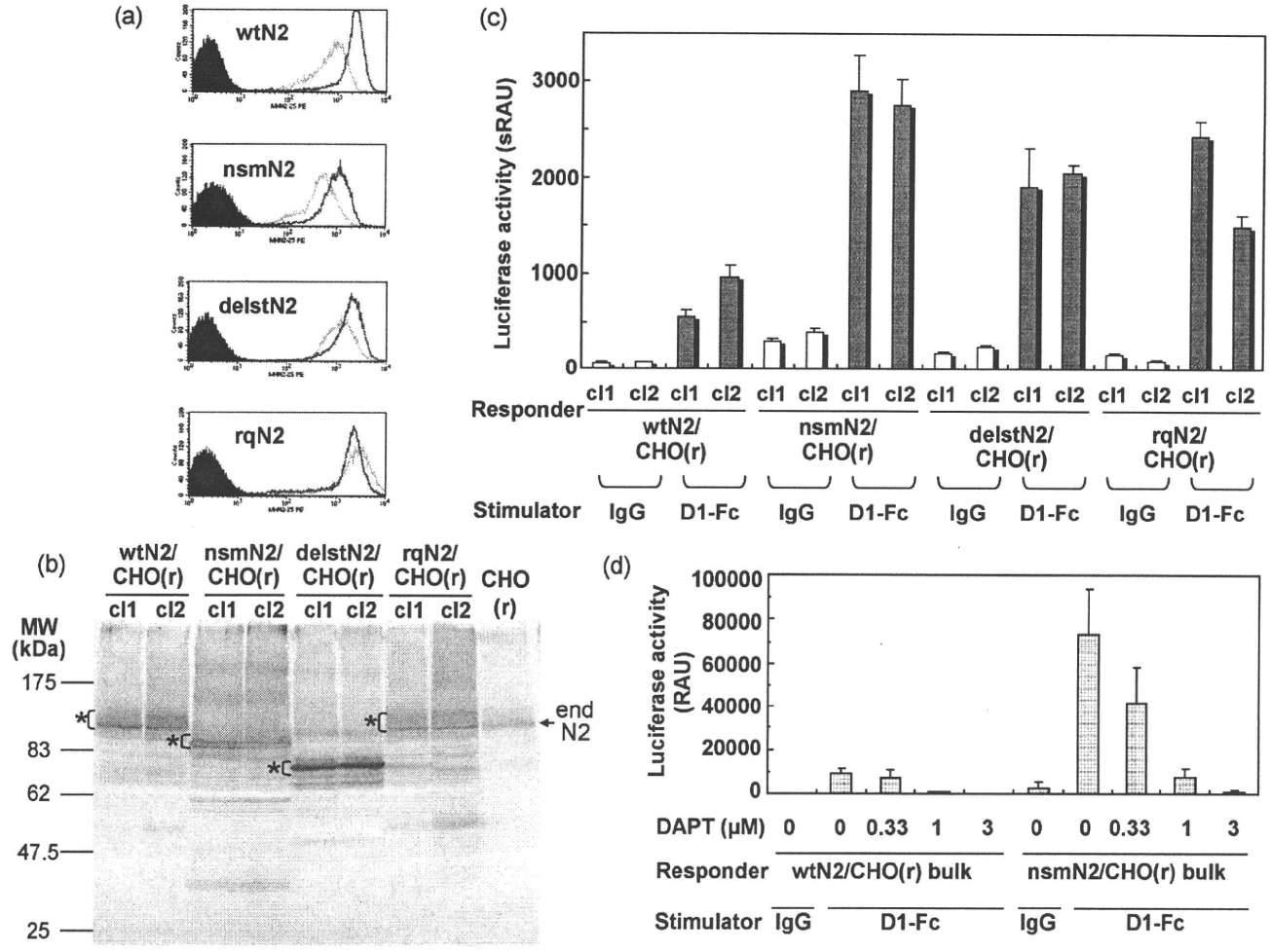

Fig. 4. Functional analysis of human full-length Notch2 cDNA (wtN2), and Notch2 with the nonsense mutation (nsmN2), single-base deletion mutation (delstN2), or R2453Q mutation (rqN2). (a) Flow cytometric analysis of CHO(r) clones expressing wtN2, nsmN2, delstN2, and rqN2 at similar expression levels. Each clone (cl1 and cl2 represented by green and red lines, respectively) of wtN2/CHO(r), nsmN2/CHO(r), delstN2/CHO(r), and rqN2/CHO(r) was analyzed by flow cytometry using the antihuman Notch2 antibody MHN2-25. Purple curves represent isotype control. (b) Western blot analysis of CHO(r) clones expressing wtN2, nsmN2, delstN2, and rqN2 using an antibody recognizing the intracellular domain of Notch2. Asterisks indicate the transmembrane species of each Notch2 protein. MW, molecular weight. (c) Reporter gene transactivation by wtN2, nsmN2, delstN2, and rqN2. Each clone (cl1 and cl2) was cultured in a dish coated with human Delta1-Fc (D1-Fc) or control IgG. Data are means of quadricate experiments. Error bars represent standard deviations. A representative experiment from repeated experiments is shown. sRAU, relative arbitrary units standardized by β-galctosidase activity. (d) Inhibition of luciferase activity by N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT), a γ-secretase inhibitor. Bulk CHO(r) cells transfected with wtN2 or nsmN2 were stimulated with D1-Fc or control IgG with graded concentrations of DAPT. RAU, relative arbitrary units.

mutations showed the same immunohistochemical staining pattern for CD10, BCL6 and MUM-1 might provide insight into this issue. DLBCL is highly heterogeneous clinically, morphologically, and genetically. The tissue microarray study based on immunostaining of the tissue samples identified three antigens (CD10, BCL6 and MUM-1) as useful markers to predict the results of mRNA expression array studies[28–30] and the staining pattern of these three antigens could be used to divide DLBCL cases into GCB and non-GCB groups.[21] Whereas all the five cases carrying *Notch2* mutations in our study belonged to the non-GCB group of DLBCL in this criterion, Troen *et al.* recently reported *Notch2* mutations in two cases of MZB-cell lymphomas.[31] Positions of these mutations are different from those that we found, and their effect on the Notch2 function is not shown. We did not find *Notch2* mutations in MZB-cell lymphomas in our cohort, yet the number of samples was not sufficient to draw conclusions. Although we were unable to find evidence that some or all the five cases carrying *Notch2* mutations in our cohort are DLBCL transformed from MZB-cell lymphoma, this might be an interesting possibility.

Enhanced activation of Notch signaling by exogenous ligand stimulation or expression of constitutively active Notch proteins supports the growth of a variety of tumor cells, including chronic lymphocytic leukemia,[32] non-Hodgkin's lymphoma, and multiple myeloma[33] cells. Alternatively, inhibition of Notch signaling by γ-secretase inhibitors suppresses the growth of those tumor cells, in which enhanced Notch signaling might be involved in tumorigenesis.[34] In contrast, a study of mice with a *Notch1* deletion in keratinocytes revealed the tumor-suppressive feature of Notch signaling.[35] In a similar context, Notch2 activation induces growth suppression in a wide range of B-cell malignancies, raising the possibility that Notch2 functions as a tumor suppressor in B cells.[36] Thus, there appears to be a controversy regarding whether Notch signaling has an oncogenic or antioncogenic role in mature B-cell malignancies. It might be possible that Notch signaling can induce both growth suppression and tumor promo-

tion in the B-cell compartment, depending on the target window within the various developmental stages of B cells.

Although it will require additional studies, including development of animal models, to draw a definitive conclusion about the role of Notch2 mutations in lymphomagenesis, our observations in this study strongly indicate that deregulation of Notch2 signaling by somatic *Notch2* gene abnormalities contributes to the development of a subset of DLBCL, the most frequent type of non-Hodgkin's lymphoma. Developing inhibitors of individual Notch molecules might provide a new strategy for the treatment of different kinds of malignancies, including T-ALL and DLBCL.

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References

1 Chiba S. Notch signaling in stem cell systems. *Stem Cells* 2006; **24**: 2437–47.
2 Wilson A, Radtke F. Multiple functions of Notch signaling in self-renewing organs and cancer. *FEBS Lett* 2006; **580**: 2860–8.
3 Bray SJ. Notch signalling: a simple pathway becomes complex. *Nat Rev Mol Cell Biol* 2006; **7**: 678–89.
4 Radtke F, Wilson A, Stark G *et al.* Deficient T cell fate specification in mice with an induced inactivation of Notch1. *Immunity* 1999; **10**: 547–58.
5 Saito T, Chiba S, Ichikawa M *et al.* Notch2 is preferentially expressed in mature B cells and indispensable for marginal zone B lineage development. *Immunity* 2003; **18**: 675–85.
6 Ellisen LW, Bird J, West DC *et al.* TAN-1, the human homolog of the Drosophila notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. *Cell* 1991; **66**: 649–61.
7 Pear WS, Aster JC, Scott ML *et al.* Exclusive development of T cell neoplasms in mice transplanted with bone marrow expressing activated Notch alleles. *J Exp Med* 1996; **183**: 2283–91.
8 Weng AP, Ferrando AA, Lee W *et al.* Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science* 2004; **306**: 269–71.
9 Lee SY, Kumano K, Masuda S *et al.* Mutations of the Notch1 gene in T-cell acute lymphoblastic leukemia: analysis in adults and children. *Leukemia* 2005; **19**: 1841–3.
10 Zhu YM, Zhao WL, Fu JF *et al.* NOTCH1 mutations in T-cell acute lymphoblastic leukemia: prognostic significance and implication in multifactorial leukemogenesis. *Clin Cancer Res* 2006; **12**: 3043–9.
11 Breit S, Stanulla M, Flohr T *et al.* Activating NOTCH1 mutations predict favorable early treatment response and long-term outcome in childhood precursor T-cell lymphoblastic leukemia. *Blood* 2006; **108**: 1151–7.
12 Sanchez-Irizarry C, Carpenter AC, Weng AP, Pear WS, Aster JC, Blacklow SC. Notch subunit heterodimerization and prevention of ligand-independent proteolytic activation depend, respectively, on a novel domain and the LNR repeats. *Mol Cell Biol* 2004; **24**: 9265–73.
13 Oberg C, Li J, Pauley A, Wolf E, Gurney M, Lendahl U. The Notch intracellular domain is ubiquitinated and negatively regulated by the mammalian Sel-10 homolog. *J Biol Chem* 2001; **276**: 35847–53.
14 Murakami Y, Hayashi K, Hirohashi S, Sekiya T. Aberrations of the tumor suppressor p53 and retinoblastoma genes in human hepatocellular carcinomas. *Cancer Res* 1991; **51**: 5520–5.
15 Nannya Y, Sanada M, Nakazaki K *et al.* A robust algorithm for copy number detection using high-density oligonucleotide single nucleotide polymorphism genotyping arrays. *Cancer Res* 2005; **65**: 6071–9.
16 Wang L, Ogawa S, Hangaishi A *et al.* Molecular characterization of the recurrent unbalanced translocation der (1;7) (q10;p10). *Blood* 2003; **102**: 2597–604.
17 Shimizu K, Chiba S, Kumano K *et al.* Mouse jagged1 physically interacts with notch2 and other notch receptors. Assessment by quantitative methods. *J Biol Chem* 1999; **274**: 32961–9.
18 Karanu FN, Murdoch B, Miyabayashi T *et al.* Human homologues of Delta-1 and Delta-4 function as mitogenic regulators of primitive human hematopoietic cells. *Blood* 2001; **97**: 1960–7.
19 Suzuki T, Yokoyama Y, Kumano K *et al.* Highly efficient *ex vivo* expansion of human hematopoietic stem cells using Delta1-Fc chimeric protein. *Stem Cells* 2006; **24**: 2456–65.
20 Hangaishi A, Ogawa S, Imamura N *et al.* Inactivation of multiple tumor-suppressor genes involved in negative regulation of the cell cycle, MTS1/p16INK4A/CDKN2, MTS2/p15INK4B, p53, and Rb genes in primary lymphoid malignancies. *Blood* 1996; **87**: 4949–58.
21 Hans CP, Weisenburger DD, Greiner TC *et al.* Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood* 2004; **103**: 275–82.
22 James C, Ugo V, Le Couedic JP *et al.* A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature* 2005; **434**: 1144–8.
23 Kralovics R, Passamonti F, Buser AS *et al.* A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med* 2005; **352**: 1779–90.
24 Shimizu K, Chiba S, Hosoya N *et al.* Binding of Delta1, Jagged1, and Jagged2 to Notch2 rapidly induces cleavage, nuclear translocation, and hyperphosphorylation of Notch2. *Mol Cell Biol* 2000; **20**: 6913–22.
25 Bedford MT, Richard S. Arginine methylation an emerging regulator of protein function. *Mol Cell* 2005; **18**: 263–72.
26 Blanchet F, Cardona A, Letimier FA, Hershfield MS, Acuto O. CD28 costimulatory signal induces protein arginine methylation in T cells. *J Exp Med* 2005; **202**: 371–7.
27 Avanzi GC, Giovinazzo B, Saglio G *et al.* Duplication of Ph and of $9q^+$ chromosomes during the blastic transformation of a CML case. *Cancer Genet Cytogenet* 1987; **29**: 57–63.
28 Alizadeh AA, Eisen MB, Davis RE *et al.* Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 2000; **403**: 503–11.
29 Shipp MA, Ross KN, Tamayo P *et al.* Diffuse large B-cell lymphoma outcome prediction by gene-expression profiling and supervised machine learning. *Nat Med* 2002; **8**: 68–74.
30 Rosenwald A, Wright G, Chan WC *et al.* The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. *N Engl J Med* 2002; **346**: 1937–47.
31 Troen G, Wlodarska I, Warsame A, Hernandez Llodra S, De Wolf-Peeters C, Delabie J. NOTCH2 mutations in marginal zone lymphoma. *Haematologica* 2008; **93**: 1107–9.
32 Hubmann R, Schwarzmeier JD, Shehata M *et al.* Notch2 is involved in the overexpression of CD23 in B-cell chronic lymphocytic leukemia. *Blood* 2002; **99**: 3742–7.
33 Jundt F, Probsting KS, Anagnostopoulos I *et al.* Jagged1-induced Notch signaling drives proliferation of multiple myeloma cells. *Blood* 2004; **103**: 3511–15.
34 Leong KG, Karsan A. Recent insights into the role of Notch signaling in tumorigenesis. *Blood* 2006; **107**: 2223–33.
35 Nicolas M, Wolfer A, Raj K *et al.* Notch1 functions as a tumor suppressor in mouse skin. *Nat Genet* 2003; **33**: 416–21.
36 Zweidler-McKay PA, He Y, Xu L *et al.* Notch signaling is a potent inducer of growth arrest and apoptosis in a wide range of B-cell malignancies. *Blood* 2005; **106**: 3898–906.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Specific binding of the mouse antihuman Notch2 monoclonal antibody (MHN2-25). MHN2-25 was added to individual CHO(r) cells and analyzed by fluorescence-activated cell sorting. CHO(r), parental CHO(r); wtN1/CHO(r), CHO(r) cells stably transfected with pTracerCMV/wild-type human Notch1; wtN2/CHO(r), CHO(r) cells stably transfected with pTracerCMV/wild-type human Notch2. Broken lines, biotin-conjugated mouse IgG2a/k (isotype control); solid lines, biotin-conjugated MHN2-25.

