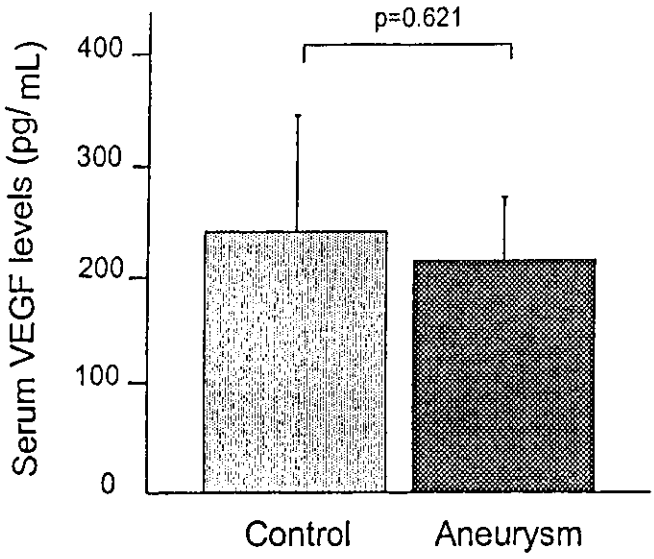

Fig. 3. Baseline serum vascular endothelial growth factor (*VEGF*) levels in the peripheral blood of patients with aortic aneurysm and control subjects. The VEGF levels were measured using an enzyme-linked immunosorbert assay kit. Results are shown as mean ± SEM

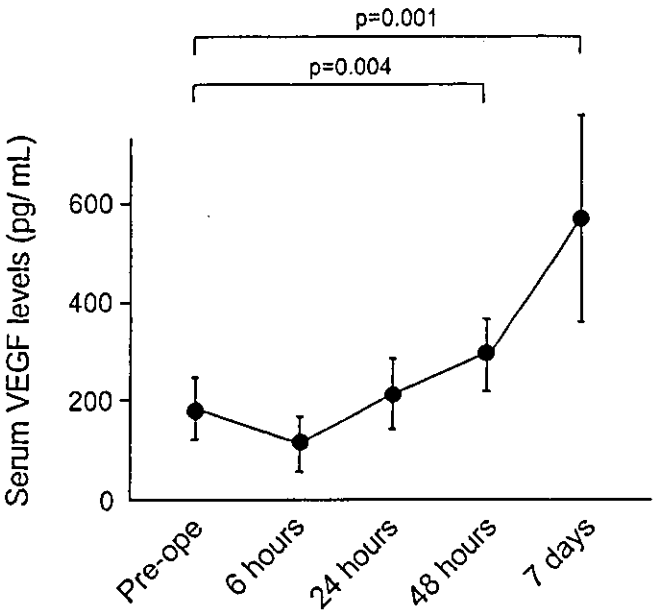

Fig. 4. Time course of serum VEGF levels after vascular prosthesis replacement. Samples were taken preoperatively (*Pre-ope*), 6, 24, and 48 h, and on the 7th day after surgery. There was a significant increase in the serum VEGF levels 48 h after surgery. Results are shown as mean ± SEM

formation.[1,12,13] It has been shown that EPCs have the capacity to be recruited into ischemic tissues or growing tumors.[3,13,14] In the present study, we demonstrated the mobilization of EPCs after vascular prosthesis replacement in patients with an aortic aneurysm.

In general, a neointima is not formed on vascular prostheses except at the anastomotic sites. However, Noishiki et al.[15,16] demonstrated that neointima formation was effectively accelerated by autologous bone marrow transplantation in experimental models. Rafii et al.[17] demonstrated evidence for EPCs originating from patients implanted with a left ventricular assist device (LVAD), where the surface of the titanium housing of the LVAD was colonized with bone marrow-derived circulating $CD34^+$ cells. Given also that several experimental studies have indicated a significant contribution of EPCs in adult neovascularization,[18–20] the increase in the number of $CD34^+$ cells after aortic aneurysm repair might contribute to the rapid endothelialization of vascular prostheses.

We also observed that the serum VEGF levels were significantly increased 48 h after the vascular surgery. Previously, Kobayashi et al.[21] reported that VEGF expression was widely detected in macrophages, monocytes, and smooth muscle cells of atherosclerotic aortic aneurysms. Tilson et al.[22] also reported upregulated VEGF mRNA expression in aortic aneurysms. Although there have been no reports on circulating VEGF levels after aortic aneurysm repair, Gill et al.[9] reported increased plasma VEGF levels after coronary artery bypass grafting. Also, in a study by Cotton et al.,[23] plasma VEGF levels were measured in patients undergoing coronary artery bypass grafting and cardiac valve replacement. Following surgery, there was a significant rise in VEGF in the former patients, but not in the latter, reaching a maximum (approximately two-fold increase) after 24 h.

It is known that VEGF mobilizes EPCs into the peripheral blood.[14,24,25] In this study, we observed that a rise in the circulating VEGF levels was followed by an increase in $CD34^+$ cell counts. These results suggest that the rise in circulating VEGF may contribute to the mobilization of EPCs after vascular repair. However, as there was no positive correlation between VEGF levels and $CD34^+$ cell counts (data not shown) and vascular surgery could promote the release of numerous known or as yet unrecognized chemocytokines, other factors in addition to VEGF may also contribute to the mobilization of $CD34^+$ cells.

Study limitations

We measured only CD34 as a marker of EPCs. Measurements of additional markers, such as VEGF receptor-2, further characterize the cells of interest. In addition, we do not have data for time points longer than 7 days after surgery and do not know whether the increase in $CD34^+$ cell counts was sustained over a longer term. In conclusion, the present study demonstrated that circulating EPC numbers as well as VEGF levels are increased after vascular repair with prosthetic vessels, which might be related to endothelialization after vascular repair.

Acknowledgments This study was supported in part by research grants from the Ministry of Education, Science and Culture, the Ministry of Health, Labor and Welfare, the Kato Memorial Trust for Nambyo Research, Mitsui Life Social Welfare Foundation, and the Vehicle Racing Commemorative Foundation.

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SCIENTIFIC LETTER

Decrease in circulating endothelial progenitor cells in patients with stable coronary artery disease

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The bone marrow derived endothelial progenitor cells (EPCs) are considered to originate from haematopoietic stem cells, which are positive for CD34.[1] Human CD34 expressing cells ($CD34^+$ cells) injected into nude mice and rats undergoing neovascularisation caused by hindlimb ischaemia are incorporated into the neovasculature and express endothelial antigens. We recently reported that transplantation of autologous bone marrow cells, including $CD34^+$ cells, improved ischaemia in patients with critical limb ischaemia.[2] Moreover, increased neovascularisation by bone marrow derived $CD34^+$ cells was shown to improve cardiac function.

Recently, a mobilisation of EPCs into circulation from bone marrow was reported in patients with acute myocardial infarction and acute coronary syndrome,[3 4] but little is known about the regulation of EPC mobilisation in patients with stable coronary artery disease (CAD). In the present study, we investigated the number of circulating $CD34^+$ cells in patients with CAD and the influence of atherosclerotic risk factors on this number.

METHODS

Thirty four patients (mean (SEM) age 62.5 (1.7) years) with angiographically documented stable CAD, and 36 healthy control subjects (mean (SEM) age 53.6 (2.0) years) without any evidence of CAD by history and physical examination, were enrolled in the present study. Risk factors for CAD were defined as: a history of hypertension for more than one year that required the initiation of antihypertensive treatment; a history of smoking more than one pack per year and currently smoking; hyperlipidaemia, defined as total cholesterol concentrations exceeding 5.70 mmol/l; and diabetes mellitus, defined as the need for oral anti-diabetic drug treatment or insulin use. The study protocol was approved by the ethics committee of Jichi Medical School, and informed consent was obtained from all patients and control subjects.

