Table 4.
Transcripts Decreased in K562/ADM Cells*

Tag	Abundance, K562	Abundance, K562/ADM	Fold Decrease	Unigene Cluster	Matched Gene
CCGGGCGTGG	48	0	48	503546	Fatty acid desaturase 1
CGCCGCCGGC	64	2	32	182825	Ribosomal protein L35
GGGACTGGGC	20	1	20	117848	Hemoglobin, ε 1
AGCTCTCCCT	16	1	16	374588	Ribosomal protein L17
GTGGCGGGCG	15	1	15	22926	KIAA0795 protein
AAGGAGATGG	11	0	11	375921	Ribosomal protein L31
CACAAACGGT	10	1	10	504524	Ribosomal protein S27
GTGGCAGGCG	10	1	10	385548	DKFZP434B168 protein
AGCACTGCAG	9	1	9	346743	*N*-myristoyltransferase 1
CTTCTTGCCC	9	1	9	398636	Hemoglobin, α 2
GCGAGACCCT	9	1	9	124962	Hypothetical protein BC004895
GTGGCACACG	9	1	9	192023	Eukaryotic translation initiation factor 3, subunit 2β, 36 kDa
				34114	ATPase, Na^+/K^+ transporting, α 2 (+) polypeptide
TGGATCCTGA	9	0	9	117848	Hemoglobin, ε 1
CGGTTACTGT	8	0	8	408257	NADH dehydrogenase Fe-S protein 6
GGCTCCCACT	8	0	8	74335	Heat shock 90-kDa protein 1, β
GTACTGTATG	8	0	8	439683	Karyopherin β1

*The tag sequence represents the 10–base pair SAGE tag. Unigene cluster matches are listed.

nase Fe-S protein 6 (*NDUFS6*), *N*-myristoyltransferase 1 (*NMT1*), heat shock 90-kDa protein, and karyopherin (importin) β1 (*KPNB1*), was also observed. These findings suggest that doxorubicin-resistant leukemic cells may reduce protein importation into the nucleus.

Prior to the present investigation, the differential expression in doxorubicin-resistant K562 cells and parental K562 cells of the genes described above was unknown. One of the mechanisms of multidrug resistance involves the overexpression of P-glycoprotein, a large transmembrane glycoprotein capable of adenosine triphosphate–dependent cellular efflux of a variety of drugs across the membrane [12,13]. The reason why the *MDR1* gene was not listed in our SAGE expression profile of doxorubicin-resistant K562 cells might be explained by its low expression levels. Although we remain uncertain of the roles of the genes identified in the present study and although we cannot exclude the possibility that the altered gene expression profile may not be related to drug resistance because the K562/ADM line was established more than 10 years ago, a number of the differentially expressed genes should provide valuable information for exploring the mechanisms of drug resistance in human leukemia.

Another interesting finding of the present study is that quantitative real-time RT-PCR analysis revealed differences in gene expression patterns for *EIF1A* and *FADS1* between K562/ADM and K/eto cells. Etoposide-resistant K/eto cells have been reported to not overexpress the MDR1 gene. This feature of K/eto cells is distinctively different from that of doxorubicin-resistant K562/ADM cells [14]. Therefore, although we do not have SAGE data on K/eto cells, we assume that the gene expression profiles of drug-resistant leukemia cells may be different, depending on the drugs for which they show resistance. SAGE analysis may provide further insight into our understanding of drug resistance in human leukemia and offer a perspective different than that offered by cDNA microarray analysis.

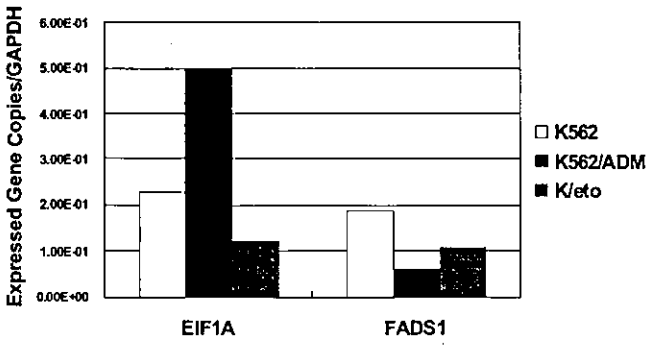

Figure 2. Quantitative real-time reverse transcription–polymerase chain reaction analysis for *EIF1A* and *FADS1* gene expression in K562/ADM and K/eto cells. Data are shown as the numbers of expressed gene copies relative to the expression of the glyceraldehyde phosphate dehydrogenase (GAPDH) gene.

Acknowledgments

The authors are grateful to Dr. K. Kinzler (Johns Hopkins University) for providing us with SAGE software. We also thank Dr. T. Tsuruo (University of Tokyo) and Dr. H. Fujii (Nihon Kayaku) for the kind gift of the cell lines.

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Powerful Graft-Versus-Leukemia Effects Exerted by HLA-Haploidentical Grafts Engrafted with a Reduced-Intensity Regimen for Relapse Following Myeloablative HLA-Matched Transplantation

The outcome of patients with refractory leukemia who undergo nonmyeloablative stem cell transplantation (NST) from matched-sibling donors is poor (*1*). In the present report, we assess whether NST from an HLA-2-antigen-mismatched (haploidentical) donor could exert appreciable graft-versus-leukemia (GVL) effects.

With the use of a conditioning regimen consisting of cyclophosphamide and total body irradiation, two patients with acute myelogenous leukemia (AML) first underwent myeloablative allogeneic stem cell transplantation from an HLA-matched sibling donor, developed no graft-versus-host disease (GVHD) clinically, and had a hematologic relapse on days 63 and 535, respectively. For the second transplantation, both donors had 2-antigen-mismatches in the graft-versus-host (GVH) direction. The conditioning regimen consisted of: fludarabine 30 mg/m^2 on days −9 to −4; busulfan 4 mg/kg on days −6 and −5; and ATG (Fresenius) 2 mg/kg on days −4 to −1 (*2*, *3*). GVHD prophylaxis was performed with tacrolimus and 1 mg/kg methylprednisolone. Peripheral blood stem cells were infused without T-cell depletion. The protocol was approved by the institutional review board of Osaka University, and informed consent was obtained from each patient. Minimal residual disease (MRD) in bone marrow was monitored frequently by quantitating *WT1* gene expression levels (*4*).

Both patients achieved rapid engraftment. Patient 1 (Fig. 1A) had acute GVHD on day 9, which was controlled by an increase in the dose of steroids. The *WT1* level decreased to the background level at engraftment, began to increase on day 90, went over the background level on day 103, and ultimately the patient had a hematologic relapse on day 132. He died of pneumonia on day 147. Patient 2 (Fig. 1B) had no acute GVHD. The *WT1* expression level, which was abnormally high before the second transplantation, decreased to background level at engraftment. Afterwards, the *WT1* expression level gradually increased, and reached 1.2×10^{-3} on day 237. However, the *WT1* level decreased again to the background level after the tapering of the dose of immunosuppressants and infusion of donor leukocytes, and continued to be at the background level until the patient died of pneumonia that was associated with chronic GVHD on day 379.