Table S1. Primers for polymerase chain reaction–single-stranded conformational polymorphism

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CASE REPORT

Monobactam and aminoglycoside combination therapy against metallo-β-lactamase-producing multidrug-resistant Pseudomonas aeruginosa screened using a 'break-point checkerboard plate'

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Abstract

Metallo-β-lactamase-producing multidrug-resistant Pseudomonas aeruginosa (MDR P. aeruginosa) is a cause of life-threatening infections. With parenteral colistin not available in Japan, we treated MDR P. aeruginosa sepsis with monobactam and aminoglycoside combination therapy, with screening using a 'break-point checkerboard plate'.

Introduction

Multidrug-resistant Pseudomonas aeruginosa (MDR P. aeruginosa), defined as P. aeruginosa resistant to aminoglycosides, carbapenems, and fluoroquinolones, has emerged as an increasingly problematic cause of hospital-acquired infection. The outcome of MDR P. aeruginosa sepsis in severely immunocompromised patients is usually poor. With parenteral colistin not available in Japan, effective antimicrobial options are severely limited. Therefore, combination therapy involving available antimicrobial agents is expected. We treated MDR P. aeruginosa sepsis with monobactam and aminoglycoside combination therapy, with screening using a 'break-point checkerboard plate' [1].

Case reports

Patient 1

A 58-y-old woman was admitted to Toranomon Hospital, Tokyo (890 beds) for the treatment of malignant lymphoma. She was treated with an unrelated donor bone marrow transplantation. The patient suffered from grade IV acute graft-versus-host disease (GVHD). On day 74 of transplantation, peritonitis due to perforation was suspected. MDR P. aeruginosa was cultured from the blood, and treatment with intravenous aztreonam (1 g i.v. every 6 h) and amikacin (400 mg i.v. every 24 h) were started according to the break-point checkerboard plate results. The patient subsequently recovered from MDR P. aeruginosa sepsis.

Patient 2

A 54-y-old man was admitted to Toranomon Hospital for the treatment of malignant lymphoma. He received chemotherapy (R-HyperCVAD/MA) and pelvic radiation therapy. He became febrile 9 days after the most recent course of chemotherapy, with a neutrophil count of 176/μl. Treatment with meropenem and vancomycin was ineffective and the high fever persisted. MDR P. aeruginosa was isolated from blood culture, and combination therapy with aztreonam (2 g i.v. every 12 h) and amikacin (400 mg i.v. every 24 h) was selected for MDR P. aeruginosa according to the break-point checkerboard plate results. The patient recovered successfully from MDR P. aeruginosa sepsis.

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Patient 3

A 63-y-old man was admitted to Toranomon Hospital for the treatment of acute myelogenous leukaemia (AML; World Health Organization classification M1). He was treated with cord blood transplantation. The patient developed redness and swelling in the right eyelid on day 1 of transplantation. He became febrile on day 3 of transplantation. On day 5 of transplantation, MDR P. aeruginosa was cultured from the eye discharge, and his neutrophil count was 0/μl. On day 8 of transplantation, MDR P. aeruginosa was cultured from the blood, and treatment with intravenous aztreonam (1 g i.v. every 6 h) and arbekacin (600 mg i.v. every 24 h) was started. The break-point checkerboard plate results suggested synergistic effects of amikacin in combination with aztreonam and piperacillin. The patient subsequently recovered from MDR P. aeruginosa sepsis.

The clinical characteristics of these 3 patients with MDR P. aeruginosa sepsis are shown in Table I.

Discussion

We have reported cases for which monobactams and aminoglycosides were successfully used concomitantly for MDR P. aeruginosa infection. The production of metallo-β-lactamase was demonstrated by the 3 MDR P. aeruginosa strains using the SMA disc method employing sodium mercaptoacetate (SMA), ceftazidime, and imipenem disks (EIKEN CHEMICAL). Pulsed-field gel electrophoresis was also conducted. The strains from patients 1 and 2 were closely related. The strain from patient 3 was different from those of patients 1 and 2 [2]. In Japan, where intravenous colistin cannot be used, combination antimicrobial therapy is expected to be effective for the treatment of MDR P. aeruginosa. A break-point checkerboard plate is used to evaluate the effect of combination therapy in reference to the breakpoint concentration established from the correlation with clinical efficacy, allowing simultaneous evaluation of the effect of combination antimicrobial therapy using 8 clinically important agents (ceftazidime, piperacillin, imipenem, aztreonam, gentamicin, ciprofloxacin, polymyxin B, and rifampicin) on a single plate. In Japan, a break-point checkerboard plate is commercially available as a BC plate 'EIKEN' from EIKEN CHEMICAL, which includes amikacin, meropenem, and colistin instead of gentamicin, imipenem, and polymyxin B.

Most MDR P. aeruginosa patients remain in a carrier state. Usually, MDR P. aeruginosa seldom causes infection. Therefore, no treatment is recommended for carriers. However, MDR P. aeruginosa is associated with a very poor prognosis when it causes infection, particularly sepsis. Thus, when MDR P. aeruginosa is detected in monitoring cultures for immunocompromised patients, it is important to predict the effect of concomitant use on a break-point checkerboard plate to conduct appropriate early antimicrobial therapy for the infection.

In Japan, the production of metallo-β-lactamase is often involved in the high-level resistance of P. aeruginosa. IMP encoded by the bla_{IMP} gene on a plasmid has been reported [3,4]. Effective combinations of antibacterial drugs seem to vary with strains. Strains producing IMP-type metallo-β-lactamase often remain susceptible to monobactams [5]. The concomitant use of monobactams and aminoglycosides seems to be promising. This regimen is considered to provide a promising second drug of choice for patients in whom intravenous colistin cannot be used.

Aminoglycosides for concomitant use with monobactams will be examined in the future. In Japan, a major drug resistance mechanism against aminoglycosides is inactivation of the antibacterial drugs via acetylation, phosphorylation, etc., by aminoglycoside-modifying enzymes produced by resistant bacteria [6,7]. Other known mechanisms include the methylation of 16S rRNA [8] and increased expression of drug-efflux pumps [9]. Arbekacin is characterized by

Table I. Clinical characteristics of patients with multidrug-resistant Pseudomonas aeruginosa sepsis.

Patient	Sex, age	Underlying disease	Predisposing conditions	Neutropenia	Site of infection	Combination selected by BC plate	Treatment	Clinical response
1	Female, 58 y	Malignant lymphoma	U-BMT, GVHD, diarrhoea	No	Blood, peritonitis	AZT/AMK, PIPC/AMK	AZT/AMK	Recovered
2	Male, 54 y	Malignant lymphoma	Chemotherapy, radiation	Yes (176/μl)	Blood, intestinal tract	AZT/AMK	AZT/AMK	Recovered
3	Male, 63 y	AML	CBT	Yes (0/μl)	Blood, eyelid cellulitis	AZT/AMK, PIPC/AMK	AZT/ABK	Recovered

AML, acute myelogenous leukaemia; U-BMT, bone marrow transplantation from an unrelated donor; GVHD, graft-versus-host disease; CBT, cord blood transplantation; BC plate, break-point checkerboard plate; AZT, aztreonam; AMK, amikacin; PIPC, piperacillin; ABK, arbekacin.

the effect against Gram-negative bacilli, including P. aeruginosa, as well as methicillin-resistant Staphylococcus aureus (MRSA) [8,10,11]. Like amikacin, arbekacin is regarded as a strong candidate for concomitant use with monobactams [12].

Declaration of interest: There is no conflict to declare.

References

[1] Tateda K, Ishii Y, Matsumoto T, Yamaguchi K. 'Break-point Checkerboard Plate' for screening of appropriate antibiotic combinations against multidrug-resistant Pseudomonas aeruginosa. Scand J Infect Dis 2006;38:268–72.

[2] Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995;33:2233–9.

[3] Nishio H, Komatsu M, Shibata N, Shimakawa K, Sueyoshi N, Ura T, et al. Metallo-β-lactamase producing Gram-negative bacilli: laboratory based surveillance in cooperation with 13 clinical laboratories in the Kinki region of Japan. J Clin Microbiol 2004;42:5256–63.

[4] Sekiguchi J, Asagi T, Miyoshi-Akiyama T, Kasai A, Mizuguchi Y, Araake M, et al. Outbreaks of multidrug-resistant Pseudomonas aeruginosa in community hospitals in Japan. J Clin Microbiol 2007;45:979–89.