The number of $CD34^+$ cells in white blood cells (WBCs) was quantified by FACScan (Becton-Dickinson). In brief, WBCs were stained with a fluorescein isothiocyanate conjugated anti-CD34 monoclonal antibody (Becton-Dickinson, Franklin Lakes, New Jersey, USA). The samples were subjected to a two dimensional side scatter fluorescence dot plot analysis. After appropriate gating, the number of $CD34^+$ cells with low cytoplasmic granularity (low sideward scatter) was quantified and expressed as the number of cells per 10^3 WBCs.

Values were expressed as mean (SEM). Significance was evaluated using unpaired Student's *t* test for comparisons between two means. The interaction between the number of $CD34^+$ cells and risk factors was examined by multivariate analysis using the multiple stepwise logistic regression model. Differences of $p < 0.05$ were considered significant.

RESULTS

The number of $CD34^+$ cells in the peripheral blood of patients with CAD and control subjects was determined by flow cytometry. As shown in fig 1, circulating $CD34^+$ cells were significantly reduced, by approximately 30%, in patients with CAD compared with age matched control subjects.

We then investigated the influence of risk factors on the number of circulating $CD34^+$ cells. As shown in fig 2, univariate analysis identified diabetes mellitus as a significant predictor of a reduced $CD34^+$ cell count. In contrast, the number of $CD34^+$ cells did not significantly differ when patients were stratified according to sex, hypertension, hyperlipidaemia, and smoking.

Multivariate analysis also demonstrated that diabetes mellitus is a significant independent predictor of a reduced circulating $CD34^+$ cell count (standard coefficient −0.285, $p = 0.027$), whereas other factors were not significant.

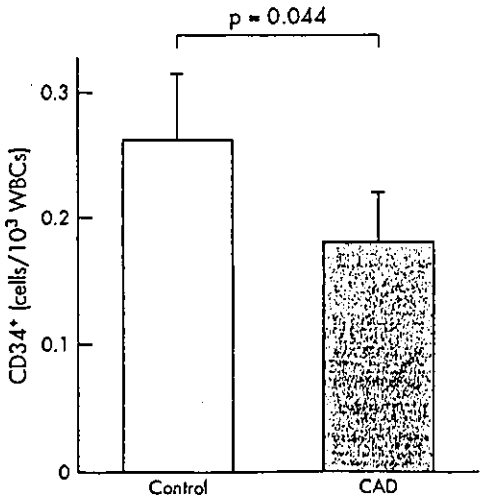

Figure 1 The number of circulating $CD34^+$ cells. $CD34^+$ cells were detected in peripheral blood from patients with CAD (n = 34) and healthy control subjects (n = 36).

DISCUSSION

The results of the present study demonstrate that the number of EPCs, as measured by the number of cells expressing CD34, was significantly reduced in patients with CAD compared with control subjects. Analysis of the individual risk factors indicated that subjects with diabetes mellitus had reduced numbers of circulating $CD34^+$ cells. Given that several experimental studies indicate a significant contribution of EPCs for adult neovascularisation, the reduction in the number of $CD34^+$ cells might contribute to reduced vascularisation in patients with CAD.

Abbreviations: CAD, coronary artery disease; EPC, endothelial progenitor cell; WBC, white blood cell

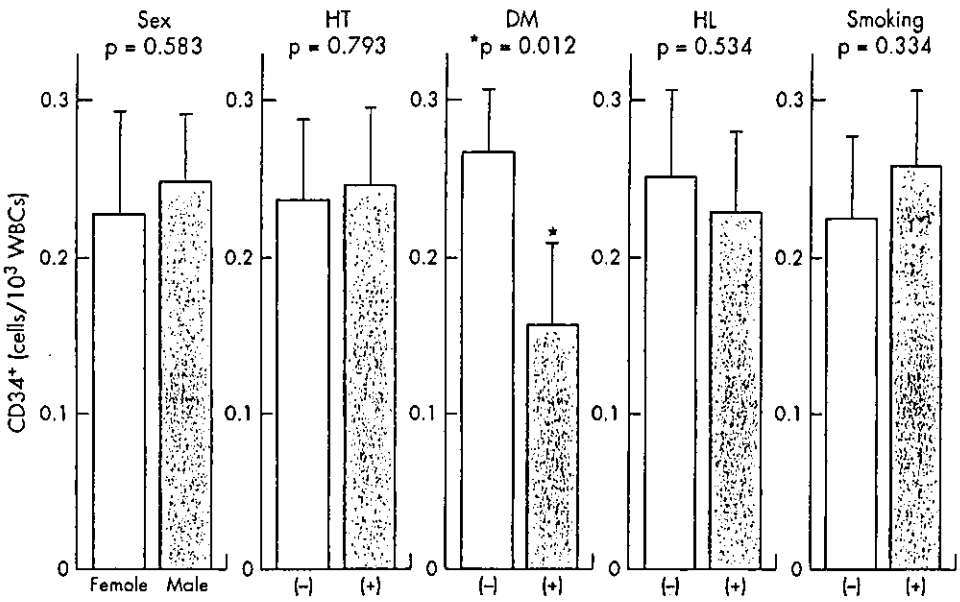

Figure 2 Effect of individual risk factors on the number of $CD34^+$ cells. DM, diabetes mellitus; HL, hyperlipidaemia; HT, hypertension.

The mechanisms by which diabetes mellitus reduces $CD34^+$ cell numbers remain to be determined. There are several possible scenarios by which diabetes mellitus could reduce the number of circulating $CD34^+$ cells. One explanation might be increased apoptosis of premature progenitor cells. Indeed, $CD34^+$ cells were shown to be very sensitive to apoptosis induction[5] and diabetes mellitus is known to increase oxidative stress, a well established stimulus for apoptotic cell death. Alternatively, diabetes mellitus may interfere with the signalling pathways that regulate EPC differentiation or mobilisation. The present results might be related to premature atherosclerosis and impaired collateralisation in diabetes mellitus, although further studies are required to prove this hypothesis.

In conclusion, the present study demonstrates that $CD34^+$ cell numbers are impaired in patients with CAD and this impairment is related to diabetes mellitus.

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Severe Hepatitis and Complete Molecular Response Caused by Imatinib Mesylate: Possible Association of Its Serum Concentration with Clinical Outcomes

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A 40-year-old female with chronic myelogeneous leukemia (CML) in the chronic phase was treated with imatinib mesylate (STI571) because of interferon resistance. She achieved complete cytogenetic response but not complete molecular response 3 months after STI571 administration. Six months later, she developed severe liver damage without evidence of actively infectious hepatitis A, B, C, G, E, TT virus, Epstein-Barr virus or cytomegalovirus. A significant serum level of STI571 (107 ng/ml) was detected, although she had not taken the drug for 6 days. Liver biopsy demonstrated massive hepatic necrosis, consistent with drug-induced hepatitis. She achieved complete molecular response, although she did not take STI571 for 47 days after the development of hepatitis. These results suggest that both hepatitis and molecular response were associated with the serum STI571 concentration.

Keywords: STI571; Serum concentration; Hepatitis; Molecular response

INTRODUCTION

STI571, an inhibitor of BCR-ABL tyrosine kinase, shows clinical activity in the treatment of CML in the chronic phase and less activity in the treatment of CML in blastic transformation [1–4]. Although STI571 has been well tolerated in clinical trials, various non-hematological adverse effects such as nausea, fluid retention, edema, muscle cramps and rash, most of which are mild, have been reported [5]. Recently, it has been reported that 3 female patients with CML treated with STI571 developed severe hepatitis [6,7]. We report a CML patient demonstrating both severe hepatitis and complete molecular response caused by STI571.