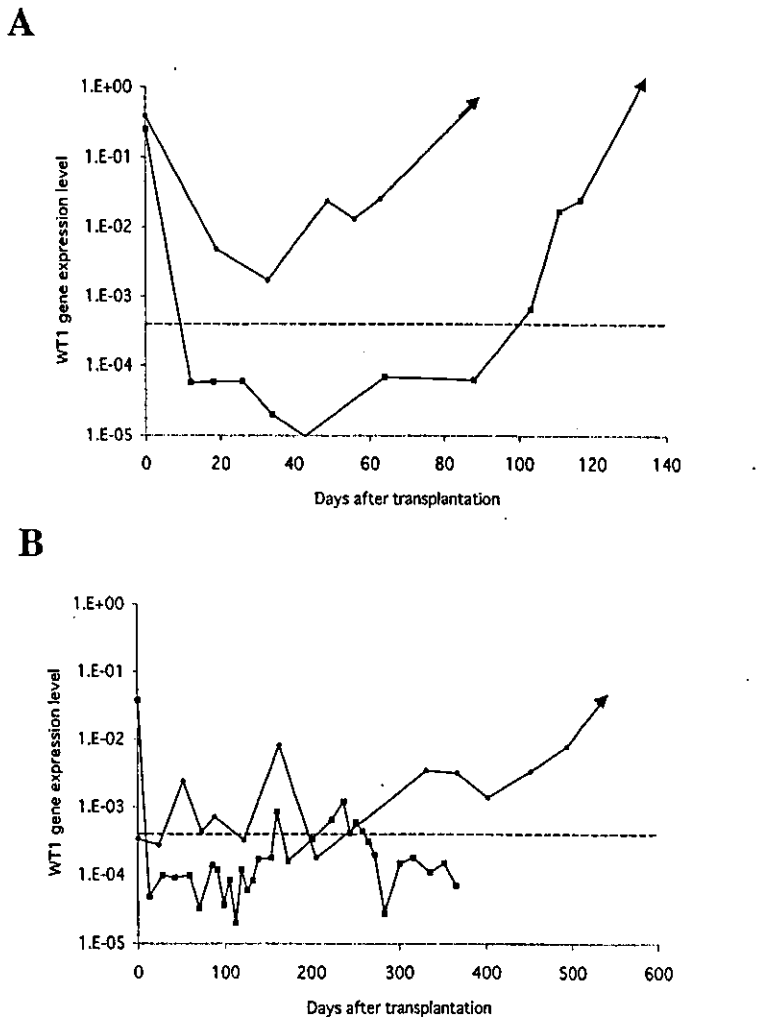

FIGURE 1. Monitoring of minimal residual disease (MRD) by quantitation of WT1 gene expression levels in two patients with acute myelogenous leukemia (AML) who underwent transplantation twice. (A) Patient 1. The HLA profiles of the patient and donor were: patient, A 26 B 35 DR 15 / A 24 B 60 DR 12; donor (aunt), A 24 B 62 DR 15 / A 24 B 60 DR 12. (B) Patient 2. The HLA profiles of the patient and donor were: patient, A 24 B 54 DR 4 / A 24 B 35 DR 11; donor (daughter), A 24 B 61 DR 14 / A 24 B 35 DR 11. ●, *WT1* gene expression levels following the first myeloablative stem cell transplantation from an HLA-matched sibling; ■, *WT1* gene expression levels following the second nonmyeloablative stem cell transplantations from an HLA-mismatched relative; *arrows*, *WT1* expression levels at the time of the occurrence of hematologic relapse; *broken lines*, upper limit of the background levels of *WT1* expression in the bone marrow.

These results show that donor lymphocytes engrafted in HLA-haploidentical NST exerted a strong GVL effect even for patients with AML who had a relapse after conventional myeloablative bone marrow transplant from a matched sibling donor. For Patient 1, the *WT1* levels were abnormally high to similar extents before the two transplantations. MRD was more intensely suppressed and hematologic complete remission lasted longer in the second transplantation than in the first transplantation. For Patient 2, although the *WT1* level before the second transplantation was higher by more than 2 orders than that before the first transplantation, it rapidly decreased by 3 orders by the time of engraftment, and was consistently lower than that in the first transplantation for 6 months after transplantation.

In conclusion, these findings strongly suggest that HLA disparity is more important for obtaining strong antileukemia effects than the intensity of the conditioning regimen, at least in the early stage of transplantation.

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ERRATA

In the article titled, "A Comparative Prospective Study of Two Available Solutions for Kidney and Liver Preservation," by P. Pedotti et al., published in the May 27, 2004 issue of *Transplantation* (volume 77, pp. 1540–1545), an author was inadvertently omitted. Domenico Montanaro should have been included as the eighteenth author. He is affiliated with Azienda Ospedaliera S. Maria della Misericordia in Udine, Italy.

In the article titled, "Preliminary Experience with Alemtuzumab (Campath-1H) and Low-Dose Tacrolimus Immunosuppression in Adult Liver Transplantation," by A. Tzakis et al., published in the April 27, 2004 issue of *Transplantation* (volume 77, pp. 1209–1214), there is an error in the Methods section. It was incorrectly stated that:

> "Beginning in December 2001, candidates for liver transplantation at the Jackson Memorial Hospital and University of Miami Medical Center were offered the option to participate in a study using C1H and Tac immunosuppression. The study was previously approved by the institutional review board. The study group (group 1, n=40) was composed of all patients who entered the study and underwent transplantation between December 2001 and April 2003."

In fact, among the 40 patients reported, 20 were treated on the IRB-approved protocol. The other 20 patients were treated using exactly the same treatment protocol with "off-label" use of Campath. IRB approval for a 20 patient on-protocol study was granted in April 2002, and the on-protocol patients began accruing into the study in October 2002. Thus, 15 patients were treated off-protocol prior to the inclusion of on-protocol patients, and an additional 5 off-protocol patients were treated after the accrual of the 20 on-protocol patients.