[5] Docquier JD, Riccio ML, Mugnaioli C, Luzzaro F, Endimiani A, Toniolo A, et al. IMP-12, a new plasmid-encoded metallo-β-lactamase from a Pseudomonas putida clinical isolate. Antimicrob Agents Chemother 2003;47:1522–8.

[6] Shaw KJ, Rather PN, Hare RS, Miller GH. Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. Microbiol Rev 1993;57:138–63.

[7] Shimizu K, Kumada T, Hsieh WC, Chung HY, Chong Y, Hare RS, et al. Comparison of aminoglycoside resistance patterns in Japan, Formosa, and Korea, Chile, and the United States. Antimicrob Agents Chemother 1985;28:282–8.

[8] Yokoyama K, Doi Y, Yamane K, Kurokawa H, Shibata N, Shibayama K, et al. Acquisition of 16S rRNA methylase gene in Pseudomonas aeruginosa. Lancet 2003;362:1888–93.

[9] Masuda N, Sakagawa E, Ohya S, Gotoh N, Tsujimoto H, Nishino T. Substrate specificities of MexAB–OprM, MexCD–OprJ, and MexXY–OprM efflux pumps in Pseudomonas aeruginosa. Antimicrob Agents Chemother 2000;44:3322–7.

[10] Tanaka N, Matsunaga K, Hirata A, Matsuhisa Y, Nishimura T. Mechanism of action of habekacin, a novel amino acid-containing aminoglycoside antibiotic. Antimicrob Agents Chemother 1983;24:797–802.

[11] Watanabe T, Ohashi K, Matsui K, Kubota T. Comparative studies of the bactericidal, morphological and post-antibiotic effects of arbekacin and vancomycin against methicillin-resistant Staphylococcus aureus. J Antimicrob Chemother 1997;39:471–6.

[12] Kobayashi Y, Uchida H, Kawakami Y. Synergy with aztreonam and arbekacin or tobramycin against Pseudomonas aeruginosa isolated from blood. J Antimicrob Chemother 1992;30:871–2.

●原　著●

腸管GVHD患者に対する経口ベクロメタゾン投与時の血中濃度に関する検討

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Analysis of Blood Concentrations Following Oral Administration of Beclomethasone Dipropionate for Gut GVHD

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Summary

In this study, we investigated the level of gut absorption following oral beclomethasone dipropionate (BDP) administration by measuring the blood concentration of its metabolites measured by LC-MS/MS using the HPLC method. Five patients who were administered BDP orally for gut GVHD were included. The blood concentrations of beclomethasone-17-monopropionate (17BMP), which is one of the active metabolites of BDP, were 618～1,749 pg/mL in 4 of the studied 5 patients, which was comparable to that after inhalation of BDP; however, it was relatively higher in one patient (2,439±161 pg/mL). As the blood concentration of 17BMP in this study patient was higher compared with healthy volunteers administered a single oral BDP 4 mg, GVHD patients might have a higher concentration than healthy volunteers.

Given that a higher grade of gut GVHD was associated with a higher blood level of 17BMP, BDP absorption might be associated with gut mucosal injury. Thus, the systemic adverse effect following oral BDP administration might not be negligible especially in gut GVHD patients. **Key words**: Beclomethasone dipropionate, Graft-versus-host disease, Stem cell transplantation (*Received Jul. 6, 2009/Accepted Aug. 20, 2009*)

要旨　腸管GVHDに対してベクロメタゾン（BDP）を経口投与した患者を対象として，BDPおよびその代謝物の血中濃度を高速液体クロマトグラフィを用いてLC-MS/MSで測定し，経口BDPの消化管からの吸収について検討した。全対象症例5例よりBDPの主要活性代謝物である17BMPが検出された。5例中4例における17BMPの血中濃度は618～1,749 pg/mLであり，吸入剤を投与した時と同程度あるいはそれ以下であった。1例は17BMPの血中濃度が2,439±161 pg/mLを示し，吸入剤投与時以上に血中濃度が上昇した。本症例における17BMPの血中濃度は，健常人にBDP 4 mgを単回経口投与した際の最高血中濃度と比較して高値を示したことから，GVHD患者では健常人よりも血中濃度が上昇する症例が存在することが示唆された。腸管GVHDのstageが高い症例に17BMPの血中濃度が高値である症例が認められたことから，腸管粘膜障害とBDP吸収の亢進との関連が示唆された。以上の成績から，腸管GVHDに対する経口BDP投与は，必ずしも全身的副作用が無視できるものではない点に留意すべきと考えられた。

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伊藤　忠明

はじめに

移植片対宿主病（graft-versus-host disease: GVHD）は，同種造血幹細胞移植後の重篤な合併症の一つである。ステロイド薬の全身投与はGVHDに対する標準的な治療法であるが，感染症の増加など副作用が問題となる。

ジプロピオン酸ベクロメタゾン（beclomethasone dipropionate: BDP）は，本邦において気管支喘息・アレルギー性鼻炎に対する吸入剤として一般に使用されている。BDPは肺や腸管から吸収後[1]，エステラーゼにより急速に分解され主要活性代謝物である17-モノプロピオン酸ベクロメタゾン（beclomethasone-17-monopropionate: 17BMP）に代謝され，17BMPはステロイド活性をもたないベクロメタゾン（beclomethasone: BOH）へ代謝される[2]。BDPは初回通過効果により大部分が代謝されることから，全身的な作用は少ないことが報告[3-5]されている。こうした体内動態の特性から，経口BDPは消炎活性が局所に限定される[6]ステロイド薬として期待され，腸管GVHDに対する有効性が報告[7-12]されてきた。BDPは局所作用が大部分であり全身的な副作用は少ないと考えられているが，腸管GVHD患者を対象に経口BDPの血中濃度を測定した報告はない。

今回われわれは，腸管GVHD患者における経口BDPの吸収の程度を確認するため，BDPおよびその代謝物の血中濃度について検討したので報告する。

Ⅰ．対象と方法

1．対　象

対象は2006年10月～2007年1月まで同種造血幹細胞移植後，腸管GVHD（急性，慢性を含む）を発症し，院内製剤経口ベクロメタゾンを投与した患者である。血中濃度の測定については，文書により十分な説明に従い同意が得られた患者を対象とした。

本研究における経口BDPの投与および血中濃度測定は虎の門病院倫理審査委員会において承認を得ている。

2．院内製剤経口ベクロメタゾンと規格

院内製剤経口ベクロメタゾンはMP Biomedical社のジプロピオン酸ベクロメタゾンの原末を使用し，院内製剤した。院内製剤経口ベクロメタゾンはカプセル剤とシロップ剤の二つの剤型とし，それぞれ名称をBDPカプセルとBDP内服液とした。BDPカプセルは小腸で溶解後，小腸および大腸に活性薬物が供給され下部消化管で作用することを期待して腸溶カプセルとした。BDP内服液は，上部消化管に作用することを期待してシロップ剤とした。院内製剤経口ベクロメタゾンの規格に関しては，BDPカプセル1カプセル中1 mgのBDP，BDP内服液30 mL中1 mgのBDPを含有するように院内製剤した。

3．BDPカプセルとBDP内服液の投与方法と血中濃度測定時間

投与方法は，全対象症例においてBDPカプセルは1回1カプセルを1日4回（6，11，16，21時），BDP内服液は1回30 mLを1日4回（カプセル服用の15分後），経口投与した。

血中濃度測定は，BDP投与開始後3日目以降でBDPカプセル服用約4時間後（BDP内服液服用後3時間45分）を目安とした。

4．BDP，17BMP，BOHの血中濃度測定方法

BDP，17BMP，BOHの血中濃度測定は，Applied Biosystems/MDS SCIEX社のAPI 3200™ LC-MS-MS system（LC-MS/MS）で行った。

高速液体クロマトグラフィ（high-performance liquid chromatography: HPLC）のカラムはSymmetry Shield™ RP8 5 μm 2.1×150 mm Column（Waters Corps.）を用い，LC-MS/MSで測定を行った。BDP，17BMP，BOHの検出範囲は250～5,000 pg/mLとし，検出限界は250 pg/mLとした。

5．17BMPの血中濃度と腸管GVHDのstageの関連

腸管GVHDの程度と経口BDPの吸収を検討した。腸管GVHDの重症度分類は造血細胞移植ガイドラインに従って行った。腸管GVHDの評価は血中濃度測定日に行った。

Ⅱ．結　果

1．患者背景と採血時間

対象患者は5例であった。患者背景をTable 1に示した。患者の年齢は20歳台から60歳台であり，原疾患は急性骨髄性白血病3例，急性リンパ性白血病1例，慢性骨髄性白血病1例であった。移植細胞源は，臍帯血2例，骨髄1例，同種末梢血2例であった。

採血時間は，症例2～5ではBDPカプセル服用4.5時間後であったが，症例1ではBDPカプセル服用1.5時間後であった。

2．BDP，17BMP，BOHの血中濃度

対象患者の血中濃度測定結果をTable 2に示した。BDPカプセル服用1.5時間後に測定した症例1は，17BMPの血中濃度が2,439±161 pg/mLまで上昇した。BDPカプセル服用4.5時間後に測定した4例全例で，17BMPは618～1,749 pg/mLの範囲の血中濃度で検出された。BDPは全例，BOHは2例で検出感度以下であった。

Table 1 Patients' background

Patient No.	Age	Sex	Diagnosis	Stem cell source	Onset of gut GVHD	Start day of BDP	Stage of gut GVHD at BDP starting	Day of blood sampling	Stage of gut GVHD at blood sampling
1	65	female	ALL	CB	day 136	day 141	3	day 159	1
2	32	male	AML	BM	day 93	day 94	2	day 120	3
3	62	female	AML	PBSC	day 120	day 122	3	day 156	3
4	68	male	AML	CB	day 31	day 29	2	day 70	2
5	28	male	CML	PBSC	day 46	day 46	2	day 52	1

ALL: acute lymphoblastic leukemia, AML: acute myelogenous leukemia, CML: chronic myelogenous leukemia, CB: cord blood, BM: bone marrow, PBSC: peripheral blood stem cell

Table 2 Blood concentration of BDP, 17BMP, and BOH

Patient No.	Interval between blood sampling and BDP administration (hour)	BDP	17BMP	BOH
		mean±SD (pg/mL)		
1	1.5	nd	2,439±161	751±63
2	4.5	nd	1,166±184	358±32
3	4.5	nd	1,749±208	339±22
4	4.5	nd	618±19	nd
5	4.5	nd	696±74	nd

nd: not detectable

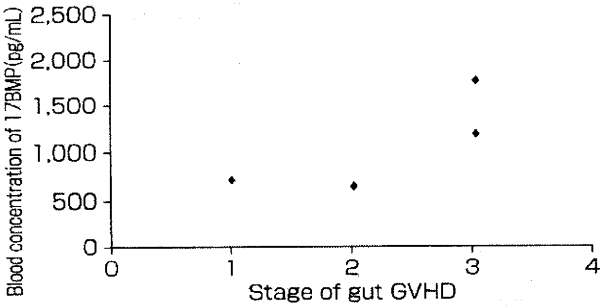

Fig. 1 Blood concentration of 17BMP and stage of gut GVHD at blood sampling

3. 17BMPの血中濃度と腸管GVHDのstageの関連

腸管GVHDの重症度と経口BDPの吸収を検討するため，17BMPの血中濃度と腸管GVHDのstageの関連をFig. 1に示した。症例1は服用1.5時間後に採血したため解析対象から除外した。解析症例4例のうち，腸管GVHDに伴う症状は全例下痢でありstage 1は1名，stage 2は1名で，17BMPの血中濃度はそれぞれ696，618 pg/mLであった。stage 3は2名で，17BMPの血中濃度はそれぞれ1,166，1,749 pg/mLであった。腸管GVHDのstageと17BMPの血中濃度の関連を検討したところ，腸管GVHDのstageが高い症例では17BMPの血中濃度が高値である傾向が認められた。