CASE REPORT

A 40-year-old female was diagnosed as having Philadelphia chromosome (Ph1)-positive CML in the chronic phase in December 1995 and received interferon soon after the diagnosis. Four months after interferon therapy, major cytogenetic response (Ph1, 1/29 cells) was obtained. Complete or major cytogenetic response was maintained from April 1996 to October 2000 by interferon alone. In November 2002, Ph1 in the bone marrow increased to 23/24 cells, although the interferon dose was increased. Therefore, STI571 at a daily dose of 400 mg started on January 2, 2003. At this time, there were no biochemical abnormalities including those of liver enzymes detected. The clinical course was uneventful and she had no adverse effects except slight leukocytopenia and anemia. On March 26, 2003, she achieved complete cytogenetic response in the bone marrow again. Although BCR-ABL messages were still detected by RT-PCR assay, 400 mg of STI571 was continuously given. On May 21, slight increase in aspartate aminotransferase (AST, 96U/l; normal range, 11–30) and alanine aminotransferase (ALT, 152 U/l; normal range, 4–30) but not total bilirubin (0.86 mg/dl; normal range, 0.29–1.03) was noticed. From June 5 to 7, she took STI571 but immediately vomited it. Between June 8 to 10, she did not take STI571 because of nausea. On June 11, she was admitted because of nausea and general fatigue (Fig. 1).

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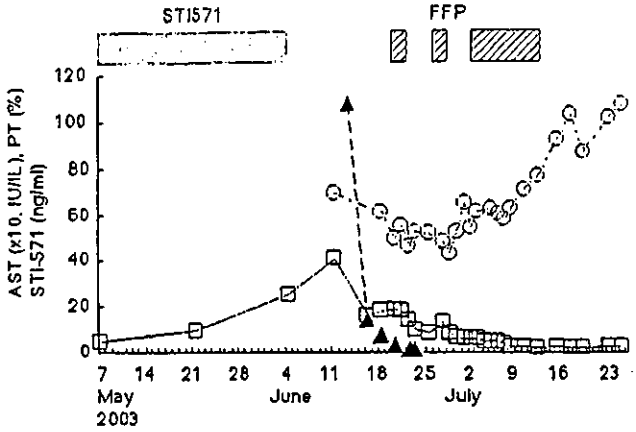

FIGURE 1 Clinical course. Open circles, open squares and filled triangles indicate percentage of prothrombin time, serum aspartate aminotransferase level and serum STI571 concentration, respectively. FFP, fresh frozen plasma.

Laboratory examination demonstrated increased levels of AST (406 U/l), ALT (559 U/l) and total bilirubin (2.7 mg/dl) and prolongation in prothrombin time (PT) (14.0 s, 69%; normal range, 10.4–12.2 s). The peripheral blood showed a hemoglobin level of 11.4 g/dl, a platelet count of 121 × 10^9/l, and a white blood cell count of 4.7 × 10^9/l with 57% neutrophils, 3% eosinophils, 1% basophils, 6% monocytes and 33% lymphocytes. Serological tests for IgM anti-hepatitis A virus (HAV) antibody, HBV surface antigen, HCV antibody and HEV antibody were negative. HGV, TT virus, HBV, HCV, cytomegalovirus and Epstein-Barr virus were not detected by the PCR method. Autoantibodies including antimitochondrial antibody and antismooth muscle antibody were negative. Ultrasonography of the abdomen showed nonspecific liver damage without gallstones. Computed tomography of the abdomen showed low-density lesions in the periportal areas of the liver, suggesting hepatitis.

She was suspected of having STI571-induced hepatitis [6], although drug lymphocyte-stimulating test for STI571 was negative. Residual STI571 in serum was demonstrated by liquid-chromatography tandem mass spectrometry [8] as follows: 107.0 ng/ml on June 13, 13.8 ng/ml on June 16, 7.4 ng/ml on June 18 and 3.6 ng/ml on June 20. STI571 had been discontinued since admission, however, total bilirubin increased to the maximum level of 9.98 mg/dl on June 27 and production of blood coagulation factors was impaired to 46% of PT, 39% of antithrombin III activity, 32% of protein C activity, and 139 mg/dl of fibrinogen on June 22. A total of 72 units of fresh frozen plasma were infused to replace coagulation factors produced in the liver. On July 23, percutaneous liver biopsy demonstrated severe centrolobular hepatic necrosis without evidence of veno-occlusive disease, consistent with drug-induced hepatitis [7]. Bone marrow aspiration the next day showed continuous complete cytogenetic response by conventional karyotypic analysis and there were no detectable BCR/ABL messages on RT–PCR. The patient was discharged on June 26 after recovery of hepatic function. On October 15, 2003, she still maintained normal blood counts with normal differential. A rechallenge test of STI571 has not been performed.

DISCUSSION

Our patient showed 2 important events during STI571 administration. One is drug-related liver toxicity. STI571 is not only metabolized by the cytochrome P450 enzymes, CYP3A4, CYP2C9 and CYP2D6 but also competitively inhibits CYP3A4 [9]. Drugs and foods inhibiting CYP3A4 such as erythromycin, clarithromycin, itraconazole and grapefruit juice increase the serum concentration of STI571 and lead to enhance STI571 toxicity in patients concurrently taking both STI571 and 1 of the CYP3A4-inhibiting agents [9]. Our patient took STI571 alone but not any agents, foods or supplements including herbs that affect CYP3A4. We do not know whether severe liver damage caused by STI571 is the result of immunologic idiosyncrasy (hypersensitivity reaction) or injury from a toxic metabolite (metabolic idiosyncrasy) [10]. Recently, Gambacorti-Passerini *et al.* have reported the pharmacokinetic analysis of STI571 in CML patients [11]. STI571 plasma concentrations were measured in 8 CML patients treated with 400 mg of the drug. After administration on day 1, peak concentration (C_{max}) of STI571 was achieved between 1 and 3 h; then in all of the patients, the drug was slowly cleared from plasma, being still detectable at 24 h. In 11 patients with CML treated with 400 mg, C_{max}, area under the curve in a 24-h period (24-h AUC) and half-life were 2.35 ± 1.0 μg/ml, 24.66 ± 8.5 μg.h/ml, and 12.5 ± 2.4 h, respectively. Similar analysis in healthy subjects treated with 400 mg of STI571 were shown in the CSTI571B2102 study conducted by Novartis Pharmaceuticals [12]: C_{max}, 1.56 ± 0.29 μg/ml; 24-h AUC, 16.30 ± 3.48 μg.h/ml; and half-life, 16.7 ± 3.1 h. Since a significant serum level of STI571 was detected 7 days after cessation of the drug administration in our patient, metabolic idiosyncrasy of STI571 in the liver is suggested. Interestingly, in our patient and others [6,7], severe liver damage caused by STI571 involved females in all cases. Therefore, we should carefully monitor STI571 administration to female patients with CML.

Another important finding in our patient is complete molecular response following severe liver damage caused by STI571. Before sustaining liver damage, the patient had achieved complete cytogenetic response but not complete molecular response by STI571. Complete molecular response was obtained followed by severe liver damage, although STI571 had not been given to the patient since the development of liver damage. As discussed above, severe liver damage in our patient was suggested to be associated with increased serum concentration of STI571. The achievement of complete molecular response may also have been caused by the

increased serum concentration of STI571. It is necessary to clarify the relationship between serum STI571 concentrations and clinical outcomes in CML patients.

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The authors wish to thank doctors in the Division of Gastroenterology for useful suggestion on drug-induced hepatitis.

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□ CASE REPORT □

Involvement of the Esophagus and Stomach as a First Manifestation of Varicella Zoster Virus Infection after Allogeneic Bone Marrow Transplantation

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Abstract

A 46-year-old man with myeloproliferative disorder received a stem cell transplant from an HLA-identical unrelated donor. Eight months status post transplantation, during the course of tacrolimus therapy, the patient developed severe epigastric pain and fever. FGS findings showed eruptions with blisters in the esophagus and ulcers in the stomach. Biopsy specimens revealed acidophilic inclusion bodies in the nuclei. Varicella zoster virus (VZV) DNA copies were detected in the serum. No skin lesions were observed prior to hospital admission. The diagnosis of visceral VZV infection was made and the gastric and esophageal lesions were successfully healed with acyclovir (ACV). Severe abdominal pain is one of the most important signs of VZV infection for recipients of stem cell transplantation.
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Key words: visceral varicella zoster, gastric ulcer, BMT, FGS, acyclovir

Introduction

VZV infection is the most common viral disease in the late post-transplant period following allogeneic bone marrow transplantation (BMT), occurring with a frequency of 17% to 50% (1–4). Typically, the infection remains cutaneous, although visceral dissemination is well-recognized, and carries an appreciable risk of mortality. Visceral disease nearly always occurs after skin lesions have been noted (5, 6), although there have been occasional reports of the disease occurring without any cutaneous manifestations.