Both perforin and Fas ligand are required for the regulation of alloreactive CD8$^+$ T cells during acute graft-versus-host disease

Yoshinobu Maeda, Robert B. Levy, Pavan Reddy, Chen Liu, Shawn G. Clouthier, Takanori Teshima, and James L. M. Ferrara

Fas ligand (FasL) and perforin pathways not only are the major mechanisms of T cell–mediated cytotoxicity but also are involved in homeostatic regulation of these T cells. In the present study, we tested whether CD8$^+$ donor T cells that are deficient in both perforin and FasL (cytotoxic double deficient [cdd]) could induce graft-versus-host disease (GVHD) in a major histocompatibility complex class I–mismatched lethally irradiated murine model. Interestingly, recipients of cdd CD8$^+$ T cells demonstrated significantly greater serum levels of interferon gamma and tumor necrosis factor alpha and histopathologic damage from GVHD than wild-type (wt) T cells on day 30 after allogeneic bone marrow transplantation ($P < .05$). Wt and either perforin-deficient or FasL-deficient CD8$^+$ T cells expanded early after transplantation followed by a contraction phase in which the majority of expanded CD8$^+$ T cells were eliminated. In contrast, cdd CD8$^+$ T cells exhibited prolonged expansion and reduced apoptosis to alloantigen stimulation in vivo and in vitro. Together these results suggest that donor cdd CD8$^+$ T cells expand continuously and cause lethal GVHD, and that both perforin and FasL are required for the contraction of alloreactive CD8$^+$ T cells. (Blood. 2005;105: 2023-2027)

Introduction

Graft-versus-host disease (GVHD) is the major complication of allogeneic bone marrow transplantation (BMT). GVHD occurs when donor T cells recognize major histocompatibility complex (MHC) and their associated peptides on host-derived antigen-presenting cells (APCs).[1-3] The target organs of GVHD are the skin, gut, liver, and lymphohematopoietic compartments. We recently demonstrated that alloantigen expression on host target epithelium is not necessary to initiate GVHD in mouse models of BMT, and inflammatory cytokines play a central role in the CD4$^+$-mediated GVHD.[4] Interestingly, single cytotoxic-deficient (perforin or Fas ligand [FasL]) donor T cells can induce CD4$^+$-mediated GVHD,[5,6] and recently, studies have demonstrated that cytotoxic double-deficient (cdd) CD3$^+$ and CD4$^+$CD8$^-$ T cells can also effect GVHD in allogeneic minor histocompatibility antigen (mHA) or MHC-disparate BMT models.[5-7] CD8$^+$-dependent GVHD is reportedly induced by both cytokine- and cytolytic T lymphocyte (CTL)–mediated cytotoxicity as several studies using FasL- or perforin/granzyme-deficient mice suggested that CTL-mediated cytotoxicity plays a role in the CD8$^+$-mediated, MHC class I–mismatched GVHD model.[5,8,9]

Recent studies have demonstrated that FasL and perforin not only are the principal cytotoxic effector molecules, but also are involved in homeostatic regulation of CD8$^+$ T cells. The role of Fas/FasL in lymphocyte homeostasis was clearly established with the recognition that functional null mutations in these proteins were associated with exacerbated autoimmune disease.[10,11] Perforin-deficient mice have essentially normal immune homeostasis. However, considerable evidence indicates the existence of a perforin-dependent mechanism to regulate the magnitude of CD8$^+$ T cell expansion in models as diverse as GVHD, viral and bacterial infections, and dendritic cell (DC) immunization.[12-19] The present studies demonstrate that CD8$^+$ T cells lacking the major cytotoxic pathways can induce GVHD across an MHC class I–only disparity. Moreover, we also found that both perforin and FasL contribute to the regulation of alloreactive CD8$^+$ T cell expansion and contraction during the acute period of GVHD in this model.

Materials and methods

Mice

Female C57BL/6 (B6, H-2^b), B6.C-H2^{bm1}/ByJ (bm1), C57BL/6-*Prf1*^{tm1Sdz}/J (pfp$^{-/-}$, H-2^b), and B6Smn.C3-*Tnfsf6*^{gld}/J (*gld*, H-2^b) mice were purchased from the Jackson Laboratories (Bar Harbor, ME). The bm1 mice possess a mutant class I allele that differs from B6 mice. C57BL/6 cytotoxic double-deficient (perforin and FasL) mice (cdd, H-2^b) were generated from the breeding of *pfp*$^{+/-}$*gld* pairings as described previously.[7] The offspring were then screened for homozygous perforin deficiency by polymerase chain reaction (PCR) as previously reported[20,21] to select for *pfp*$^{-/-}$*gld* (ie, cdd). The cdd mice (6 to 8 weeks old) were maintained in pathogen-free conditions in the Department of Microbiology and Immunology at the University of Miami School of Medicine. *gld* and cdd mice were used as donors before the age of 9 weeks after a flow cytometric analysis confirmed

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T.T. and J.L.M.F. contributed equally to this work.

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that there was no evidence of accumulation of $CD3^+CD4^-CD8^-B220^+$ T cells in their spleens. The age range of mice used as other bone marrow (BM) transplant donors and recipients was between 8 and 12 weeks.

BMT

Mice underwent transplantation according to a standard protocol described previously.[4] Briefly, mice were irradiated again with 13 Gy total body irradiation (TBI), split into 2 doses, and injected with 2×10^6 $CD8^+$ splenic T cells with 5×10^6 T-cell–depleted (TCD) BM cells from wild-type (wt) B6 donors after 13-Gy total body irradiation (TBI). $CD8^+$ T cells were negatively isolated by using CD4, DX5, MHC class II, and CD11b Micro Beads and the auto magnetic-activated cell sorter (MACS) following nylon wool purification of T cells from splenocytes.

Systemic and histopathologic analysis of GVHD

Survival after BMT was monitored daily and the degree of clinical GVHD was assessed weekly by a scoring system that sums changes in 5 clinical parameters: weight loss, posture, activity, fur texture, and skin integrity (maximum index = 10) as described.[22] This score is a more sensitive index of GVHD severity than weight loss alone in multiple murine models.[22] Acute GVHD was also assessed by detailed histopathologic analysis of liver, intestine, and skin, as described.[23,24] The degree of cell infiltration in tissue was assessed by a scoring system incorporating 4 parameters: cell infiltration in portal triada, bill ducts/ductules, vascular, and hepatocellular area. Slides were coded without reference to mouse type or prior treatment status and examined systematically by a single pathologist (C.L.) using a semiquantitative scoring system.[23,24]

Mixed leukocyte reactions

Splenic $CD8^+$ T cells and dendritic cells (DCs) were isolated using CD8 and CD11c Micro Beads, respectively. The purity of the CD8 T cell and DC suspension was more than 90%. $CD8^+$ T cells were used as responders at 2×10^5/well against irradiated (20 Gy) DCs (1×10^4/well) for 2 to 4 days. During the final 12 hours of culture, cells were pulsed with 1 μCi (0.037 MBq) [^{3}H] thymidine (2 Ci/mmol [74.0 GBq]; Perkin Elmer, Billerica, MA) and proliferation was determined on a Top Count NTX (Packard Instrument, Meriden, CT).