Ⅲ. 考 察

全対象患者5例でBDPの主要活性代謝物である17BMPが血中で検出された。1995年のMcDonaldらの研究[7]では，経口BDPを投与した腸管GVHD患者20例中11例（55%）に副腎抑制を認めたことより経口BDPは吸収され，全身的な副作用が発症し得ることが示唆されている。2001年に報告[2]された健常人を対象とした研究において，BDPの経口投与によりわずかながら全身的な吸収を有することが報告されている。今回，われわれの研究結果は，McDonaldらが副腎抑制により間接的にBDPの吸収を示唆した報告と健常人でBDPが吸収された報告を支持する結果となった。

健常人でのBDP 1,000 μgの単回吸入投与の報告[13]によると，17BMPの血中濃度は2,103 pg/mLであった。また，BDPの吸入剤であるキュバール®において，BDP 400 μgを軽度から中程度の気管支喘息患者に単回吸入投与した際の17BMPの血中濃度は1,419 pg/mL[14]であった。今回の研究では1回2 mg（カプセル1 mg，内服液1 mg）を1日4回投与したが，5例中4例において17BMPの血中濃度は618～1,749 pg/mLであった。これはBDP 1回400～1,000 μgを単回吸入投与した時（1,419～2,103 pg/mL）と同程度あるいはそれ以下の血中濃度であることが確認された。吸入剤投与時のBDP 1日投与量が1,500 μgまでの場合，副腎皮質系抑制の有意な危険性はないとの報告[15]があることより，吸入剤と

同程度の血中濃度であれば副腎皮質系抑制の危険性は低いと考えられる。1例は，17BMPの血中濃度は2,439±161 pg/mLで吸入剤投与時以上の血中濃度の上昇がみられた。

健常人を対象とした研究[2]で経口BDP服用後の17BMPの最高血中濃度到達時間が4時間であったことより，本研究は血中濃度測定時期をBDPカプセル服用約4時間後としたが，実際の測定時間は，症例1はBDPカプセル服用1.5時間後，症例2～5は4.5時間後であった。BDPカプセル服用1.5時間後に測定した症例1の17BMPの血中濃度は2,439 pg/mLと5症例のうち最高値を示した。症例1は，BDP開始時の腸管GVHDのstageが3であり高度の腸管粘膜障害を呈していた。腸管輸送（運動）能が保持されていて，粘膜障害も高度でないstage 1および2の患者では，健常者と同程度のTmaxである可能性があるものの，stage 3以上の腸管GVHD患者では，最高血中濃度到達時間は健常人と異なる可能性を有すると考えられる。

腸管GVHDのstage 1とstage 2の症例における17BMPの血中濃度はそれぞれ696，618 pg/mLであり，健常人にBDP 4 mgを単回経口投与し服用4時間後の最高血中濃度（703 pg/mL）[2]と比較して同程度の血中濃度であった。腸管GVHDのstage 3の症例2名の17BMPの血中濃度は1,166，1,749 pg/mLであり，健常人の血中濃度と比較しそれぞれ1.7，2.5倍と高値を示したことから，GVHDのstageが高い患者では健常人よりも血中濃度が上昇する可能性が示唆された。また，腸管GVHDのstageが高い症例で17BMPの血中濃度が高値である傾向が認められた原因として腸管GVHDの重症度，つまり腸管粘膜障害の程度が高まるにつれて経口BDPの吸収が亢進し，血中濃度が上昇した可能性がある。特に重度の腸管GVHDが発症している症例は，経口BDPによる全身的な副作用の発現の可能性を考慮すべきである。

文　献

1) 相良博典：吸入ステロイド薬の吸収特性と安全性．アレルギー科　19(5)：421-428，2005.
2) Daley-Yates PT, Price AC, Sisson JR, *et al*: Beclomethasone dipropionate: absolute bioavailability, pharmacokinetics and metabolism following intravenous, oral, intranasal and inhaled administration in man. *Br J Clin Pharmacol*　**51**: 400-409, 2001.
3) Brattsand R: Airways-lung selectivity of inhaled steroids. アレルギーの領域　**5**(10): 1410-1415, 1998.
4) Phillipps GH: Structure-activity relationships of topically active steroids: the selection of fluticasone propionate. *Respir Med*　**84**(*suppl* A): 19-23, 1990.
5) Hubner M, Hochhaus G, Derendorf H, *et al*: Comparative pharmacology, bioavailability, pharmacokinetics, and pharmacodynamics of inhaled glucocorticosteroids. *Immunol Allergy Clin North Am*　**25**(3): 469-488, 2005.
6) 大石孝義，出口隆志，丸茂博大：Beclomethasone 17, 21-dipropionateの代謝．応用薬理　**22**(5)：717-727，1981.
7) Baehr PH, Levine DS, Bouvier ME, *et al*: Oral beclomethasone dipropionate for treatment of human intestinal graft-versus-host disease. *Transplantation*　**60**(11): 1231-1238, 1995.
8) McDonald GB, Bouvier M, Hockenbery DM, *et al*: Oral beclomethasone dipropionate for treatment of intestinal graft-versus-host disease: a randomized, controlled trial. *Gastroenterology*　**115**: 28-35, 1998.
9) Castilla C, Perez-Simon FA, Sanchez-Guijo FM, *et al*: Oral beclomethasone dipropionate for the treatment of gastrointestinal acute graft-versus-host disease (GVHD). *Biol Blood Marrow Transplant*　**12**: 936-941, 2006.
10) Hockenbery DV, Cruickshank S, Rodell TC, *et al*: A randomized placebo-controlled trial of oral beclomethasone dipropionate as a prednisone-sparing therapy for gastrointestinal graft-versus-host disease. *Blood*　**109**(10): 4557-4563, 2007.
11) 山本久史，内田直之，内田ゆみ子・他：同種造血幹細胞移植後の消化管GVHDに対する経口ベクロメタゾン療法．臨血　**47**(9)：239，2006.
12) Miura Y, Narimatsu H, Kami M, *et al*: Oral beclomethasone dipropionate as an initial treatment of gastrointestinal acute graft-versus-host disease after reduced-intensity cord blood transplantation. *Bone Marrow Transplant*　**38**: 577-579, 2006.
13) Lipworth BJ and Jackson CM: Pharmacokinetics of chlorofluorocarbon and hydrofluoroalkane metered-dose inhaler formulations of beclomethasone dipropionate. *Br J Clin Pharmacol*　**48**: 866-868, 1999.
14) キュバールエアゾール®医薬品インタビューフォーム，第10版，大日本住友製薬株式会社，大阪，2006，p16.
15) Ip M, Lam K, Ng M, *et al*: Decreased bone mineral density in premenopausal asthma patients receiving long-term inhaled steroids. *Chest*　**105**(6): 1722-1727, 1994.

Possible graft-versus-host disease involving the central nervous system soon after cord blood transplantation

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The concept that central nervous system (CNS) could be a target of graft-versus-host disease (GVHD) is controversial. There are a few case reports which support the possibility of CNS-GVHD [1,2]. Here, we describe a patient who developed unique CNS symptoms soon after cord blood transplantation with reduced-intensity conditioning (RI-CBT). On Day 7 post-transplant, a high fever, slight skin eruption, moderate diarrhea, and liver damage suddenly developed. Three days later, her white blood cell (WBC) count rapidly increased to 1,700 μl^{-1} and consisted mostly of mature lymphocytes. Generalized convulsions developed on the same day. An analysis of the cerebrospinal fluid (CSF) revealed elevated proteins and pleocytosis comprising mostly mature lymphocytes. The lymphocytes found in the peripheral blood (PB) and CSF were phenotypically polyclonal T-cells that were donor derived. Extensive investigations did not detect any microorganisms or other causes for the T-cell proliferation and CNS symptoms. Considering the coexistence of CNS and systemic GVHD-like symptoms, proliferation of donor-derived polyclonal T-cells in the CSF and PB, and no microorganisms or other factors detected, CNS GVHD seems to be the most likely explanation for her clinical course.

Cord blood (CB) has been increasingly applied as a viable source of stem cells for allogeneic hematopoietic stem cell transplantation (allo-SCT) [3,4]. The incidence and severity of GVHD following cord blood transplantation (CBT) are lower than those after allo-SCT using bone marrow or peripheral blood stem cells from either matched siblings or unrelated donors [5–7]. On the other hand, unique immune-mediated complications, such as pre-engraftment immune reaction (PIR) and hemophagocytic syndrome (HPS), have been observed early after RI-CBT [8,9]. Thus, the spectrum of immune-mediated reactions after RI-CBT has not yet been fully clarified.

CNS complications have been described following allo-SCT [10]. Infections, drug toxicity, and metabolic and cerebrovascular disorders are the major causes, and there have been rare cases of apparent immune-mediated reaction to CNS [1,2].

Here, we present an interesting case of a patient who developed unique CNS symptoms soon after RI-CBT. A 40-year-old woman with follicular lymphoma that was refractory to chemotherapy was admitted to our hospital in September 2006. Her clinical stage was IV B at diagnosis in 2002. Six cycles of rituximab (R)-CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) resulted in complete remission, and rituximab therapy was maintained for 1 year. A relapse occurred in 2005 and was treated with R-ACES (high-dose Ara C, carboplatin, etoposide, and steroids), R-ICE (ifosfamide, carboplatin, etoposide), cladribine, and R-COP (cyclophosphamide, vincristine, and prednisone), which resulted in a partial response at each cycle. However, the disease gradually progressed thereafter, with the development of systemic lymphadenopathy, pleural effusion, and ascites. Since no suitable related or unrelated donors from the Japan Marrow Donor Program were available, unrelated CB was considered as an alternative graft, and she was referred to our hospital. The patient and graft were sex-mismatched and phenotypically two and genotypically three-loci mismatches in HLA-A, HLA-B, and DRB1 loci. The types of the HLA-A, HLA-B, and DRB1 loci were *A01* (*0101*)/A31 (3101), B35 (3501)/*B48* (*4801*), and DRB1*04

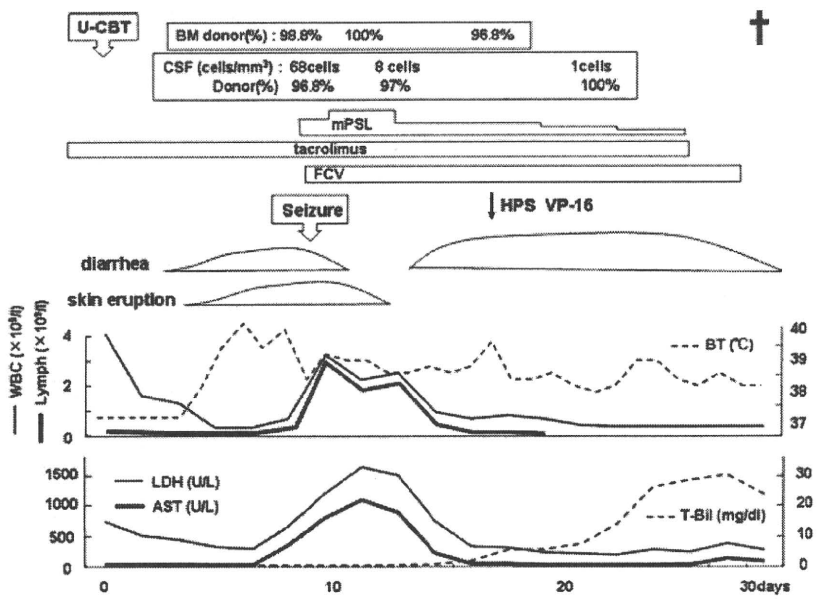

Figure 1. Clinical course of the patient. Abbreviations: U-CBT, unrelated cord blood transplantation; BM, bone marrow; CSF, cerebrospinal fluid; mPSL, methylprednisolone; FCV, foscarnet; HPS, hemophagocytic syndrome; VP-16, etoposide; WBC, white blood cell; BT, body temperature; LDH, lactate dehydrogenase; AST, aspartate aminotransferase; T-bil, total bilirubin.