In our case, we were able to diagnose visceral VZV infection via fiber-gastroscopy (FGS) prior to the appearance of the classical skin rash. Immediate therapy with ACV led to a successful outcome for the patient's visceral VZV infection.

Case report

A 46-year-old man was diagnosed with a severe myeloproliferative disorder in 1997. The patient developed leukemic cell infiltration in the pulmonary and anal regions in November 1999. Due to a lack of HLA-identical siblings, he received an allogenic bone marrow transplant from an unrelated HLA-identical male donor on February 23, 2001. DRB1 genotype was identical in both the donor and the recipient. The conditioning regimen consisted of total body irradiation at 1,200 cGy in six fractions from days –10 to –8; cytarabine, 1 g/m^2 twice daily from days –6 to –4; and cyclophosphamide, 60 mg/kg from days –3 and –2. GVHD prophylaxis involved short-term methotrexate and tacrolimus. Serum VZV titer was positive.

The following complications arose during the post-transplant course. On day 26 post-BMT, adult respiratory distress syndrome (ARDS) was diagnosed and was subsequently successfully treated with prednisolone. On day 82, mild GVHD (grade I) developed, and was responsive to a short course of prednisolone. On day 87, steroid and tacrolimus-induced diabetes developed, requiring treatment with insulin. On day 119, pneumocystis carinii pneumonia developed, which was treated with sulfamethoxazole/trimethoprim. On day 140, serum IgG and IgA levels were 652 mg/dl and 98 mg/dl, respectively, and CD4/CD8 ratio was 0.8.

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The patient was readmitted on November 4, 2001 (day 255), after reporting a four-day history of progressively worsening severe epigastric pain radiating to the back, and a moderate grade fever (below 39°C). The presence of mild stomatitis strongly suggested chronic GVHD. Tacrolimus had been continued at 1.4 mg/day for treatment of chronic GVHD, and the serum trough level was 9.9 ng/ml the morning after admission. Prior to the onset of abdominal pain, no history of exposure to VZV was reported.

Physical examination revealed marked epigastric tenderness without rigidity. Back pain was exacerbated in the standing position. No eruptions were observed on the face or trunk. Laboratory results were as follows: WBC $4.9\times10^9/l$, Hb 14.0 g/dl, and platelet count $42\times10^9/l$. Liver and renal function were normal. C-reactive protein titer was elevated (2.6 mg/dl). VZV DNA was detected in serum at 900 copies/10^6 cells by PCR, while HSV and CMV DNA were not detected. On admission, FGS examination was performed. Several blistering eruptions were noted on the esophageal mucosa (Fig. 1A) and active stage (A2) ulcers were visualized in the mid-portion of the stomach (Fig. 1B). On the same day, initial pathologic examination of biopsied mucosa from the gastric ulcers demonstrated acidophilic nuclear inclusion bodies and cytoplasmic edema (Fig. 1C), typical features of VZV-infected tissue. Serum VZV DNA results were obtained the morning after admission, confirming a diagnosis of visceral dissemination of VZV. Intravenous ACV (5 mg/kg three times daily) was therefore commenced immediately and eruptions appeared on the patient's face, scalp, trunk, and oral mucosa on the evening of the same day. Shortly after ACV treatment was commenced, the epigastric pain resolved. On the seventh day of admission, the skin lesions had dried and crusted over and by the tenth day healing (H1 stage) of the gastric ulcers was observed (Fig. 1D). The patient was discharged 14 days after admission.

Discussion

Various viral diseases (including CMV, adenovirus, and EBV) are among the important complications that may present months after hematopoietic stem cell transplantation (7). However, as the clinical course is not uniform, it can be very challenging to arrive at a prompt diagnosis from the presenting clinical symptoms seen in the early phase. Although early treatment following immediate diagnosis is very important, diagnosis is often only possible after significant clinical progression of the infectious disease.

In the present case, fever and intense abdominal pain without cutaneous symptoms appeared eight months after allogenic bone marrow transplantation. At that time, the differential diagnosis was considerable, including acute gastric ulcer, acute enteritis, ileus, mesenteric artery thrombosis, acute pancreatitis, acute cholecystitis, and urinary calculi. In order to evaluate the abdominal distress, we initially performed physical examination, routine laboratory tests, and abdominal roentgenography. It was subsequently recognized that FGS should be urgently performed for diagnosis. On endoscopy, biopsies of the gastric lesions were taken, enabling immediate diagnosis of VZV infection.

The differential diagnosis of abdominal pain after stem cell transplantation is vast and varied, including gram-positive bacterial, fungal, or viral (VZV, HSV, EBV, CMV) infectious disease; enteritis due to chronic GVHD; TMA (thrombotic microangiopathy); and ischemia. In many cases, serologic examination is useful for differentiating between these causes of abdominal pain. While in some cases serum virus antibody titer, ultrasound imaging, or roentgenography are useful for diagnosis, these tests may not enable definitive diagnosis in all cases. When questions remain, digestive tract endoscopy is a very useful modality for quickly arriving at a diagnosis, because it allows direct observation and biopsy of lesions.

The important differential diagnoses are CMV gastritis and Herpes simplex infection. In the present case, ulcer tissue demonstrated VZV-specific findings such as nuclear acidophilic inclusion bodies, cytoplasmic bullous edema, and large cells with multiple nuclei. On November 17, the final pathologic report indicated that immunopositivity was only seen for VZV, with no tissue staining for CMV or Herpes simplex 1. These results thus confirmed the initial diagnosis.

The frequency of VZV infection in recipients of allogeneic BMT ranges from 17% to 50% (1–4). VZV is one of the most common late infections, typically occurring 3–6 months after transplantation. In the majority of patients, VZV infection occurs as a result of reactivation of latent virus, and usually presents as a dermal infection with subsequent cutaneous dissemination (1). Approximately 10–15% of patients progress to further visceral dissemination, which carries a reported mortality of 9% (2). In the present case, VZV infection occurred during tacrolimus administration, on day 255 after BMT. This was unusual, as activation of latent virus generally occurs earlier in the post-transplant course. It is unclear which patients have a high risk of VZV visceral dissemination following BMT. Nevertheless, there is no evidence that chronic GVHD prophylaxis with tacrolimus increases the risk of visceral VZV infection (2, 8).

Recently, Yagi et al (8) reported that seven of nine patients with VZV infection presented with abdominal pain as their initial symptom, prior to the appearance of skin lesions. However, FGS was not performed during the initial diagnostic workup in any of these cases. In this report, we describe a case of visceral VZV infection in which FGS enabled direct diagnosis prior to the manifestation of skin lesions. We believe that the immediate institution of ACV treatment following the prompt diagnosis of visceral VZV infection resulted in attenuation of this infection, which is generally associated with a high risk of mortality.