Flow cytometric analysis

A flow cytometric analysis was performed using fluorescein isothiocyanate (FITC)–, phycoerythrin (PE)–, or allophycocyanin-conjugated monoclonal antibodies (mAbs) to mouse CD45.1, CD3, CD4, CD8, B220, and CD11c (BD Pharmingen, San Diego, CA). Cells were preincubated with 2.4G2 mAbs to block Fcγ receptor, and were then incubated with the relevant mAbs for 30 minutes at 4°C. Finally, cells were washed twice with 0.2% bovine serum albumin in phosphate-buffered saline (PBS), fixed with 1% paraformaldehyde in PBS and analyzed by EPICS Elite ESP cell sorter (Beckman-Coulter, Miami, FL). Irrelevant immunoglobulin $G_{2a/b}$ mAbs were used as a negative control. For analysis of donor cell apoptosis, spleens from recipient mice were harvested 10 days after transplantation and stained with PE-CD8, then washed with PBS, and then stained with FITC-conjugated annexin (R&D Systems, Minneapolis, MN) in the dark for 15 minutes at room temperature. Donor cell apoptosis was identified based on double staining for CD8 and annexin.

Enzyme-linked immunosorbent assay (ELISA)

ELISA for interferon-gamma (IFN γ) and tumor necrosis factor-alpha (TNF α) (BD Pharmingen) was performed as described.[25] Samples and standards were run in duplicate.

Statistical analysis

The Mann-Whitney *U* test was used for the statistical analysis of in vitro data, while the Wilcoxon rank test was used to analyze survival data. Linear regression and analysis of covariance were used to quantify the relationship between 2 variables. *P* less than .05 was considered statistically significant.

Results

Cytotoxic double-deficient $CD8^+$ T cells cause acute GVHD

Previous studies have shown that lack of either the perforin ($pfp^{-/-}$) or FasL (*gld*) in donor T cells significantly reduced GVHD following nonmyeloablative conditioning in a murine model.[26] We evaluated the role of each pathway in donor $CD8^+$ T cells during GVHD following myeloablative conditioning in a donor-recipient strain combination that differs at a single MHC class I antigen.[27] bm1 mice received 13 Gy total body irradiation (TBI) on day 0 and then received transplants of 5×10^6 TCD BM from wt B6 and 2×10^6 $CD8^+$ T cells from either wt, *gld*, or $pfp^{-/-}$ B6 donors; survival at day 30 after BMT was similar in all donor groups (8/10, 8/10, and 9/10, respectively). We analyzed histologic changes of GVHD at day 30 in the liver and intestine using a semiquantitative pathology index ("Materials and methods"). As shown in Figure 1, GVHD damage in target organs did not differ among donor groups, although histologic damage in all groups was relatively high due to the young age of recipients. Therefore, the deficiency of a single cytotoxic effector molecule in $CD8^+$ donor T cells did not reduce GVHD in this BMT model following myeloablative conditioning.

We next evaluated cytotoxic double-deficient (cdd) $CD8^+$ T cells in this GVHD model. The bm1 mice received transplants as before from wt or cdd B6 donors. Surprisingly, cdd $CD8^+$ T cells caused more rapid mortality than wt donor cells (Figure 2A). Histologic GVHD damage in the liver and intestine was also significantly greater on day 30 after BMT in recipients of cdd T cells (Figure 2B). We next evaluated inflammatory cytokines associated with acute GVHD. Compared with wt cells, cdd donor T cells caused dramatic increases in serum levels of IFN γ and TNF α on day 30 even though single cytotoxic deficient donor cells did not (Figure 2C).

Cdd T cells exhibit prolonged expansion to alloantigen stimulation in vivo and in vitro

Because recipients of cdd cells showed dramatic increase in serum levels of inflammatory cytokines and recipients of single deficient cells did not, we hypothesized that a mechanism in addition to CTL toxicity might be operative in the induction of GVHD. FasL and perforin not only are important effector molecules of CTL, but they also are involved in homeostatic regulation of $CD8^+$ T cells.[17-19] In this light, we hypothesized that cdd T cells proliferated to a greater extent than wt cells, causing increased cytokine production and more severe GVHD. We thus compared in vivo expansion of donor T cells in recipients of allogeneic $CD8^+$ T cells from wt, *gld*,

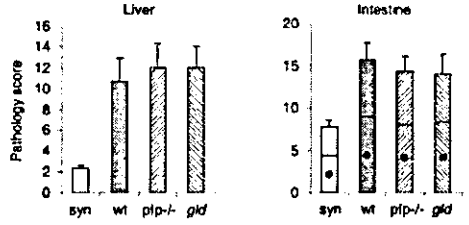

Figure 1. Effects of perforin- or FasL-deficient $CD8^+$ T cells on acute GVHD. Lethally irradiated bm1 mice received transplants as described in "Materials and methods" from wt B6 (wt), perforin-deficient B6 ($pfp^{-/-}$), or Fas ligand–deficient B6 (*gld*) donors. Syngeneic B6 BM transplant recipients (syn) served as no-GVHD controls. At 30 days after BMT, liver and intestine (bottom column [●], small intestine; upper column, large intestine) were harvested and scored semiquantitatively (n = 4 mice/group). Data represent mean ± SD.

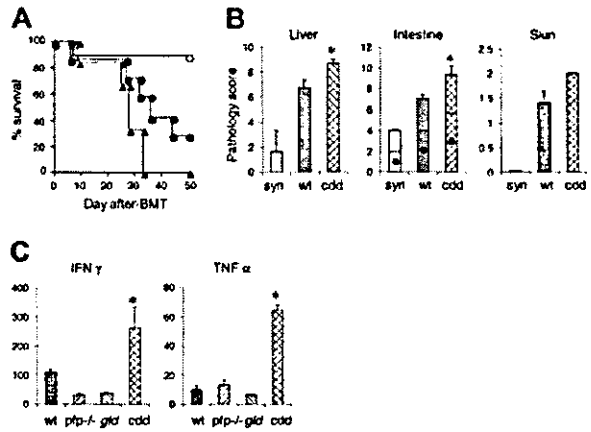

Figure 2. Cdd CD8⁺ T cells cause acute GVHD. The bm1 cells were transplanted from wt B6 (●, n = 11) or cdd B6 (▲, n = 6) donors as in Figure 1. B6 syngeneic BM transplant recipients (○, n = 9) served as no-GVHD controls. (A) Survival. (B) Histology. At 30 days after BMT, liver, intestine (bottom column [●], small intestine; upper column, large intestine), and skin were harvested and scored semiquantitatively (n = 4/group). B6 → B6 (syn; □), wt B6 → bm1 (wt; ▨), and cdd B6 → bm1 (cdd; ■). (C) Serum cytokines. Mice received transplants of wt, pfp$^{-/-}$, *gld*, or cdd as in Figure 1 and were killed on day 30 (n = 3 mice/group). Serum was harvested and analyzed for TNF α and IFN γ by ELISA (pg/mL). Each graph represents 1 of 3 similar experiments. Data represent mean ± SD. wt versus cdd, $^*P < .05$ by Mann-Whitney *U* test.