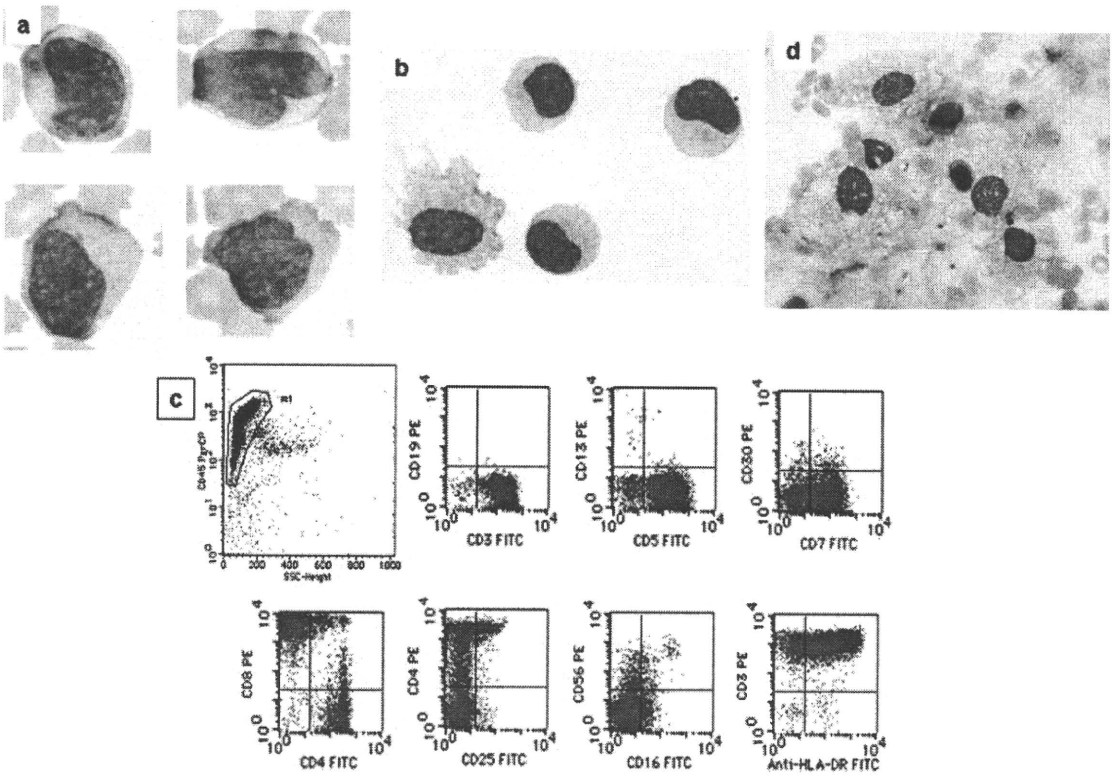

Figure 2. Activated lymphocytes in peripheral blood (a) and in cerebrospinal fluid (b) on Day 10 post-transplant. Flow cytometry of peripheral blood on Day 10 post-transplant (c). Activated macrophages in bone marrow on Day 17 post-transplant (d). May-Giemsa staining ×1000 (a, b) ×400 (c). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

(*0404*)/DRB1*09 (0901), respectively, in the recipient, and *A26* (*2601*)/A31 (3101), B35 (3501)/*B51* (*5101*), and DRB1*04 (*0401*)/DRB1*09 (0901), respectively, in the donor. The graft contained 2.4×10^7/kg total nucleated cells and 0.92×10^5/kg $CD34^+$ cells. The pretransplant conditioning regimen consisted of fludarabine (25 mg/m^2/day) for 5 days, melphalan (40 mg/m^2/day) for 2 days, and 4 Gy of total body irradiation. Tacrolimus alone was administered as GVHD prophylaxis. Granulocyte colony-stimulating factor was started from Day 1. Pretransplant viral serology was positive for HSV, HVZ, CMV, and EBV, and negative for HIV and HTLV-1. She received 600 mg/day of oral acyclovir, 400 mg/day of oral tosufloxacin, 200 mg/day of oral itraconazole, and trimethoprim-sulfamethoxazole (160 mg/day of the trimethoprim component) as for antimicrobial prophylaxis. Figure 1 shows her entire clinical course following RI-CBT. On Day 7 post-transplant, a high fever, slight skin eruption, and moderate diarrhea developed with a slightly increased WBC count (from 10 μl^{-1} on Day 6 to 30 μl^{-1} on Day 7). Her WBC count rapidly increased on Day 10 to 1,700 μl^{-1} and comprised 90% lymphocytes (Fig. 2a). Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels increased to 715 and 359 IU/l, respectively, and serum lactate dehydrogenase (LDH) levels increased to 1,101 IU/l. The patient suddenly lost consciousness along with generalized convulsions on the same day and required mechanical ventilation. Cerebrospinal fluid (CSF) analysis revealed an extremely elevated protein level of 675 mg/dl (normal range: 15–40 mg/dl) and pleocytosis (68 cells/μl), consisting mainly of lymphocytes (98%) (Fig. 2b). Magnetic resonance imaging scans of the brain revealed no specific abnormalities typically seen in cerebrovascular disorders, tacrolimus encephalopathy, thrombotic microangiopathy, or other CNS complications, and schistocytes were undetectable in the PB. Flow cytometry revealed that the excessive lymphocytes in both PB and CSF comprised polyclonal mature T-lymphocytes expressing CD3, CD4, CD5, CD8, and HLA-DR. The expression of CD4 and CD8 was variable, in which $CD4^-CD8^+$, $CD4^+CD8^-$, and $CD4^+CD8^+$ cells accounted for 65, 25, and 9%, respectively, of the cells in PB, and 38, 56, and 6%, respectively, of those in the CSF (Fig. 2c). Y chromosome-based fluorescence in situ hybridization analysis showed that most of these cells were derived from the donor (98.8% in PB and 96.8% in CSF). Furthermore, 98% of BM cells obtained on Day 10 were also donor derived. Routine cultures of PB and CSF for bacteria and fungi were negative. Analyses by real-time polymerase chain reactions were negative for HSV-1, HHV-6, VZV, CMV, and EBV in PB and CSF, and, for HSV-2, HSV-7, HSV-8, JCV, BKV, ADV, Parvovirus B19, HBV, and HCV in PB. Southern blotting of cells from the PB showed that the genes for both T-cell receptor Cβ1 and Jδ1 were in germ-line configuration, and EBV genome clonality was undetectable. Methylprednisolone (500 mg/day) was administered for 3 days, and acyclovir was switched to foscarnet, considering the possibility of acute GVHD and viral infection insensitive to acyclovir. After the initiation of these therapies, the numbers of lymphocytes in PB and CSF gradually decreased, and her clinical symptoms and laboratory data improved, so methylprednisolone was carefully tapered. However, high fever, diarrhea, and CNS symptoms recurred around Day 17, and then pancytopenia and cholestatic liver damage rapidly progressed. On Day 17, BM aspiration revealed an increase of activated macrophages (35%) with massive hemophagocytosis (Fig. 2d). The chimeric status of the BM cells revealed sustained donor cell dominance (96.8%), indicating that the hematopoietic cells and macrophages in the BM were both donor derived. Despite the administration of etoposide (50 mg/m^2) to control the hemophagocytosis, pancytopenia and cholestatic liver damage progressed and the patient died of bacterial sepsis 32 days after transplantation. An autopsy was not performed.

Polyclonal T-cell proliferation is the principal mechanism of the antigen-specific immune response that generally occurs upon infection and/or inflammation. GVHD is also primarily a T-cell-mediated event, and the subsequent expansion of donor T-cell clones-recognizing antigens causes tissue damage either directly through T-cells encountering recipient MHC-bearing cells in target tissues or indirectly through cytokine production [11].

We previously reported higher incidence of immune-mediated complications, such as PIR, characterized by high-grade fever, skin eruption, diarrhea, jaundice, and body weight gain developing before engraftment, and HPS early after RI-CBT [8,9]. Despite the known immunological naïvety of CB cells, the exceptionally high incidence of PIR and HPS suggests that the properties of CB cells are unique and distinctly different from adult donor cells.

The most striking features of our patient were the remarkable polyclonal T-cell proliferation both in PB and CSF, followed by sudden generalized convulsions and loss of consciousness. As the coexistent CNS and systemic GVHD-like symptoms, proliferating donor-derived polyclonal T-cells in the CSF and PB, and microorganisms or other factors that might be responsible for these symptoms or T-cell proliferation were undetectable. We therefore postulated that an alloimmune reaction of the CB graft against the CNS caused the CNS symptoms in our patient.

The concept that CNS could be a target of GVHD is controversial. Some case reports support the possibility of CNS-GVHD [1,2]. All of the patients in these reports were diagnosed with CNS-GVHD only when they responded to immunosuppressive therapy and had histologically and immunophenotypically documented perivascular T-cell infiltration without evidence of other CNS diseases with overlapping features. However, uniform diagnostic approaches or criteria have not been established. Most of the reported CNS-GVHD was diagnosed at the time of chronic GVHD development. Powles et al. [12] reported that convulsions, possibly due to cerebral edema, could develop as a manifestation of severe acute GVHD after haploidentical transplantation. This could explain the events in our patient, although information about the CSF, the presence or absence of T-cell proliferation, or detectable infectious organisms was not provided in the literature. We reported that early CNS complications are more frequent after RI-CBT than after transplantation with other stem cell sources and that hypercytokinemia associated with PIR could influence the development of CNS complications [13]. T-cell proliferation in CSF along with the severe systemic symptoms in our patient might have resulted from a type of hypercytokinemia that is unique to RI-CBT.

Moreover, severe HPS developed around 10 days after T-cell proliferation, and the activated macrophages in the BM were donor derived. Although HPS is a rare complication following allo-SCT, some investigators have suggested that a severe alloimmune response could result in HPS after PB transplantation [14,15]. Furthermore, we recently reported that the incidence of HPS following RI-CBT is higher than was previously reported and that HPS is a significant risk factor for engraftment failure [9]. Hypercytokinemia associated with engrafted T-cell proliferation may have played an important role in donor-derived macrophage activation and in the development of HPS in our patient.

In conclusion, we described a patient who developed sudden generalized convulsions and lost consciousness at the same time as polyclonal T-cell proliferation soon after RI-CBT. The findings of extensive investigations indicated that the CNS can be a target of GVHD. Further accumulation of clinical and laboratory data with the awareness of this devastating complication soon after RI-CBT is warranted to precisely understand the underlying basic mechanisms and to develop optimal intervention strategies.

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References

1. Kamble RT, Chang CC, Sanchez S, et al. Central nervous system graft-versus-host disease: Report of two cases and literature review. Bone Marrow Transplant 2007;39:49–52.
2. Padovan CS, Bise K, Hahn J, et al. Angiitis of the central nervous system after allogeneic bone marrow transplantation? Stroke 1999;30:1651–1656.
3. Rocha V, Gluckman E. Clinical use of umbilical cord blood hematopoietic stem cells. Biol Blood Marrow Transplant 2006;12:34–41.
4. Brunstein CG, Setubal DC, Wagner JE. Expanding the role of umbilical cord blood transplantation. Br J Haematol 2007;137:20–35.
5. Takahashi S, Iseki T, Ooi J, et al. Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult patients with hematologic malignancies. Blood 2004;104:3813–3820.
6. Takahashi S, Ooi J, Tomonari A, et al. Comparative single-institute analysis of cord blood transplantation from unrelated donors with bone marrow or peripheral blood stem-cell transplants from related donors in adult patients with hematologic malignancies after myeloablative conditioning regimen. Blood 2007;109:1322–1330.
7. Rocha V, Labopin M, Sanz G, et al. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. N Engl J Med 2004;351:2276–2285.
8. Kishi Y, Kami M, Miyakoshi S, et al. Early immune reaction after reduced-intensity cord-blood transplantation for adult patients. Transplantation 2005; 80:34–40.
9. Takagi S, Masuoka K, Yamamoto H, et al. Risk factors for the development of hemophagocytic syndrome following reduced-intensity umbilical cord blood transplantation. EBMT Annual Meeting 2007. O368 S43 [abstract].
10. Siegal D, Keller A, Xu W, et al. Central nervous system complications after allogeneic hematopoietic stem cell transplantation: Incidence, manifestations, and clinical significance. Biol Blood Marrow Transplant 2007;13:1369–1379.
11. Morris ES, Hill GR. Advances in the understanding of acute graft-versus-host disease. Br J Haematol 2007;137:3–19.
12. Powles R, Pedrazzini A, Crofts M, et al. Mismatched family bone marrow transplantation. Semin Hematol 1984;21:182–187.
13. Kishi Y, Miyakoshi S, Kami M, et al. Early central nervous system complications after reduced-intensity stem cell transplantation. Biol Blood Marrow Transplant 2004;10:561–568.
14. Abe Y, Choi I, Hara K, et al. Hemophagocytic syndrome: A rare complication of allogeneic nonmyeloablative hematopoietic stem cell transplantation. Bone marrow Transplant 2002;29:799–801.
15. Kishi Y, Kami M, Murashige N, et al. Hyperacute GVHD and emergence of peripheral CD3+CD56+ T-cells and activated natural killer cells are useful markers for early diagnosis of post-transplant hemophagocytic syndrome. Bone Marrow Transplant 2005;35:415–417.