Severe abdominal pain is one of the most important and earliest signs of VZV infection in immunocompromised hosts, such as recipients of stem cell transplantation (8). We suggest FGS to be the most useful and definitive examina-

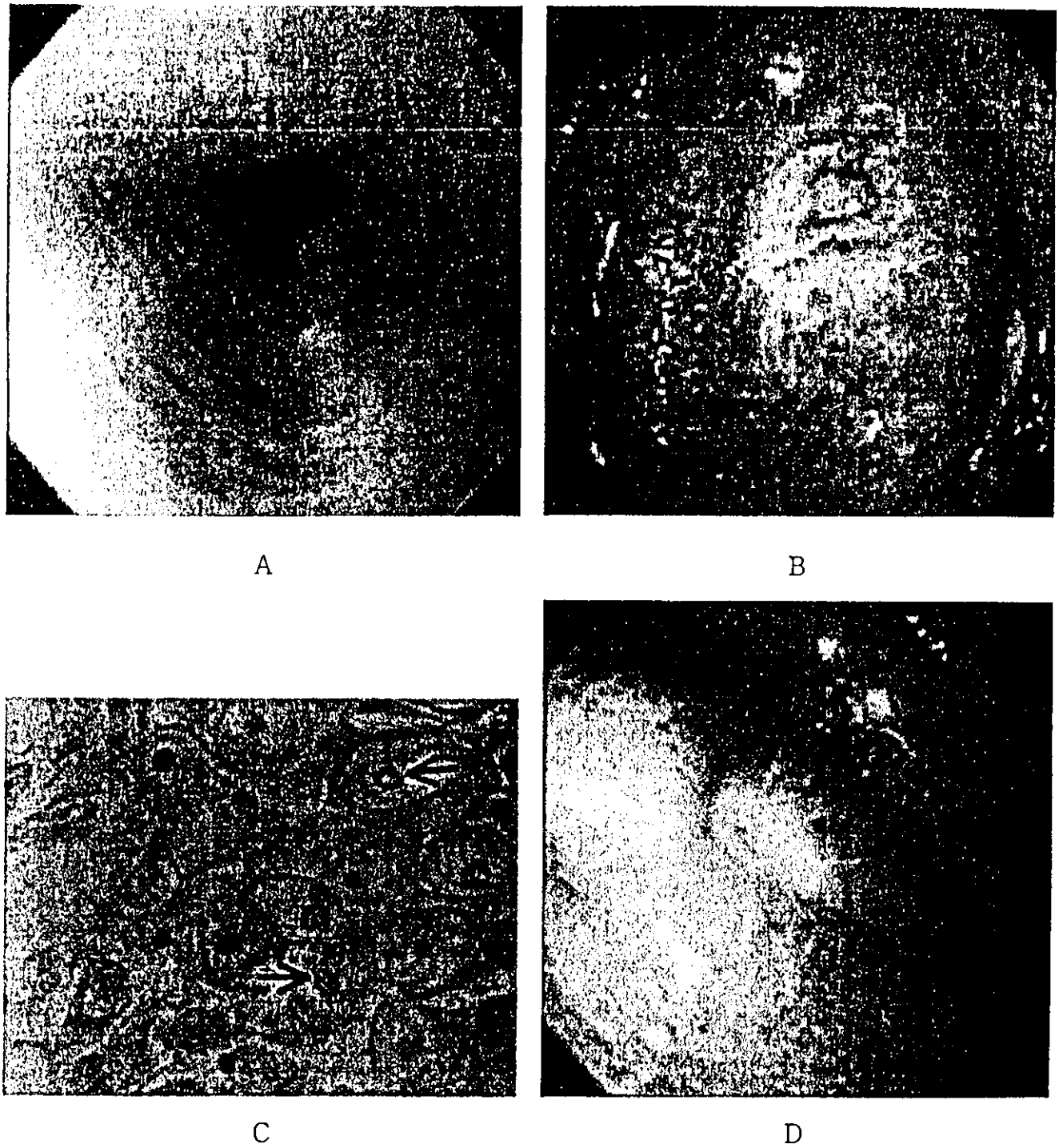

Figure 1. Fiberscope findings at admission. Eruptions with blisters in the esophagus (A) and an active stage ulcer in the midportion of the stomach (B) were observed. Cells in the stomach ulcer had nuclear acidphilic inclusion bodies (arrows) and cytoplasmic edema (C). The stomach ulcer was in a healing stage after acyclovir treatment (D).

tion in these circumstances, allowing immediate diagnosis and rapid institution of antiviral therapy to treat this life-threatening disease.

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症 例

骨髄壊死による膝関節痛が先行出現したminor bcr/abl キメラ遺伝子陽性急性リンパ性白血病

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室井一男[1,2]，小松則夫[1]，小澤敬也[1,2]

症例は16歳男性。白血病の疑いにて当科に入院したが，骨髄標本では一部にリンパ芽球の集塊を認めるのみで全体が壊死組織で占められていた。入院5カ月前より右膝関節痛を自覚しており，膝関節MRIでは大腿骨遠位端周囲に造影効果を伴う低信号域を認め，広範な骨髄壊死の所見であった。急性リンパ性白血病（ALL）と診断し化学療法にて寛解導入したところ，寛解時骨髄標本では壊死組織の消失と造血の回復を認め，膝関節痛も消失した。非血縁者間骨髄移植を施行し，26カ月間寛解を維持している。移植後に施行した膝関節MRIでは壊死組織は脂肪髄化し，その変化は可逆性であった。骨髄壊死は稀な病態であるが，本例は原疾患である白血病の治療により壊死の改善を認め，その経過を追跡できたため報告する。（臨床血液45（11）：1203～1207，2004）

Key words：Bone marrow necrosis, Acute lymphoblastic leukemia, Magnetic resonance imaging (MRI), Knee joint

緒 言

骨髄壊死は造血器腫瘍をはじめとする悪性腫瘍や感染症などに伴うまれな合併症のひとつであるが，生前に診断されることは少なく，その殆どが骨髄穿刺にて偶発的に発見される[1~3]。今回われわれは大腿骨の広範な骨髄壊死による膝関節痛が先行出現した急性リンパ性白血病を経験した。寛解を得たのちに非血縁者間骨髄移植を行い，骨髄壊死の回復を確認している。骨髄壊死による症状が先行し，経過を追跡できた報告は稀であり，文献的考察を加えて報告する。

症 例

患 者：16歳，男性。

主 訴：右膝関節痛，腹痛。

既往歴：特記すべきことなし。

現病歴：2002年11月より運動時の右膝関節痛を自覚し，2003年1月には安静時にも拍動性の疼痛を認めるようになった。整形外科にて施行された右膝関節のMRIで大腿骨遠位端に不整な信号域を指摘されたが，骨腫瘍としては典型的ではないため，鎮痛薬投与にて経過観察された。クラブ活動（野球部）を休部し運動を控えたが，症状は改善せず，3月下旬よりあらたに発熱と腹痛を認めたため4月6日に当院を受診。末梢血中に芽球を認め，白血病の疑いにて同日入院した。

入院時現症：身長162cm，体重54kg，体温38.5度，脈拍70/分，整，血圧124/70mmHg。眼瞼結膜に貧血を認めず。表在リンパ節を触知しない。心肺に異常所見なし。腹部では肝を3横指，脾を4横指触知し，腸音は減弱。疼痛のため，右膝関節は屈曲制限あり。

入院時検査所見（Table 1）：末梢血白血球数は30,500/μlと増加し，リンパ芽球を59.2％認めた。凝固異常は軽度であった。生化学検査ではLDHが2,156 IU/lと著増し，軽度の肝機能障害を認めるほかCRPが8.3mg/dlと高値であった。骨髄穿刺（胸骨）ではほぼ全体が壊死した死細胞で占められており，造血細胞がわずかに点在するのみであった（Fig. 1）。残存する細胞集塊の一部に核小体を有する中型の芽球が認められ，ペルオキシダーゼ染色は陰性であった。芽球はフローサイトメトリーによる表面抗原解析にてCD10, CD19, CD20, CD34及びHLA-DR陽性を示した。G-bandingによる染色体分析は正常男性核型であったが，RT-PCR法にてminor bcr/ablキメラ遺伝子を検出した。また腹部CTにて著明な肝脾腫を認め，腸管は圧排されてイレウスの所見を呈していた。

入院後経過：骨髄壊死を伴ったminor bcr/ablキメラ遺伝子陽性急性リンパ性白血病と診断し，prednisolone（PSL）を先行投与後，4月17日より寛解導入療法（cyclophosphamide（CY），daunorubicin, vincristine（VCR），L-asparaginase）を開始した。化学療法後の骨髄像では壊