pfp$^{-/-}$, and cdd donors. T cells from wt donors expanded in the spleen until day 10 after BMT, followed by a rapid decline (contraction phase) (Figure 3A). *gld* T cells showed similar kinetics as wt T cells, whereas the pfp$^{-/-}$ T cells showed a significantly greater peak on day 10 but also contracted by day 30. By contrast, cdd CD8⁺ T cells expanded continuously up to day 30 after BMT, peaking at 100 times the number of wt T cells. Flow cytometric analysis of intracytoplasmic cytokines revealed that a greater proportion of cdd CD8⁺ T cells produced IFN γ and TNF α compared with wt T cells ($P < .05$, Figure 3B), consistent with the increased serum levels of these cytokines. We then analyzed whether GVHD damage correlated with the number of infiltrating mononuclear cells. We chose the liver for this evaluation because of the large dynamic range of cellular infiltration in this target organ, incorporating portal triads and bile ducts/ductules as well as vascular and hepatocellular areas. As shown in Figure 3C, the amount of hepatic tissue damage correlated closely with the degree of cellular infiltration ($P < .01$). Taken together, these data demonstrate that the absence of both cytotoxic pathways led to increased donor CD8⁺ T-cell expansion and greater cytokine production, causing greater GVHD target organ damage.

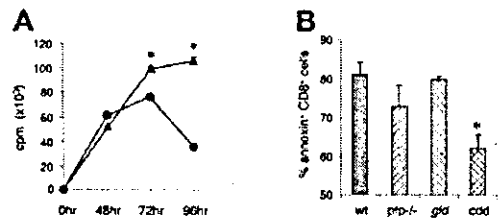

Figure 4. Deficiency of both perforin and FasL causes decreased apoptosis of donor CD8⁺ T cells after BMT. (A) Wild-type (●) and cdd (▲) CD8⁺ T cells at 2 × 10⁵/well were cultured with irradiated bm1 DCs (1 × 10⁴/well) for 2 to 4 days. During the final 12 hours of culture, cells were pulsed with [³H] thymidine and assayed for proliferation. $^*P < .05$ by Mann-Whitney *U* test. (B) Mice underwent transplantation as in Figure 1 and splenocytes were analyzed on day 10 after BMT by 2-color flow cytometry for expression of annexin and CD8⁺ cells. Gates were set for CD8⁺ cells, and the percentage of cells expressing annexin was determined. Data represent mean ± SD. wt versus cdd, $^*P < .05$ by Mann-Whitney *U* test.

Deficiency of both perforin and FasL causes decreased apoptosis of donor CD8⁺ T cells after BMT

We next evaluated whether the increased expansion of cdd T cells was due to more rapid proliferation or to decreased activation-induced cell death (AICD). CD8⁺ T cells were isolated from wt and cdd mice and were cultured with DCs isolated from bm1 mice. Proliferation of both cell types was equivalent for the first 48 hours of culture. Proliferation of wt cells peaked at 72 hours after the initiation of culture and then declined; in contrast, proliferation of cdd CD8⁺ T cells continued to expand at least up to 96 hours (Figure 4A).

We next harvested spleens on day 10 after BMT and analyzed donor T cells by 2-color flow cytometry for annexin as a measure of apoptosis. As shown in Figure 4B, percentages of apoptosis of pfp$^{-/-}$ and *gld* CD8⁺ T cells were comparable with wt cells ($P = .27$ and .51, respectively). In contrast, cdd donor CD8⁺ T cells showed significantly less apoptosis ($P < .05$), demonstrating that impaired AICD in cdd T cells results in their greater expansion.

Discussion

Little is known regarding the regulation of the contraction phase of CD8⁺ T cells after alloantigen stimulation. The present study demonstrates that both perforin and FasL are required for the normal AICD-mediated contraction of activated CD8⁺ T cells during GVHD following myeloablative conditioning. Absence of either of these pathways alone did not affect AICD and did not reduce GVHD. Our findings are consistent with those of Spaner et al[12] and others who reported that pfp$^{-/-}$ CD8⁺ T cells proliferate

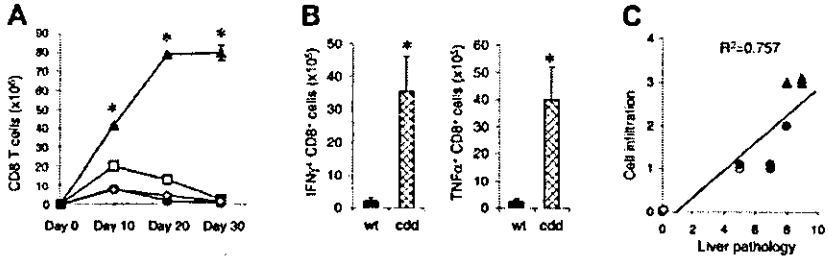

Figure 3. Deficiency of both perforin and FasL in donor CD8⁺ T cells cause prolonged expansion after BMT. (A) Mice underwent transplantation as in Figure 1. Splenocytes were harvested 10, 20, and 30 days (n = 3 mice/group) after BMT and analyzed by fluorescence-activated cell sorter (FACS). wt B6 → bm1 (●), pfp$^{-/-}$ → bm1 (□), *gld* → bm1 (◇), and cdd → bm1 (▲). wt B6 → bm1 versus cdd → bm1, $^*P < .05$ by Mann-Whitney *U* test. (B) TNF α and IFN γ production by donor CD8⁺ T cells was determined by intracytoplasmic staining. $^*P < .05$ by Mann-Whitney *U* test. (C) Overall pathologic damage of liver specimens correlated to the intensity of cell infiltration in portal triads, bile ducts/ductules, vascular, and hepatocellular areas. $P < .01$ by linear regression analysis. Symbols are same as in 3A. Error bars indicate mean ± SD.

more than wt $CD8^+$ T cells after transfer to allogeneic mice even though most $pfp^{-/-}$ $CD8^+$ T cells are eliminated in the contraction phase.[28] Unlike Fas-mediated AICD in $CD4^+$ T cells, the role of Fas/FasL on the homeostasis of $CD8^+$ T cells appears to be minimal.[29-31] The absence of both pathways leads to the unrestrained expansion of $CD8^+$ T cells, causing more severe GVHD. Although the involvement of IFN-γ and/or TNF-α in homeostasis in $CD8^+$ T cells after BMT remains to be determined,[32] these effects appear to be overshadowed by the 2 cytotoxic pathways. These findings support the findings of Marks et al, demonstrating that these cytolytic molecules must be present in donor $CD8^+$ T cells to regulate the contraction of alloreactive $CD8^+$ T cells after transplantation (L. Marks, E. R. Podack, R.B.L., Donor T cell contraction following MHC-mismatched allogeneic bone marrow transplantation requires CD8 mediated perforin and FasL dependent regulation, manuscript submitted).