Treatment with hydroxyurea in a patient compound heterozygote for a high oxygen affinity hemoglobin and β-thalassemia minor

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Compound heterozygotes for β-thalassemia and high oxygen affinity hemoglobin (Hb) have been documented, but experience in the management of such rare cases is minimal. Although hydroxyurea (HU) has never been used in a heterozygote with high oxygen affinity Hb and β-thalassemia, we hypothesized that it would decrease erythrocytosis through a lowered production of abnormal cells and increase of P_{50} by induction of fetal hemoglobin (HbF). We present the case of a patient with compound high oxygen affinity Hb mutation with β-thalassemia. PCR analysis revealed combined Hb Regina and IVS1-110 G/A mutations. Treatment with HU caused a decrease in Ht (61.1% to 38.6%) and erythrocyte volume (74.87 mL/kg to 40.65 mL/kg), as well as an increase in P_{50} (6 mmHg to 10 mmHg) and HbF level (3.6% to

CLINICAL RESEARCH

Central Nervous System Relapse of Leukemia after Allogeneic Hematopoietic Stem Cell Transplantation

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Little information is available regarding central nervous system (CNS) relapse of adult leukemia after allogeneic hematopoietic stem cell transplantation (HSCT). Therefore, we reviewed the data of 1226 patients with acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), and chronic myelogenous leukemia (CML) who received first allogeneic HSCT between 1994 and 2004, using the database of the Kanto Study Group for Cell Therapy (KSGCT), and analyzed the incidence, risk factors, and outcome of patients with CNS relapse. Twenty-nine patients developed CNS relapse at a median of 296 (9-1677) days after HSCT with a cumulative incidence of 2.3%. Independent significant factors associated with CNS relapse included ALL as the underlying diagnosis (relative risk [RR] = 9.55, 95% confidence interval [CI] = 1.26-72.2, P = .029), nonremission at HSCT (RR = 2.30, 95% CI = 1.03-5.15, P = .042), the history of CNS invasion before HSCT (RR = 5.62, 95% CI = 2.62-12.0, $P = 9.2 \times 10^{-6}$), and the prophylactic intrathecal chemotherapy after HSCT (RR = 2.57, 95% CI = 1.21-5.46, P = .014). The 3-year overall survival (OS) after CNS relapse was 18%. In 7 of 29 patients with CNS relapse, leukemia was observed only in CNS. Three of 7 patients were alive without systemic relapse, resulting in 3-year survival after CNS relapse of 46%. Although the outcome of patients with CNS relapse was generally poor, long-term disease-free survival could be achieved in some patients.

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KEY WORDS: Leukemia, Central nervous system, Relapse, Allogeneic hematopoietic stem cell transplantation

INTRODUCTION

Relapse of the original disease remains 1 of the most important causes of failure after allogeneic hematopoietic stem cell transplantation (HSCT) for leukemia. Although majority of the patients develop systemic relapse, extramedullary relapse has been also observed after HSCT. The incidence of central nervous system (CNS) relapse after allogeneic HSCT ranged from 2.9% to 11% [1-3]. Risk factors for CNS relapse identified in previous studies included CNS involvement before HSCT [2] and nonremission at HSCT [1]. Prophylactic intrathecal administration of methotrexate (MTX) was shown to decrease the incidence of CNS relapse of acute lymphoblastic leukemia (ALL) in the Seattle study [1], whereas the other 2

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studies failed to find the benefit of prophylactic intrathecal administration of MTX on CNS relapse in patients with acute leukemia [2,3]. There has been no generalized consensus on intrathecal administration of MTX, and in fact, a survey of the European Group for Blood and Marrow Transplantation (EBMT) had reported that the practice varied widely among centers [4].

We examined the incidence, risk factors, and outcome of CNS relapse after allogeneic HSCT in adult patients with acute myelogenous leukemia (AML), ALL, and chronic myelogenous leukemia (CML), and also evaluated the prophylactic effect of intrathecal administration of MTX on CNS relapse.

MATERIALS AND METHODS

Study Population

The study population consisted of 1226 patients, who underwent allogeneic HSCT for AML, ALL, and CML for the first time between January 1994 and December 2004 at 10 hospitals participating in the Kanto Study Group for Cell Therapy (KSGCT).

Transplantation Procedure

Of the 1226 patients, the sources of stem cell was bone marrow (BM) in 903, peripheral blood stem cells (PBSC) in 178, BM plus PBSC in 10, and cord blood (CB) in 134. Conventional myeloablative conditioning regimens such as total body irradiation (TBI) and cyclophosphamide (Cy), busulfan (Bu), and Cy, and their modified regimens were performed in 1168 patients. Among them, TBI of at least 10 Gy was performed in 815 patients. Reduced-intensity conditioning (RIC) regimens were conducted in 53 patients. Prophylaxis of graft-versus-host disease (GVHD) was attempted with calcineurin inhibitors (cyclosporine [CsA] or tacrolimus) with or without short-term MTX in the majority of patients.

Definition of CNS Relapse

CNS relapse was diagnosed as the presence of leukemic cells in the cerebrospinal fluid (CSF). Isolated CNS relapse was defined as CNS relapse without any other sites of relapse of leukemia.

Statistical Considerations

Overall survival (OS) was calculated using the Kaplan-Meier method. Cumulative incidence of CNS relapse was calculated using Gray's method, considering death without CNS relapse as a competing risk [5]. Cumulative incidence of isolated CNS relapse was calculated using Gray's method, treating systemic relapse and death without relapse as a competing risk [5]. The protective effect of chronic GVHD (cGVHD) on CNS relapse was evaluated among patients who developed bone marrow relapse within 100 days after HSCT. Factors associated with at least borderline significance ($P < .10$) in the univariate analyses were subjected to a multivariate analysis using backward stepwise proportional-hazard modeling. Finally, P values of $<.05$ were considered statistically significant.

RESULTS

Characteristics of the Patients

Characteristics of patients included in the study were listed in Table 1. The median age was 36 years, ranging from 15 to 69 years. The underlying diseases were AML (n = 533), ALL (n = 352), and CML (n = 341). Eighty-one patients had the history of CNS involvement before HSCT. Eight hundred and nine patients were in complete remission of acute leukemia or in chronic phase of CML at HSCT, and the remaining patients had active disease. In the following analyses, CML in the chronic phase was included in leukemia in complete remission.

CNS Relapse

Twenty-nine patients developed CNS relapse at a median of 296 days (9-1677 days) after HSCT, giving the cumulative incidence of 2.3% (Figure 1). The median age was 31 years (range: 17-47). The underlying disease was ALL in 18, AML in 9, and CML in 2. Sixteen patients had CNS involvement before HSCT and

Table 1. Characteristics of Patients

Median age (range) at transplantation	36 (15-69)
Sex	
Male	762
Female	464
Underlying disease	
AML	533
ALL	352
CML	341
Disease status	
CR	809
non-CR	416
History of CNS disease	
Yes	81
No	802
Type of conditioning	
Conventional	1168
Reduced intensity	53
TBI ≥ 10 Gy in conditioning	
Yes	815
No	404
Donor type	
Related	478
Unrelated	548
Stem cell source	
BM	902
PBSC	178
BM + PBSC	10
CB	134

CB indicates cord blood.

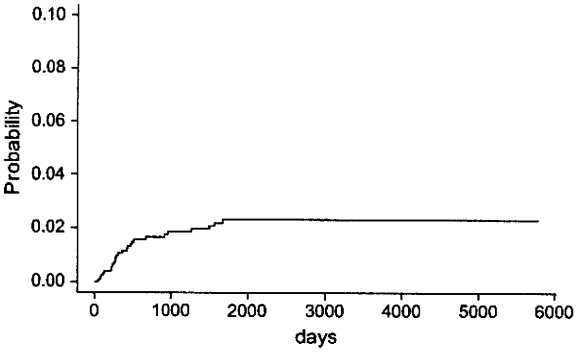

Figure 1. Cumulative incidence of CNS relapse treating death without CNS relapse as competing risk.

6 of them had active CNS disease at HSCT. OS after CNS relapse was 42% at 1 year and 18% at 3 years (Figure 2A). OS of the whole patient cohort, patients with CNS relapse, and those without CNS relapse was 59.8%, 33.2%, and 60.6%, respectively, at 3 years after transplantation.

Pretransplant factors that affected the incidence of CNS relapse after HSCT with at least borderline significance were ALL as the underlying disease, active disease at HSCT, a history of CNS leukemia, the use of TBI regimens, HSCT from an unrelated donor, and the use of prophylactic intrathecal chemotherapy after HSCT (Table 2). Among them, multivariate analysis showed that ALL as the underlying disease, active disease at HSCT, the history of CNS involvement, and the use of intrathecal chemotherapy after HSCT were independently significant (Table 2 and Figure 3). The cumulative incidences of CNS relapse in patients with and without a history of CNS involvement before HSCT were 21.3% and 1.3%, respectively (Figure 3A). Patients with ALL were at higher risk for CNS relapse even in patients in remission at HSCT without a history of CNS involvement before HSCT (ALL 2.7%, AML 0.8%, and CML 0.4%, P = .088, Figure 4A). Twenty-three patients who had active leukemia at HSCT had persistent disease after HSCT. Among these, only 2 patients developed CNS relapse after HSCT. However, median survival of this cohort was only 90 days after HSCT producing a 1-year survival of 14%, and thus, majority of the patients died very early, before developing CNS relapse.

Effect of Intrathecal Chemotherapy on the Incidence of CNS Relapse

The practice of intrathecal chemotherapy in allogeneic HSCT recipients varied among the 10 institutions of the KSGCT. Half of them never used prophylactic intrathecal chemotherapy before and after HSCT. The remaining half administered intrathecal prophylaxis routinely before HSCT, of which 2 institutions added intrathecal chemotherapy after

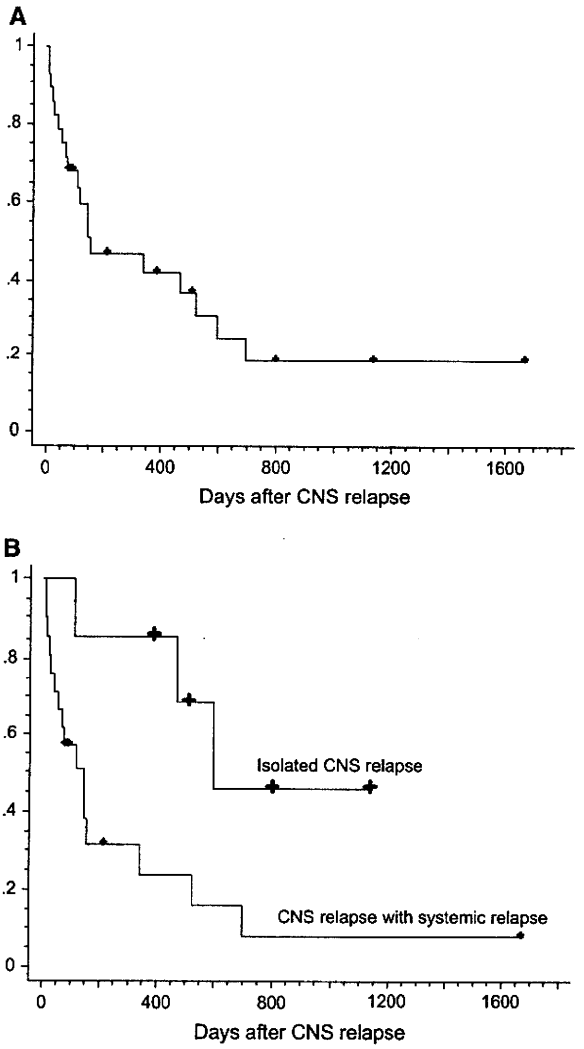

Figure 2. OS after CNS relapse (A) and that grouped according to isolated CNS relapse or CNS relapse associated with systemic relapse (B).