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Table 1 Laboratory data on admission

Peripheral blood		Chemistry		Coagulation	
WBC	30,500/μl	TP	7.4 g/dl	PT	13.8 sec
Blast	59.2 %	Alb	3.5 g/dl	APTT	33.2 sec
Pro	1.4 %	BUN	9 mg/dl	Fbg	460 mg/dl
Myelo	4.2 %	Cr	0.81 mg/dl	ATIII	88.5 %
Meta	2.2 %	ALP	483 IU/l	FDP	8.6 μg/ml
Band	6.8 %	AST	42 IU/l		
Seg	6.8 %	ALT	21 IU/l		
Eos	1.2 %	LDH	2,156 IU/l	Bone marrow	
Lymph	17.8 %	γ-GTP	48 IU/l	NCC	13.0×10^4/μl
Mono	1.8 %			Meg	0/μl
Aty-L	0.2 %	Serology		M/E ratio	22.0
		CRP	8.3 mg/dl	Blasts	50.5 %
RBC	589×10^4/μl	ESR	28 mm/hr	(excluded necrotic cells)	
Hb	15.5 g/dl				
Ht	45.8 %				
Plt	15.1×10^4/μl				

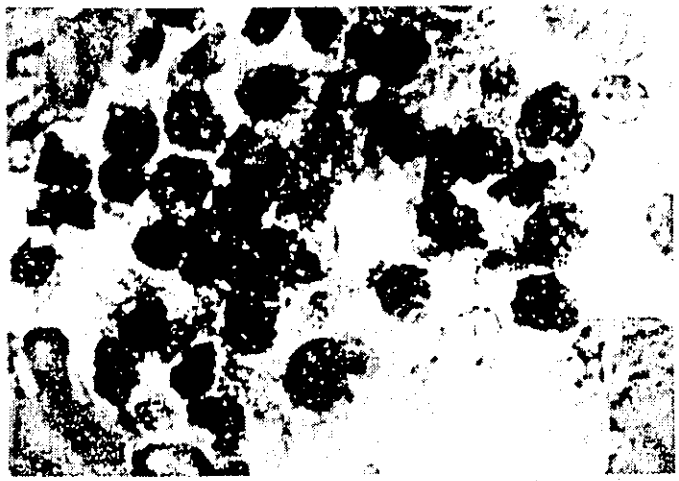

Fig. 1 Bone marrow aspiration showed extensive invasion of necrotic cells.

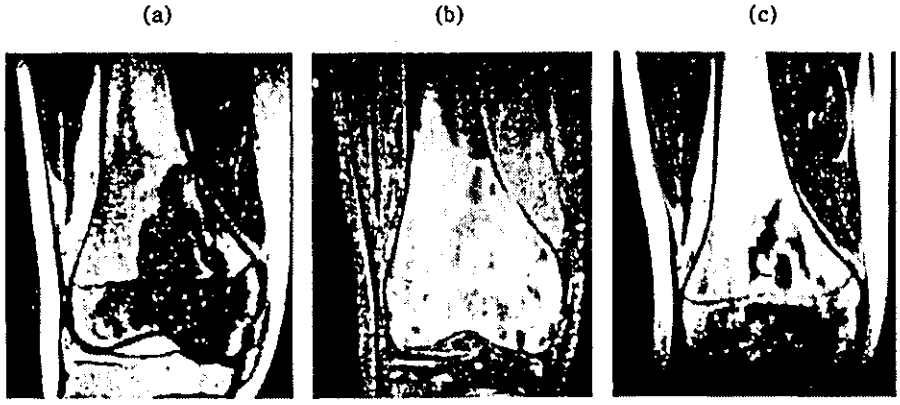

Fig. 2 MRI findings in the right knee joint at the time of diagnosis. MRI of knee joint showed irregular shaped low signal intensity in the subchondral region on T1-weighted images (a) and peripheral rim enhancement on Gd-DTPA enhanced fat suppression images (b). T1-weighted MR images sixteen months after onset of bone marrow necrosis. Low signal intensity areas in knee joint have been reduced (c).

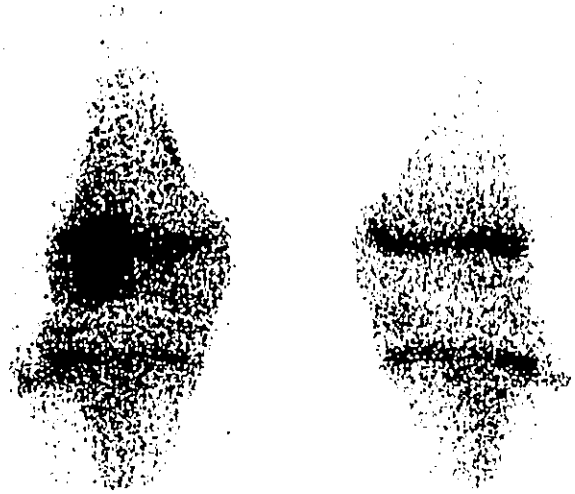

Fig. 3 ^{99m}Tc-bone scintigram revealed uptake in lateral side of right knee joint.

死組織はなく，3 系統の造血細胞の回復を認めた。骨髄芽球は2.8％でリンパ芽球を認めず，寛解後療法としてhyper-CVAD（CY, VCR, doxorubicin, dexamethasone）とHD-MTX/ Ara C（methotrexate, cytarabine, methyl PSL）による交替療法を3クール施行した。PSLの内服開始後，右膝関節痛は劇的に軽減し，寛解確認時には疼痛は消失した。5月30日に施行した右膝関節のMRIでは，T1強調画像で大腿骨遠位端に地図状の辺縁不明瞭な不均一な低信号域を認めた（Fig. 2a）。脂肪抑制造影T1強調画像で周囲に輪状の造影効果を認め，骨髄壊死の所見であると診断した（Fig. 2b）。3月に施行された^{99m}Tc骨シンチグラムで右大腿骨遠位端，膝蓋骨及び脛骨近位端に集積の亢進を認めたが，多骨性に分布していることから白血病細胞の腫瘤形成によるものは否定的で，同部にも骨髄壊死を併発していたものと推察した（Fig. 3）。血縁者のHLAを検索するも不一致のため，HLA full matchのドナーより11月26日に非血縁者間骨髄移植を施行した。grade 2の皮膚graft-versus-host disease（GVHD）を合併したが，PSLの投与にて軽快し，Day34に退院した。2004年5月に施行したMRIでは，T1強調画像で大腿骨遠位端の壊死部位は明らかに縮小し（Fig. 2c），周囲の造影効果は消失していた。現在も寛解状態を維持しており，膝関節に機能的障害を残すことなく，クラブ活動にも復帰している。

考　察

骨髄壊死は稀な病態であるが，基礎疾患としては悪性腫瘍の頻度が高く，特に造血器疾患に合併することが多いと報告されている[1~3]。発症機序として，腫瘍細胞等による微小塞栓が骨髄の虚血をきたすためと考えられている。造血器疾患の初診時の骨髄標本を検討した報告によると骨髄壊死の頻度は0.15～2.2％であり，原疾患としては急性リンパ性白血病の頻度が高い[3,4]。その他の原因として敗血症，播種性血管内凝固症候群，インターフェロンやG-CSF，亜硫酸などによる薬剤性の報告もある[5~7]。症状として骨痛と発熱が代表的で70～80％の症例に認められる[3]。壊死が広範に生じた場合には貧血や血小板減少を合併し，造血不全に陥る例も報告されている[1~3]。腫瘍細胞から分泌されるTumor Necrotic Factor（TNF）が骨髄壊死を惹起するとの報告もあるが，症例によっては必ずしもTNFは高値を示さない[8,9]。急性リンパ性白血病に骨髄壊死を合併した症例は小児に多い傾向があり，小児と成人リンパ性白血病との病態の違いを考えるうえで興味深い[9~12]。いずれの症例も無症状で，骨髄穿刺時に偶発的に発見されたものが殆どであり，本例のように骨髄壊死を広範に認め，原疾患の発症に先行して局所症状が出現することはきわめて少ない。

