi R, Rossi Z, Stipa E *et al* : *Helicobacter pylori* eradication in the management of patients with idiopathic thrombocytopenic purpura. *Am J Med* **118** : 414-419, 2005

20) Pellicano R, Touscoz GA, Smedile A *et al* : Prevalence of non-organ-specific autoantibodies in patients suffering from duodenal ulcer with and without *Helicobacter pylori* infection. *Dig Dis Sci* **49** : 395-398, 2004

21) Guariso G, Brotto F, Basso D *et al* : Organ-specific autoantibodies in children with *Helicobacter pylori* infection. *Helicobacter* **9** : 622-628, 2004

22) Ishiyama M, Teramura M, Iwabe K *et al* : Clonally expanded T-cells in the peripheral blood of patients with idiopathic thrombocytopenic purpura and *Helicobacter pylori* infection. *Int J Hematol* **83** : 147-151, 2006

23) McCrae KR : *Helicobacter pylori* and ITP : many questions, few answers. *Blood* **103** : 752-753, 2004

24) Byrne MF, Kerrigan SW, Corcoran PA *et al* : *Helicobacter Pylori* binds von Willebrand factor and interacts with GPIb to induce platelet aggregation. *Gastroenterology* **124** : 1846-1854, 2003

25) Veneri D, Gottardi M, Guizzardi E *et al* : Idiopathic thrombocytopenic purpura, *Helicobacter pylori* infection, and HLA class II alleles. *Blood* **100** : 1925-1926, 2002

WILEY InterScience
DISCOVER SOMETHING GREAT

The role of autoantibody-producing plasma cells in immune thrombocytopenic purpura refractory to rituximab

M. Kuwana,[1]* S. Iki,[2] and A. Urabe[2]

[1] Division of Rheumatology, Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan
[2] Division of Hematology, NTT Kanto Medical Center, Tokyo, Japan

Rituximab is becoming popular as a treatment for immune thrombocytopenic purpura (ITP). We report here a patient with ITP, who initially responded to rituximab, but later became refractory. In this patient, the appearance of plasma cells producing anti-platelet autoantibodies is likely to be one of the mechanisms for rituximab resistance. Am. J. Hematol. 82:846–848, 2007. © 2007 Wiley-Liss, Inc.

Introduction

Immune thrombocytopenic purpura (ITP) is an acquired hemorrhagic condition of accelerated platelet consumption caused by anti-platelet autoantibodies, which mainly target platelet surface glycoprotein IIb/IIIa (GPIIb/IIIa) [1]. The majority of ITP patients maintain a safe platelet count by taking corticosteroids and/or undergoing a splenectomy, but 9–30% require additional treatment after failing to respond to initial treatment [2]. A number of immunosuppressants and other reagents are currently used for refractory ITP, but no gold standard has been established to date. Rituximab is a chimeric monoclonal antibody directed against CD20, which is a transmembrane protein present on the surface of a broad spectrum of B cells, but not on plasma cells [3]. Rituximab has been used extensively in the treatment of B-cell lymphoma, and several studies and case series in adults and children with refractory ITP have reported that approximately half the patients show a significant platelet response [3–6]. Because of its efficacy and relative safety, rituximab is becoming popular as a treatment for refractory ITP. Patients who achieve a complete response tend to maintain it for more than one year, but the relapse rate is approximately 40% [4,6]. We report here a patient with refractory ITP who initially responded to rituximab, but later became refractory. In this patient, the appearance of plasma cells producing anti-GPIIb/IIIa antibodies is likely to be responsible for rituximab resistance.

Results

As shown in Fig. 1A, platelet-associated anti-GPIIb/IIIa antibodies and anti-GPIIb/IIIa antibody-producing cells were markedly reduced during the first rituximab treatment, but these levels did not change during the third one. Flow cytometric analysis of the patient's PBMC revealed that $CD19^+CD3^-$ and $CD20^+CD138^-$ B cells were completely lost after rituximab treatment in both the first and third courses, although the pretreatment proportion of B cells was very low (<1%) for the third rituximab treatment (Fig. 1B). Interestingly, $CD20^-CD138^+$ plasma cells were detected in the circulation before and after the third rituximab course, when the patient had become refractory. To determine whether the $CD20^-CD138^+$ plasma cells in the peripheral blood produced anti-GPIIb/IIIa antibodies, PBMC depleted of $CD19^+$, $CD20^+$, $CD138^+$, or $CD34^+$ cells were used for the ELISPOT assay. As shown in Fig. 2, in samples taken before the first rituximab treatment, cells producing anti-GPIIb/IIIa antibodies were completely lost following the depletion of $CD19^+$ or $CD20^+$ cells, but not following the depletion of $CD138^+$ cells, indicating that the anti-GPIIb/IIIa antibody-producing cells were exclusively $CD19^+CD20^+CD138^-$ B cells. In contrast, before the third rituximab treatment, when the patient had become refractory to rituximab, the depletion of the $CD19^+$ or $CD20^+$ cells had a minimal effect on the number of antibody-producing cells, but $CD138^+$ cell depletion markedly reduced them, indicating that $CD19^-CD20^-CD138^+$ plasma cells predominantly produced the anti-GPIIb/IIIa antibodies. Bone marrow cells obtained three months after the third rituximab treatment also showed plasma cells to be the dominant population responsible for anti-GPIIb/IIIa antibody production. When PBMC from 10 randomly selected ITP patients who never received rituximab were examined using the ELISPOT assay, none of them had $CD138^+$ plasma cells producing anti-GPIIb/IIIa antibodies. HACA were positive and at an intermediate titer (174 ng/mL) at the time of the third rituximab treatment, but were not detectable prior to rituximab use.

Discussion

Our patient with refractory ITP initially responded to rituximab, but after retreatment she became refractory to it. Rituximab resistance is probably not simply due to HACA induced by the repeated use of this chimeric antibody, because the third rituximab treatment still induced complete B-cell depletion, and bone marrow cells responsible for anti-GPIIb/IIIa antibody production obtained three months after the third rituximab treatment showed a plasma cells phenotype. Data on the clinical significance of HACA during rituximab therapy are limited, but HACA have been discussed primarily in association with adverse events, including infusion reactions and serum sickness [7]. Rapid clearance of the drug may be of concern, but successful rituximab retreatment of lymphoma has been reported in a patient with HACA [8]. An alternative explanation, however, was suggested by our patient's having plasma cells producing anti-GPIIb/IIIa antibodies in the circulation as well as in

Contract grant sponsor: The Japanese Ministry of Health, Welfare and Labour.

*Correspondence to: Masataka Kuwana, Division of Rheumatology, Department of Internal Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan.
E-mail: kuwanam@sc.itc.keio.ac.jp

Received for publication 17 January 2007; Accepted 26 February 2007

Am. J. Hematol. 82:846–848, 2007.

Published online 15 May 2007 in Wiley InterScience (www.interscience.wiley.com).
DOI: 10.1002/ajh.20951

© 2007 Wiley-Liss, Inc.