HSCT for high-risk patients such as those with ALL or the history of CNS involvement. In this cohort, intrathecal prophylaxis before HSCT was conducted in 701 of 887 patients and intrathecal chemotherapy after HSCT was done in 141 of 807 patients whose information about intrathecal chemotherapy was available. Antineoplastic agents used for intrathecal chemotherapy mainly consisted of MTX. The median numbers of intrathecal chemotherapy before and after HSCT were 1 (range: 1-4) and 2 (range: 1-4), respectively.

We failed to find a significant prophylactic effect of intrathecal chemotherapy for CNS relapse. The relative risk for CNS relapse was 1.52 (95% CI 0.61-3.79, P = .37) for intrathecal chemotherapy before HSCT and 3.92 (95% CI 1.80-8.51, P = .00057) for intrathecal chemotherapy after HSCT (Table 2). This adverse influence of intrathecal chemotherapy after HSCT was significant even after adjusted for the

Table 2. Impact of Pretransplant Factors on the Incidence of CNS Relapse after Transplantation

Factor		Univariate RR (95% CI)	P value	Multivariate RR (95% CI)	P value
Age		1.00 (1.00-1.00)	.15		
Sex		1.01 (0.65-1.59)	.95		
Disease	CML	1.00		1.00	
	AML	5.58 (0.70-44.5)	.10	3.60 (0.46-28.4)	.22
	ALL	17.7 (2.36-132.8)	.0052	9.55 (1.26-72.2)	.029
CR/non-CR		2.33 (1.08-5.04)	.031	2.30 (1.03-5.15)	.042
History of CNS disease		17.9 (8.30-38.6)	2.0×10^{-13}	5.62 (2.62-12.0)	9.2×10^{-6}
TBI		2.91 (1.00-8.44)	.050		
Conventional/reduced intensity		0.99 (0.47-2.07)	.97		
Related/unrelated		1.85 (1.06-3.23)	.030		
Source	BM	1.00			
	PBSC	0.24 (0.03-1.77)	.16		
	CB	0.70 (0.17-2.96)	.63		
Sex mismatch		1.06 (0.42-2.66)	.90		
HLA mismatch		0.46 (0.06-3.46)	.45		
Prophylactic IT before HSCT		1.52 (0.61-3.79)	.37		
Prophylactic IT after HSCT		3.92 (1.80-8.51)	.00057	2.57 (1.21-5.46)	.014

IT indicates intrathecal chemotherapy; CNS, central nervous system; RR, relative risk.

underlying disease, disease status at HSCT, and the history of CNS involvement before HSCT (relative risk 2.57, 95% CI 1.21-5.46, $P = .014$). Among patients without a history of CNS involvement before HSCT who were in remission at HSCT, the incidences of CNS relapse after HSCT were 3.6% and 1.6% who received and did not receive intrathecal chemotherapy after HSCT, respectively ($P = .057$, Figure 4B). In patients with a history of CNS involvement before HSCT, the incidences of CNS relapse after HSCT were 37.4% and 11.6%, respectively, who received and did not receive intrathecal chemotherapy after HSCT ($P = .018$; Figure 4C). When we limited the analysis in patients with ALL, the incidences of CNS relapse after HSCT were 6.2% and 3.7% who received and did not receive intrathecal chemotherapy after HSCT ($P = .17$), respectively, in patients without a history of CNS involvement before HSCT who were in remission at HSCT and they were 55.6% and 15.5%, respectively, in patients with a history of CNS involvement before HSCT ($P = .0081$).

Nine patients developed leukoencephalopathy with a median onset of 288 days after HSCT. The incidence of leukoencephalopathy was significantly higher in patients who underwent intrathecal chemotherapy after HSCT (3.5% versus 0.5%, $P = .0076$).

Isolated CNS Relapse

Seven patients developed isolated CNS relapse at a median of 671 days (125-1677 days) after HSCT, presenting the cumulative incidence of 0.70%. Characteristics of these 7 patients were listed in Table 3. All received bone marrow as stem cell source. Prognostic factors associated with isolated CNS relapse with at least borderline significance were age, active disease at HSCT, CNS involvement before HSCT, stem cell source, the use of intrathecal chemotherapy after HSCT, and the absence of HLA mismatch. Among these, independent significant factors for isolated CNS relapse included the history of CNS involvement before HSCT, the use of PBSC or CB as stem cell source, and the absence of HLA mismatch (Table 4). The treatment of isolated CNS relapse consisted of intrathecal chemotherapy and/or cranial irradiation and CNS disease was successfully controlled in 5 of the 7 patients. Four patients developed bone marrow relapse within 1 year. However, the remaining 3 patients were alive without systemic relapse at 518, 807, and 1149 days after CNS relapse and 1283, 1478, and 2195 days after HSCT, respectively. Survival after CNS relapse was significantly better in patients who developed isolated CNS relapse than those who developed CNS relapse with systemic relapse (46% versus 8% at 3 years, $P = .023$, Figure 2B).

Effect of cGVHD on CNS Relapse

Among the 378 patients who experienced bone marrow relapse within 100 days after HSCT but were free from CNS relapse at day 100, 21 (6.1%) showed CNS relapse later on. The incidence of CNS relapse after bone marrow relapse was 7.1% in patients with cGVHD and 2.0% in those without cGVHD ($P = .14$).

Analysis Excluding CML Patients

We repeated these analyses excluding patients with CML, because the incidence of CNS relapse was extremely low, as shown in Figure 3C. The cumulative incidence of CNS relapse was 3.2%. Independently significant pretransplant factors for CNS relapse

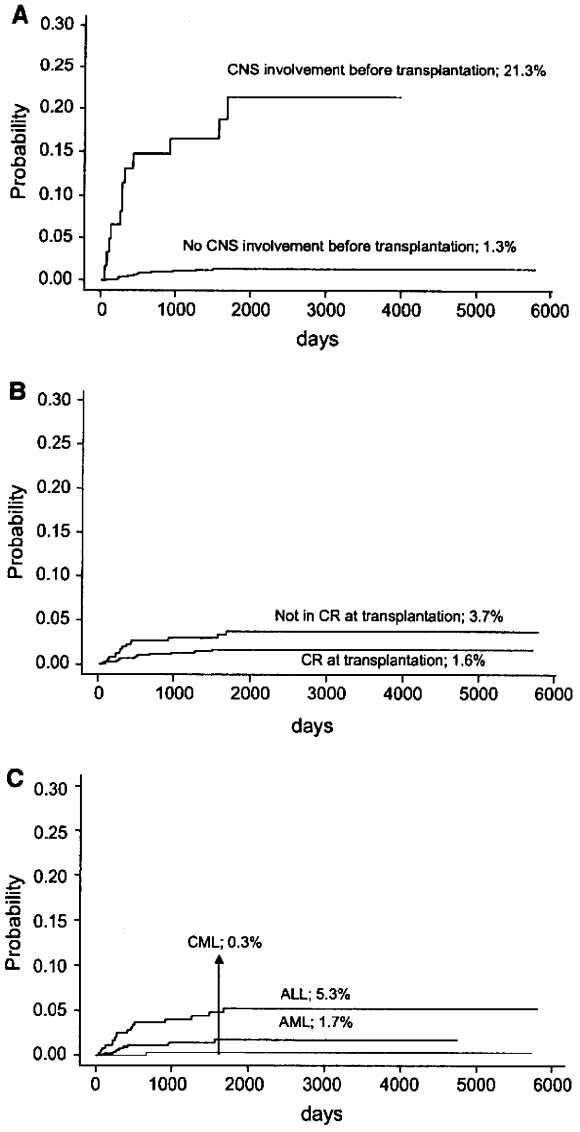

Figure 3. Cumulative incidence of CNS relapse grouped according to the history of CNS involvement before transplantation (A), disease status at transplantation (B), and underlying disease (C).

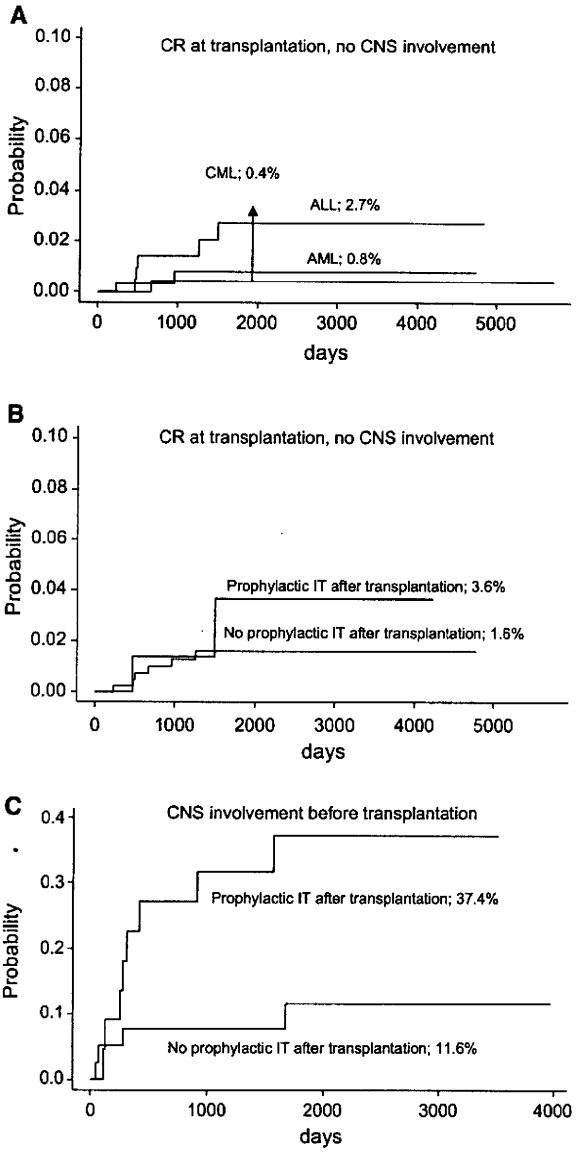

Figure 4. Cumulative incidence of CNS relapse in patients in remission at transplantation without a history of CNS involvement before transplantation grouped according to the underlying disease (A) and the use of prophylactic intrathecal chemotherapy (IT) after transplantation (B). Cumulative incidence of CNS relapse in patient with CNS involvement before transplantation grouped according to the use of prophylactic IT after transplantation (C).

were the same as the analyses including CML patients; ALL compared to AML as the underlying disease (RR 2.68, 95% CI 1.18-6.11, P = .019), active disease at HSCT (RR 2.49, 95% CI 1.08-5.73, P = .032), the history of CNS involvement (RR 5.64, 95% CI 2.60-12.3, P = .000012), and the use of intrathecal chemotherapy after HSCT (RR 2.69, 95% CI 1.25-5.81, P = .012). The cumulative incidence of isolated CNS relapse was 0.9%. Independently significant pretransplant factors for CNS relapse included ALL compared to AML as the underlying disease, the history of CNS involvement, the use of PBSC as stem cell source, the absence of HLA mismatch, and the use of intrathecal chemotherapy after HSCT.