The current results sharply contrast with the study by Braun et al[26] demonstrating that the absence of either pathway in donor T cells reduced GVHD and that the absence of both pathways completely abrogated GVHD lethality. That study used a nonmyeloablative conditioning regimen and demonstrated the important role of host immune cells containing perforin to prevent donor T cell expansion critical to the induction of GVHD.[6,26] The use of a myeloablative regimen in the current study probably reduced the host-versus-graft response to an insignificant level, magnifying the role of the cytotoxic pathways in AICD.

Perforin deficiency could enhance $CD8^+$ T cell expansion through decreased killing of APCs, resulting in prolonged stimulation of additional naive T cells.[33,34] Therefore, we determined whether the absence of cytolytic effector function permitted longer survival of host DCs, resulting in greater stimulation of donor T cells. Host DCs were not detected, however, in either the spleen, BM, or gut of any recipients of $CD8^+$ T cells on day 6 after BMT (data not shown). Recently, Merad et al[35] demonstrated that donor T cells eliminated host Langerhans cells (LCs) through a FasL-dependent cytolytic mechanism and that persistent host LCs could stimulate skin GVHD. Although host LCs might persist and play a role in skin GVHD in cdd recipients, our data suggest that the residual skin LCs are not responsible for the unrestrained expansion of only cdd cells because no such expansion was observed in recipients of gld cells (Figure 3A). In addition, we created BM chimeras (bm1 into B6) that would express allogeneic MHC class I only on BM-derived APCs and not on target epithelial cells, as described in a previous study.[4] B6 cdd T cells induced lethal GVHD in these chimeras (mean survival time [MST]: day 30) similar to wt bm1 recipients, confirming that persistent alloantigen expression on host cells did not account for unrestrained expansion of cdd cells.

In summary, our data suggest that in the $CD8^+$-mediated GVHD, the lack of both perforin and FasL impaired AICD, causing unrestrained expansion of $CD8^+$ T cells and lethal immunopathology of GVHD.

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Reduced-intensity non-T-cell depleted HLA-haploidentical stem cell transplantation for older patients based on the concept of feto-maternal tolerance

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Summary:

With the recent progress in reduced-intensity conditioning stem cell transplantation (RIST) and taking into consideration the concept of feto-maternal immunological tolerance, we carried out non-T-cell depleted HLA haploidentical RIST from noninherited maternal antigen (NIMA) complementary siblings or offspring donors for four older patients: a patient with myeloplastic syndrome (MDS) and three patients with adult T-cell leukemia (ATL) in partial remission or with progressive disease. All patients showed early, durable engraftment, and no serious toxicities were observed apart from grade III mucositis in one case. Grade II acute GVHD occurred in two cases, which was well-controlled. In one ATL patient whose donor did not have NIMA microchimerism, tacrolimus could not be continued after engraftment due to renal dysfunction, and grade III acute GVHD (gut: stage 4) occurred on day 35. A patient with MDS was free from disease (requiring no transfusions and with a normal bone marrow) for 15 months. Two cases of ATL relapsed. Feto-maternal tolerance may lead to new RIST strategies in the haploidentical reduced-intensity situation, but further evaluation is required.

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Recent progress in reduced-intensity stem cell transplantation (RIST) has expanded the use of stem cell transplantation (SCT) to older patients and patients with various underlying diseases,[1] although there are still many patients without matched donors. Haploidentical donors are available for the majority of patients, but the previously reported results of haploidentical SCT are not acceptable due to severe graft versus host disease (GVHD)[2] and an increase in graft failure, especially in T-cell depleted SCT.[3] The recently reported favorable results of haploidentical SCT based on feto-maternal microchimerism[4] suggest an attractive strategy to bypass the lack of suitable donors. Based on the hypothesis of feto-maternal immunological tolerance, favorable results of non-T-cell depleted haploidentical SCT from a noninherited maternal antigen (NIMA) complementary sibling have been reported. With the progress in RIST and taking into consideration the concept of feto-maternal tolerance, we describe the results of reduced-intensity non-T-cell depleted HLA haploidentical SCT for older patients.

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Patients and methods

Patients and donors

From January 2003 to January 2004, four older patients underwent HLA haploidentical SCT after agreement from the hospital ethics committee, and written informed consent had been given by the patients. None of the patients had suitable related or unrelated donors. Therefore, based on the hypothesis of feto-maternal tolerance, NIMA complementary siblings or offspring were selected as haploidentical donors. Feto-maternal microchimerism was examined by nested polymerase chain reaction with sequence-specific primer typing according to a previous report.[4] However, patient eligibility did not depend on the results of microchimerism.

Preparative regimen, graft-versus-host disease (GVHD) prophylaxis, and supportive care

The conditioning regimen included fludarabine at a daily dose of 30 mg/m^2 on days −8 to −3 (total dose 180 mg/m^2) and busulfan at a daily dose of 4 mg/kg on days −6 and −5 (total dose 8 mg/kg). Because of the limited evidence for maternal tolerance to an offspring and the aggressive nature of the disease, the patients with ATL with offspring donors received additional total-body irradiation (4 Gy) on day −7. Thereafter, patients received unmanipulated G-CSF-mobilized peripheral blood stem cells. As GVHD prophylaxis tacrolimus was initially administered from day −1 at a dose of 0.02 mg/kg/day as a continuous

infusion, and short-course methotrexate was administered on days 1, 3, and 5 at doses of 10, 7, and 7 mg/m^2. All patients were screened weekly for cytomegalovirus (CMV) viremia by a direct immunoperoxidase technique using a peroxidase-labeled monoclonal antibody (HRP-C7) against CMV immediate early antigen;[5] they were treated with ganciclovir if CMV viremia was detected.

Results

Patients and disease status at the time of transplantation

Four older patients ranging from 51 to 62 years of age underwent SCT (Table 1). The first patient was a 61-year-old woman with myelodysplastic syndrome (MDS), refractory anemia with ringed sideroblasts (RARS) which had been diagnosed in 1997. She had received a total of 42 red cell transfusions before the SCT, and her transfusion requirements were increasing. The second patient was a 62-year-old woman with acute type refractory adult T-cell leukemia (ATL) diagnosed in August 2002. She had undergone reduced-intensity cord blood SCT in May 2003, but the graft had been rejected. The third patient was a 59-year-old woman with acute type refractory ATL diagnosed in January 2003 which prior intensive treatment had failed to control. Because of poor renal function due to previous chemotherapy and treatment of CMV infection, the fludarabine dose was modified. The fourth patient was a 51-year-old woman with acute ATL diagnosed in September 2003. Relapse was observed during CHOP treatment. Thus, three high risk patients with ATL were included in this study. Disease status at the time of transplantation for ATL was partial remission or progressive disease with hypercalcemia (Table 1). All patients and donors were seropositive for CMV.