本例は若年男性で活発にクラブ活動を行っていた。思春期の膝関節痛の原因としてはまずスポーツ外傷や骨肉腫などの原発性骨軟部腫瘍を考えるため，初発時に骨髄壊死を鑑別することは困難であり，MRI及び^{99m}Tc骨シンチグラムなどの画像検査にも限界がある。骨髄壊死単独では必ずしも致死的合併症とならないが，原疾患の診断の遅れによっては予後不良の転帰をとることもあり[1~3]，原因不明の関節痛は慎重に経過観察すべきものと考える。骨髄壊死とその背景に存在する原疾患の診断に骨髄穿刺は不可欠であるが，塗抹標本ではいわゆるgelatinous transformationを呈し，ときとして確定診断までに頻回の穿刺を要する[1]。壊死巣は骨髄中にmultifocalに散在するため，単回の骨髄穿刺では壊死の程度を必ずしも反映せず，しばしば診断に難渋する。また骨髄壊死をきたした場合には，骨髄穿刺では十分に組織が吸引できないこと

が多く，dry tapを呈することも報告されている[1~4]。本例では大腿骨骨髄壊死の評価のためにMRIにより病変を追跡した。骨髄壊死では骨髄中の脂肪が壊死組織に置き換わることにより水濃度が増加するため，典型例ではT1強調画像で低信号域を示し，脂肪抑制造影T1強調画像で周囲に輪状の造影効果を認める。治癒過程では脂肪髄化するため，造影効果の消失とT1強調画像にて高信号領域が増加することで判断できる[13]。MRIは造血能評価の補助診断に用いられてきたが，非侵襲的かつ広範囲の骨髄が検索可能であり，骨髄壊死の評価においてもきわめて有用である[14, 15]。

骨髄壊死を併発した白血病の治療戦略については一定した見解が得られていない。本例は右膝関節への放射線照射を追加すべきか判断に迷うが，エビデンスがない。寛解後早期に疼痛の劇的改善を認め，関節可動域も正常化していることから本例は経過観察にとどめている。骨髄壊死を合併した場合には化学療法後に骨髄抑制が遷延するとの報告もあるが，本例の造血能回復はすみやかであり，白血病の治療と相関して骨髄壊死も改善を認めた。原因不明の骨髄壊死が観察される場合には，造血器疾患を念頭におき骨髄検査を実施する必要があり，早期診断，早期治療が原疾患のみならず局所の改善をもたらすと考える。

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Minor bcr/abl positive acute lymphoblastic leukemia preceded by knee joint pain due to bone marrow necrosis

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Key words : Bone marrow necrosis, Acute lymphoblastic leukemia, Magnetic resonance imaging (MRI), Knee joint

A 16-year-old male was referred to our hospital in April 2003 due to severe knee joint pain from five months previously. Lymphoblasts were identified in his peripheral blood, resulting in a diagnosis of acute lymphoblastic leukemia (ALL). Bone marrow examination revealed massive necrosis with clusters of lymphoblasts and the bcr/abl fusion gene. Magnetic resonance imaging (MRI) of the knee joint showed low signal intensity on T1-weighted images, and peripheral rim enhancement on Gd-DTPA enhanced fat suppression images, which was compatible with bone marrow necrosis. After the patient achieved complete remission (CR), the knee joint pain has disappeared. He was treated with an allogeneic bone marrow transplantation (BMT) from an HLA-identical unrelated donor and has been in CR for 26 months after the diagnosis of ALL. In the knee joint, the replacement of fatty marrow after BMT has been confirmed with MRI. Hematological malignancies including ALL should be considered in the cases of bone marrow necrosis and adequate treatment may improve necrosis.

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短 報

特発性血小板減少性紫斑病に対する *Helicobacter pylori* 除菌療法 －*Helicobacter pylori* の活動性と血小板数との相関－

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Key words : Idiopathic thrombocytopenic purpura (ITP), *Helicobacter pylori*

緒 言

特発性血小板減少性紫斑病（ITP）に対する *Helicobacter pylori*（*H. pylori*）除菌療法は，本邦では38.4～63.2％の高い奏効率が報告されている[1,2]。除菌薬剤には血小板増加作用や免疫抑制作用を示すものはなく，*H. pylori* 感染の終焉がITPの寛解に結びつくものと考えられる。われわれはITPに対する除菌療法を追試し，臨床的検討を行った。初回除菌療法不成功のため再治療を必要とした1例は，*H. pylori* 感染と血小板数の推移に強い相関を示したため，臨床経過に文献的考察を加え報告する。

対象と方法

非寛解期のITP 11症例を対象とし，*H. pylori* 感染の有無を検討した。平均罹病期間は75.4カ月で，11例中10例が発症から6カ月以上経過した慢性型のITPで，1例は発症1カ月の急性期であった。年齢中央値は51歳で，男性3例，女性8例であった。11例中7例は副腎皮質ホルモン，脾摘などの治療歴を有するも無効ないしは薬剤の減量に伴って再燃した症例であった。*H. pylori* 感染は迅速ウレアーゼ試験あるいは尿素呼気試験を用いて判定した。*H. pylori* 感染例には，インフォームドコンセントを書面にて得たうえで除菌療法（ランソプラゾール，アモキシシリン，クラリスロマイシン）を施行した。除菌療法の判定は尿素呼気試験を用いて原則的に6週間後とした。血小板数の追跡期間は6カ月以上とし，増悪例を除いてあらたな治療を追加しないこととした。

結 果

11例中9例に *H. pylori* 感染を認め（81.8％），感染が確認された全例に除菌療法を施行した（Table 1）。全例で除菌に成功したが，1例は初回除菌療法が不成功で再除菌療法により除菌に成功した。治療後早期に血小板の増加を9例中5例に認め，奏効率は55.6％であった。副作用は1例に軽度の薬疹をみたのみであり，追跡期間中に原疾患の増悪をきたした症例はなかった。

再除菌療法を必要とした症例9の血小板数の推移を示す（Fig. 1）。除菌療法1カ月後に血小板数は20.6万/μlと著明に増加したが，効果は一過性で3.4万/μlまで減少した。尿素呼気試験は除菌療法前の59.3‰から治療後は29.6‰と低下したものの依然として陽性であった。再除菌療法を施行したところすみやかな血小板数の増加を認め，尿素呼気試験は1.5‰と陰性化した。現在は21カ月間の長期寛解を維持している。

考 察

症例数は少ないものの，われわれの臨床経験ではITPに対する除菌療法の奏効率は55.6％と従来の報告とほぼ同等の高い治療成績を挙げている[1~4]。非侵襲的で安全性の高い治療としても評価は高い。一方でJarqueやMichelらの報告では奏効率は7～13％と低く，少なくとも初回治療として *H. pylori* 感染ITP患者に除菌療法を行うことに否定的な見解を示すものもある[5,6]。また除菌療法の有効性や病態への関与は，いわゆる急性型においては検討されておらず，二次性血小板減少症での治療効果は否定的である[1]。今回われわれは発症1カ月のITP症例に除菌療法を施行した。除菌不成功のため一過性であったものの明らかな血小板増加を認め，発症早期においても除菌療法が有効であった。但し急性型は成人では稀であり，本症例も未治療であれば慢性型の経過を辿った可能性が高い。従って必ずしも急性型に対する *H. pylori* の関与や

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Table 1 Clinical and laboratory characteristics in 11 idiopathic thrombocytopenic purpura patients