DISCUSSION

The cumulative incidences of CNS relapse and isolated CNS relapse were 2.3% and 0.70% in this cohort, respectively, which were almost comparable with those in previous studies (Table 5) [1-3]. The history of CNS leukemia before HSCT was identified as the strongest predictive factor for CNS relapse after HSCT in our study as previously reported [1,2].

We could not show a beneficial effect of prophylactic intrathecal chemotherapy on the incidence of

Table 3. Characteristics of Patients Who Developed Isolated CNS Relapse after Transplantation

Patient No.	1	2	3	4	5	6	7
Age	23	31	24	35	26	41	18
Sex	M	M	M	M	F	M	M
Disease	CML	CML	ALL	AML	ALL	ALL	ALL
Disease status	CP2	BC	RL2	RL1	RL2	CR1	RL2
History of CNS disease	Yes	Yes	Yes	Yes	Yes	No	No
Stem cell source	BM	BM	BM	BM	BM	BM	BM
Donor type	R	R	U	R	R	R	U
HLA mismatch	No	Yes	No	No	No	No	Yes
Conditioning regimem	Bu+Cy	CA+Cy+TBI	ETP+Cy+TBI	CA+Cy+TBI	ETP+Cy+TBI	Cy+TBI	CA+Cy+TBI
Days to an isolated CNS relapse	671	134	125	1565	276	1265	1677
CNS treatment	IT+RT	IT+RT	IT	IT+DLI	RT	IT+RT	IT
Systemic relapse	No	No	Yes	Yes	Yes	Yes	No
Days from HSCT to systemic relapse	164	1680	444	1572			
Day from CNS relapse to systemic relapse	39	115	168	307			
Outcome	Alive	Alive	Dead	Dead	Dead	Alive	Alive
Follow-up duration (days)	1478	1283	236	2031	870	1661	2195

IT indicates intrathecal chemotherapy; RT, radiation; DLI, donor lymphocyte infusion; BU, busulfan; CY, cyclophosphamide; CA, cytarabine; ETP, etoposide; CNS, central nervous system; HSCT, hematopoietic stem cell transplantation.

CNS relapse after HSCT. The incidence of CNS relapse was rather higher in patients who received intrathecal chemotherapy after HSCT. This was probably biased by the fact that significantly higher proportion of patients received intrathecal chemotherapy after HSCT among patients with CNS involvement before HSCT than those without CNS leukemia (47.4% versys 13.4%, $P < .0001$). However, intrathecal chemotherapy after HSCT significantly adversely affected the incidence of CNS relapse even after adjusted for the underlying disease, disease status at HSCT, and the history of CNS involvement before HSCT. Also, a benefit of intrathecal chemotherapy after HSCT was not shown in patients with ALL, in contrast with the previous reports [1,6]. This discrepancy might have resulted from the difference in the intensity of the intrathecal chemotherapy. Intrathecal chemotherapies were administered 6 times after HSCT in the Seattle group, whereas the medium number of intrathecal chemotherapy in the current study was only 2 (range: 1-4). Therefore, the intensity of intrathecal chemotherapy might be important to sufficiently prevent CNS relapse after HSCT. However, they observed the development of leukoencephalopathy in 7 of the 415 patients and we also observed leukoencephalopathy significantly more frequently in patients who received intrathecal chemotherapy after HSCT than those who did not. Therefore, such an intensive intrathecal chemotherapy should be avoided for patients at low risk for CNS relapse. We had a concern that the use of intrathecal chemotherapy after HSCT might delay immune recovery and thereby

Table 4. Impact of Pretransplant Factors on the Incidence of Isolated CNS Relapse after Transplantation

Factor		Univariate RR (95% CI)	P-Value	Multivariate RR (95% CI)	P-Value
Age		0.99 (0.98-1.00)	.055		
Sex		1.05 (0.47-2.34)	.90		
Disease	CML	1.00			
	AML	0.73 (0.04-11.9)	.82		
	ALL	5.31 (0.61-45.9)	.13		
CR/non-CR		4.98 (0.97-25.7)	.055		
History of CNS disease		48.3 (9.37-249.4)	3.6×10^{-6}	48.1 (9.40-245.9)	3.3×10^{-6}
TBI		3.21 (0.38-26.8)	.28		
Conventional/reduced intensity		1.08 (0.26-4.49)	.92		
Related/unrelated		1.45 (0.65-3.24)	.37		
Source	BM	1.00		1.00	
	PBSC	N.A.	<.0001	N.A.	<.0001
	CB	N.A.	<.0001	N.A.	<.0001
Sex mismatch		1.58 (0.26-9.41)	.62		
HLA mismatch		N.A.	<.0001	N.A.	<.0001
Prophylactic IT before HSCT		1.09 (0.21-5.61) 0.92			
Prophylactic IT after HSCT		7.11 (1.62-31.2) 0.0094			

N.A. indicates not assessable because no events were observed in the group; IT, intrathecal chemotherapy; RR, relative risk.

Table 5. Cumulative Incidence of CNS Relapse after HSCT in Prior Studies and Our Study

	n	Underlying Disease (AML/ALL/CML)	History of CNS Leukemia (%)	CR at Transplant (%)	Allogeneic Transplant (%)	Incidence of CNS Relapse (%)	Reference
1	415	217/198/0	23.4	47.7	100	2 in AML, 13 in ALL	1
2	92	0/92/0	22.8	100	71.7	11	2
3	487	366/121*/0	3.5	100	67.6	2.9	3
4	1226	533/352/341	9.2	65.8	100	2.3	Present report

*Including 5 patients with acute unclassified leukemia.

increase the risk of systemic relapse, but the incidence of systemic relapse was not significantly different between those who received intrathecal chemotherapy and those who did not (relative risk 1.11, 95% CI 0.79-1.55, $P = .56$). The use of total body irradiation (TBI) in the conditioning regimen has been considered to prevent CNS relapse, because irradiation is effective for so called sanctuary sites of chemotherapy. However, the incidence of CNS relapse was also rather higher in patients who received the TBI regimen. This may be again because of the fact that significantly higher proportion of patients received the TBI regimen among patients with CNS involvement before HSCT than those without CNS leukemia (81.5% versus 57.9%, $P < .0001$).

As for stem cell source, isolated CNS relapse was observed exclusively after BMT. A possible explanation for this may be the year effect, because allogeneic PBSCT and CBT started after 2000 in Japan. However, the year of HSCT of patients who developed isolated CNS relapse evenly ranged between 1997 and 2002. Another possible explanation is the presence of graft-versus-CNS relapse effect enhanced by increased incidence of cGVHD after allogeneic PBSCT and the presence of HLA-mismatch in CBT. The significantly higher incidence of CNS relapse after autologous HSCT than that after allogeneic HSCT suggested the existence of such an immunologicprotection against CNS relapse [2]. Isolated extramedullary relapse was also reported to be observed earlier in autologous HSCT than in allogeneic HSCT [7]. Furthermore, successful treatment of CNS relapse with reduced-intensity transplantation may suggest the presence of graft-versus-leukemia CNS leukemia effect [8], although the other reports doubted such effect against for CNS lesions [9-12]. The observed tendency toward a lower CNS relapse incidence after bone marrow relapse in patients with cGVHD than those without cGVHD in the current study might support this speculation, although we have no immunologic evidence.

The prognosis of patients who developed relapse after allogeneic HSCT has been reported to be extremely poor [13,14]. Also, survival after isolated CNS relapse was reported to be no better than that after bone marrow relapse in pediatric patients with AML and adult patients with ALL [15,16]. However, in the current study, 3 of the 7 patients who developed isolated CNS relapse were alive for more than a year without leukemia, resulting in the significantly better survival than those who developed CNS relapse after or simultaneously with systemic relapse. We could not identify the reason for this discrepancy, but the age and underlying disease of the study population differed between our study and the previous report. We consider that an intensive treatment against CNS leukemia is warranted for adult patients with isolated CNS relapse.

In conclusion, we confirmed that ALL as the underlying disease, active disease at HSCT, and the history of CNS involvement before HSCT were significant predictors for CNS relapse after HSCT. We failed to show a significant prophylactic effect of intrathecal chemotherapy to prevent CNS relapse and such a prophylactic treatment should be avoided for patients at low risk for CNS relapse. The prognosis for isolated CNS relapse was surprisingly good.

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REFERENCES

1. Thompson CB, Sanders JE, Flournoy N, et al. The risks of central nervous system relapse and leukoencephalopathy in patients receiving marrow transplants for acute leukemia. *Blood*. 1986;67: 195-199.
2. Ganem G, Kuentz M, Bernaudin F, et al. Central nervous system relapses after bone marrow transplantation for acute lymphoblastic leukemia in remission. *Cancer*. 1989;64:1796-1804.
3. Singhal S, Powles R, Treleaven J, et al. Central nervous system relapse after bone marrow transplantation for acute leukemia in first remission. *Bone Marrow Transplant*. 1996;17:637-641.
4. Ruutu T, Corradini P, Gratwohl A, et al. Use of intrathecal prophylaxis in allogeneic haematopoietic stem cell transplantation for malignant blood diseases: a survey of the European Group for Blood and Marrow Transplantation (EBMT). *Bone Marrow Transplant*. 2005;35:121-124.
5. Gooley TA, Leisenring W, Crowley J, et al. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med*. 1999;18:695-706.
6. Nagatoshi Y, Kawano Y, Nagayama J, et al. Treatment of isolated central nervous system relapse in high-risk lymphoid malignancy with allogeneic bone marrow transplantation and extended intrathecal therapy. *Br J Haematol*. 2004;125:766-768.

7. Potenza L, Luppi M, Riva G, et al. Isolated extramedullary relapse after autologous bone marrow transplantation for acute myeloid leukemia: case report and review of the literature. *Am J Hematol.* 2006;81:45-50.
8. Ostronoff M, Domingues MC, Ostronoff F, et al. Reduced intensity conditioning allogeneic bone marrow transplantation following central nervous system (CNS) relapse of acute promyelocytic leukemia: evidence for a graft-versus-leukemia effect in the CNS. *Am J Hematol.* 2006;81:387-388.
9. Au WY, Lie AK, Liang R, et al. Isolated extramedullary relapse of acute lymphoblastic leukaemia after allogeneic bone marrow transplantation. *Bone Marrow Transplant.* 1999;24:1137-1140.
10. Seo S, Kami M, Honda H, et al. Extramedullary relapse in the so-called "sanctuary" sites for chemotherapy after donor lymphocyte infusion. *Bone Marrow Transplant.* 2000;25:226-227.
11. Salutari P, Sica S, Micciulli G, et al. Extramedullary relapse after allogeneic bone marrow transplantation plus buffy-coat in two high risk patients. *Haematologica.* 1996;81:182-185.
12. Wilson DB, Michalski JM, Grossman WJ, et al. Isolated CNS relapse following stem cell transplantation for juvenile myelomonocytic leukemia. *J Pediatr Hematol Oncol.* 2003;25:910-913.
13. Bosi A, Laszlo D, Labopin M, et al. Second allogeneic bone marrow transplantation in acute leukemia: results of a survey by the European Cooperative Group for Blood and Marrow Transplantation. *J Clin Oncol.* 2001;19:3675-3684.
14. Breems DA, Van Putten WL, Huijgens PC, et al. Prognostic index for adult patients with acute myeloid leukemia in first relapse. *J Clin Oncol.* 2005;23:1969-1978.
15. Johnston DL, Alonzo TA, Gerbing RB, et al. Risk factors and therapy for isolated central nervous system relapse of pediatric acute myeloid leukemia. *J Clin Oncol.* 2005;23:9172-9178.
16. Sancho JM, Ribera JM, Oriol A, et al. Central nervous system recurrence in adult patients with acute lymphoblastic leukemia: frequency and prognosis in 467 patients without cranial irradiation for prophylaxis. *Cancer.* 2006;106:2540-2546.