Table 1 Patient and donor characteristics

Case	*Age*	*Sex*	*Diagnosis*	*Donor (age)*	*Disease status at SCT*
1	61	F	MDS/RARS	Sister (59)	Transfusion dependent
2	62	F	ATL	Son (38)	Partial remission
3	59	F	ATL	Son (28)	PD with hypercalcemia
4	51	F	ATL	Son (28)	Partial remission

SCT = stem cell transplantation; F = female; M = male; MDS = myelodysplastic syndrome; RARS = refractory anemia with ringed sideroblasts; ATL = adult T-cell leukemia; PD = progressive disease.

HLA compatibilities and microchimerism

Various patterns of HLA diversity were observed between patients and donors (Table 2). Microchimerism of recipient HLA was demonstrated in the peripheral blood of three of four donors.

Engraftment and early toxicities

Total numbers of CD34+ cells infused are shown in Table 3. Although patients had two or three mismatched HLA loci in the host versus graft direction, all showed early engraftment. Severe early toxicities were not observed with the exception of grade III mucositis[6] in case 3. Complete donor T-cell chimerism was confirmed by day 30 in cases 1–3. In case 4, mixed chimerism remained, due to residual ATL.

GVHD, late complications and survival

Acute GVHD occurred in three of the four cases as shown in Table 3, and was well-controlled in cases 1 and 2. In case 3, whose donor did not have NIMA microchimerism in his peripheral blood, tacrolimus could not be continued after engraftment due to renal dysfunction, and stage 4 gut GVHD occurred on day 35. Methylprednisolone at a dose of 10 mg/kg/day was initiated, but did not control the problem. CMV-viremia was detected in all cases and CMV esophagitis occurred in case 2 on day 64.

Case 1 (MDS/RARS) was disease-free (no transfusions and normal bone marrow findings) for 437 days of follow-up with extensive chronic GVHD. The CD4+ lymphocyte count and serum IgG level recovered to normal 7 months after transplantation. Relapse of ATL was observed in case 2 on day 74 but spontaneously resolved following withdrawal of tacrolimus and the re-emergence of skin GVHD. However, relapse occurred in the central nervous system on day 120, and she died of disease on day 134. Case 3 died of gut GVHD on day 58. In case 4, tacrolimus was stopped on day 22 because residual leukemia cells were detected in the peripheral blood. A donor leukocyte infusion was given on

Table 2 HLA diversities and microchimerism

Case		*HLA type*	*No. of MM*		*MC*	*(HLA)*
			GvH	*HvG*		
1	Patient	A24, B7, DR1/A26, B7, DR1	1	3	—	(B-60)
	Donor	A24, B7, DR1/A2, B60, DR12			1/1000	(A-26)
2	Patient	A24, B7, DR1/A31, B70, DR4	3	2	1/10 000	(B-62)
	Donor	A24, B7, DR1/A24, B62, DR6			1/10 000	(A-31)
3	Patient	A24, B15, DR4/A33, B44, DR13	3	3	—	(B-51)
	Donor	A24, B15, DR4/A2, B51, DR15			—	(A-33)
4	Patient	A11, B61, DR14/A24, B59, DR4	2	1	1/10 000	(B-35)
	Donor	A11, B61, DR14/A24, B35, DR14			1/1000	(B-59)

MC = frequency of microchimerism; (HLA) = target antigen for detection of microchimerism; MM = mismatch; GvH = graft versus host direction; HvG = host versus graft direction.

Table 3 Results of transplantation

Case	*Infused CD34+cells (× 10^6/kg)*	*Engraftment (days post-SCT)*		*aGVHD (skin, liver, gut)*	*Result, day*
		N	*P*		
1	2.38	*	17	II (1;0;1)	Alive in CR, day 440+
2	5.41	11	15	II (1;0;1)	Died of relapse, day 143
3	3.97	12	17	III (1;0;4)	Died of GVHD, day 57
4	5.14	13	13	0	Alive in relapse, day 60+

aGVHD = acute graft versus host disease; N = day of neutrophil engraftment ($>0.5 \times 10^9$/l); P = day of platelet engraftment ($>50 \times 10^9$/l); * = never fell below 0.5×10^9/l; CR = complete remission (free from transfusion with normal findings of bone marrow).

day 38; however, further chemotherapy was needed to control the ATL.

Discussion

The effect of tolerance to NIMA on transplantation was first demonstrated in the field of renal transplantation.[7] With this in mind, retrospective analysis of Japanese nationwide surveys for adult conventional SCT demonstrated a higher overall survival in patients receiving maternal hematopoietic stem cell (HSC) compared with those receiving paternal HSC.[8] A retrospective analysis by the International Bone Marrow Transplantation Registry (IBMTR) revealed the effect of tolerance to NIMA on occurrence of GVHD in the setting of conventional transplantation.[9]

Although the duration of follow-up is too short for reliable evaluation in the setting of an aggressive disease, favorable engraftment and control of GVHD were observed in our study. In RIST for older patients, their siblings are in a similar age group and often have health problems which prevent them from providing stem cells. Our results in haploidentical SCT from offspring to mother are encouraging, making RIST feasible for older patients without appropriate donors. Patient 3, whose donor did not have NIMA microchimerism in his peripheral blood, developed severe gut GVHD. However, this severe GVHD was most likely related to the cessation of tacrolimus because of renal dysfunction. The significance of feto-maternal microchimerism as an index for feto-maternal tolerance needs to be evaluated.

Despite the frequent detection of feto-maternal microchimerism,[10] the results in renal transplantation,[7] and the retrospective analysis of conventional SCT[8,9] all of which strongly support the concept of feto-maternal tolerance, immunological and clinical evidence has been limited. Furthermore, long-term problems, such as slow immune reconstitution in mismatched SCT[11] and late-onset GVHD in RIST[12] may influence outcome, especially in older patients. Further evaluation is needed to clarify this concept. However, feto-maternal immunological tolerance might lead to new SCT strategies with reduced-intensity conditioning for older patients without a matched donor.

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臨　床

両側肺浸潤影と末梢血好酸球増多を呈した原発性肺コクシジオイデス症の1例

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Key words：　coccidioidomycosis, imported mycosis, eosinophilia

序　文

コクシジオイデス症は，土壌中に棲息する真菌の *Coccidioides immitis* (*C. immitis*) の吸入によって発症する全身感染症である[1]．本菌はアメリカ大陸の乾燥地域にのみ棲息するため，コクシジオイデス症はわが国では稀な輸入感染症であるが，海外渡航者の増加と伴に報告例が最近増加の傾向にある[2]．

本稿では米国アリゾナ州への旅行歴と末梢血好酸球増多を伴う急性肺炎の臨床像を呈し，胸腔鏡下肺生検で確定診断を得た原発性肺コクシジオイデス症の1例を経験したので報告する．