No.	Patient Sex	Disease duration (month)	Previous treatment	*H.pylori* infection	Urea breath test		Platelets ($\times 10^4/\mu l$)	
					before	after	before	after
1.	52F	6	PSL, m-PSL	Yes	9.2	1.2	1.9	26.4
2.	25M	18	None	Yes	47.4	0.7	9.6	14.9
3.	71F	72	PSL	Yes	23.8	0.7	5.5	17.0
4.	31F	38	PSL, IVIG	Yes	RUT3+	0.9	3.9	3.9
5.	64F	288	PSL, splenectomy	Yes	26.6	1.2	1.9	1.6
6.	53F	144	None	Yes	RUT3+	1.5	6.9	5.0
7.	42F	12	None	Yes	39.3	0.7	6.1	8.0
8.	50M	86	PSL, splenectomy	Yes	58.3	1.5	3.8	14.4
9.	57F	1	None	Yes	59.3	29.6	2.7	24.4
10.	53F	32	None	No	2.1	ND	3.7	ND
11.	61F	132	PSL,splenectomy, AZT	No	0.7	ND	1.0	ND

PSL, prednisolone; m-PSL, methyl-prednisolone; IVIG, intravenous immunoglobulin
AZT, azathiopurine; RUT, rapid urease test; ND, not done

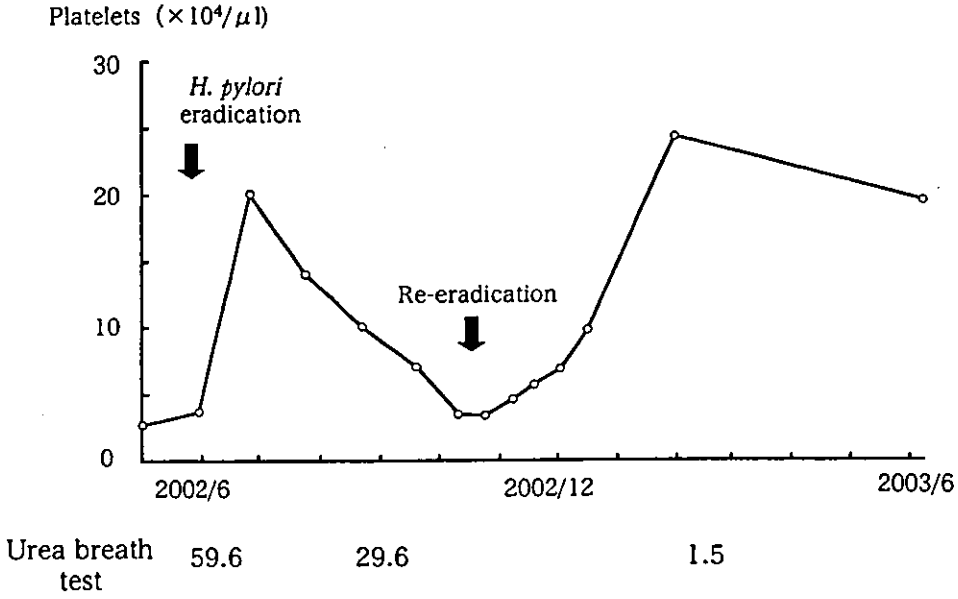

Fig. 1 Clinical course
Although platelet recovery was transient after first eradication therapy, complete *H. pylori* eradication led to long time complete remission.

除菌療法の有効性を示唆するものではないが，重度の出血傾向を伴わない発症早期の症例においても除菌療法は検討すべき治療となる可能性がある。現在本邦で策定中のITPの新規治療ガイドラインにおいて除菌療法は第一選択としての位置づけが検討されている。

血小板減少の機序としてGasbarriniらは*H. pylori*と血小板膜蛋白との共通抗原に対する交差反応の可能性を報告している。また本邦ではTakahashiらがPAIgGと*H. pylori*のCagA蛋白との分子相同性を報告し，地域によって除菌療法の奏効率にばらつきがあるのはCagA蛋白の有無によると推察している[7,8]。今回われわれが再除菌療法を施行した症例は初回除菌療法が不成功にもかかわらず一過性の血小板増加を認めた。不十分ながら治療により*H. pylori*がある程度死滅したことによって血小板が増加し，経時的な*H. pylori*の菌量の増加によって再燃したものと考えられる。従って分子相同性による交差抗原抗体反応のみでは本例のような血小板数と*H. pylori*除菌との迅速かつ定量的な相関は説明できず，*H. pylori*が直接ないしは間接的に血小板に作用している可能性が示唆される。

われわれの臨床成績の検討では除菌療法の効果は完全寛解か，或いは全く無効であるかのいずれかであり，*H. pylori* 関連血小板減少症といった独立した疾患群が示唆された。一方で本邦の調査研究では部分寛解例も多く報告されており，必ずしも単一の病態では説明できない血小板減少症も存在している。病態については検討の余地が残るものの，従来の報告とわれわれの臨床経験と併せて，少なくとも本邦では一部の症例における除菌療法の有効性は確立されたものといえる。今回の知見から発症早期の除菌療法及び初回除菌不成功で一過性に血小板増加をみた場合の再治療についても効果が期待され，積極的に検討する必要があるものと考える。

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Helicobacter pylori eradication in patients with idiopathic thrombocytopenic purpura —The associateion between the activity of *Helicobacter pylori* and platelet recovery—

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Key words : Idiopathic thrombocytopenic purpura (ITP), *Helicobacter pylori*

We investigated the prevalence of *Helicobacter pylori* infection and the effect of eradication therapy in patients with idiopathic thrombocytopenic purpura (ITP). *H. pylori* infection was found in 9 of 11 patients (81.8%). *H. pylori* eradication was obtained in all patients and significant platelet recovery was found in 5 of 9 patients (55.6%). One patient required re-eradication therapy because the urea breath test remained positive after the first therapy. After complete eradication, long time remission was obtained. The results suggested that the platelet count might be correlated closely with the amount of *H. pylori* in some patients with *H. pylori* positive ITP. (Jpn J Clin Hematol **45** (11) : 1252 ～ 1254, 2004)

8．間葉系幹細胞の造血幹細胞移植への応用

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Summary　骨髄細胞中の間葉系幹細胞（MSC）は，容易に分離し体外で増幅させることができる。MSCは，非上皮細胞への多分化能，造血幹細胞の支持能，強い免疫修飾作用を有するユニークな細胞である。これらのMSCの機能に基づき，造血幹細胞移植に関連したMSCを用いた臨床試験が行われており，有望な結果が得られつつある。MSCを用いた臨床試験では，MSCの投与に関連した重篤な副作用がみられていないことも大きな特徴である。

はじめに

間葉系幹細胞（mesenchymal stem cell：MSC）は，骨髄細胞中に存在するplastic dishに付着する線維芽様の細胞で，*in vitro*で容易に増殖させることが出来る[1]。MSCは造血幹細胞に発現するCD45，CD34，CD117，CD133が陰性で，CD73（SH3），CD105（SH2），SSEA4等が陽性であり，骨，軟骨，脂肪細胞，筋細胞，血管内皮や神経へ分化する能力を有する細胞である[1]。血液疾患に対するMSCを用いた臨床試験では，①MSCの分化能を利用して，障害された細胞の修復に用いる，②MSCの造血幹細胞支持能を利用して，自家または同種造血幹細胞移植時の生着と造血促進に用いる，③MSCの免疫修飾作用を利用して，同種造血幹細胞移植時の移植片対宿主病（graft-versus-host disease：GVHD）予防や治療に用いる3つが行われている。本稿では，これらの臨床試験について順次解説する。

1．先天性代謝異常症と骨形成不全症に対するMSCの投与

先天性代謝異常症の中でもlysosome病は，必要な酵素の先天的欠損によってlysosome内に不要な，あるいは有害な物質が蓄積する疾患である。造血幹細胞移植はlysosome病に対して有効であるが，造血幹細胞移植後に産生された正常なリンパ球や単球が損傷された組織に到達し，欠損している酵素を細胞外に分泌し，分泌された酵素

《略語一覧》
MSC（mesenchymal stem cell；間葉系幹細胞）
GVHD（graft-versus-host disease；移植片対宿主病）