症　例

症例：51歳，男性．

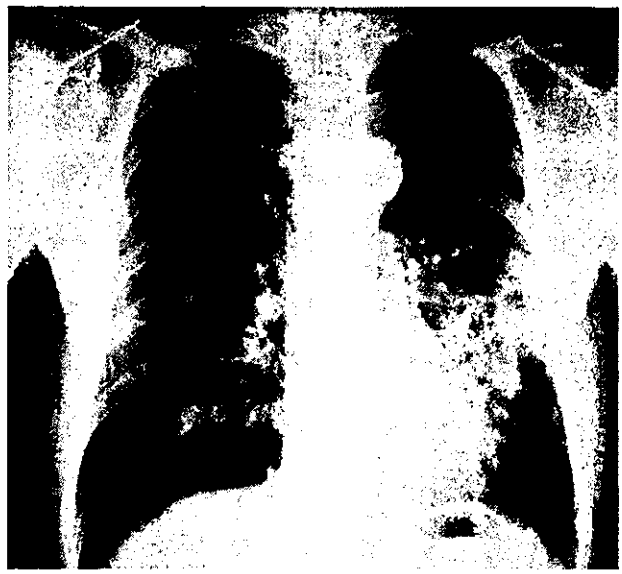
主訴：発熱，咳嗽，労作時呼吸困難．

既往歴・家族歴：特記事項なし．

生活歴：職業は建築デザイナー．20歳より30歳までの10年間，1日約20本の喫煙歴あり．粉塵吸入歴およびペット飼育歴はない．

現病歴：1998年9月10日よりアメリカ合衆国カリフォルニア州およびアリゾナ州へ約2週間旅行した．この間アリゾナ砂漠に数日滞在し，渓谷散策や乗馬などの機会があった．9月23日帰国後，同月30日より悪寒を伴う38℃台の発熱が出現し，その後咳嗽および労作時呼吸困難を自覚するようになった．10月4日近医を受診し感冒と診断され，総合感冒薬，鎮咳薬，抗菌薬 cefpodoxime proxetil を処方された．しかしながら，発熱，咳嗽が持続するため，10月12日に当院を受診し，肺炎

Fig. 1　Posteroanterior chest radiograph taken on admission reveals patchy infiltrations in the left middle lung field and multiple small consolidations in both lungs.



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虎の門病院呼吸器センター内科
川畑　雅照

Fig. 2　Chest CT scans showing dense air space consolidation in the left lingular segment, and multiple nodular infiltrative opacities with ill-defined margins spreaded in the segmental distribution in both lungs. A. Mid portion at the bifurcation level. B. Lower portion.

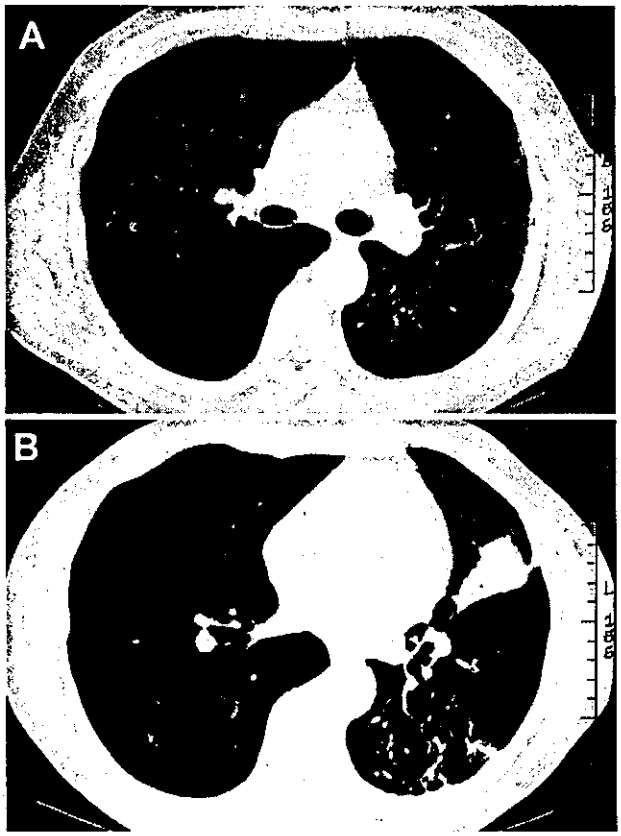
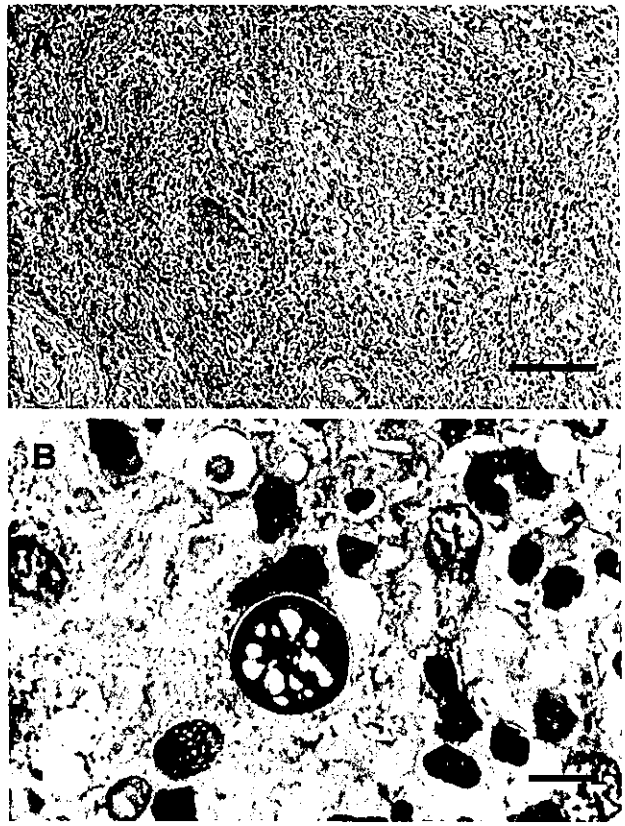


Fig. 3　Histology of the VATS-biopsied lung specimen. A. Granulomatous inflammation consisted of multinuclear giant cells, and infiltrated small mononuclear cells and eosinophils. Stained with hematoxylin and eosin. The bar indicates 100μm. B. A coccidioidal spherule filled with endopores observed within the granuloma. PAS staining. The bar indicates 10μm.



を疑われ精査加療目的で入院となった.

身体所見：身長 176cm, 体重 74.8kg, 血圧 140/70mmHg, 脈拍 72/分, 整, 体温 36.9℃, 意識清明. 結膜に異常はなく, 瞳孔は両側同大で眼球運動は正常, 咽頭に軽度の発赤を認めた. 甲状腺腫あるいは表在リンパ節腫脹は認めなかった. 胸部では, 心音は正常であったが, 左上背部に吸気時の coarse crackle を聴取した. 腹部は触診, 聴診ともに異常を認めなかった. 皮膚にはチアノーゼを含め異常所見はなく, 四肢にもバチ指および浮腫はなかった. また, 神経学的所見は正常であった.

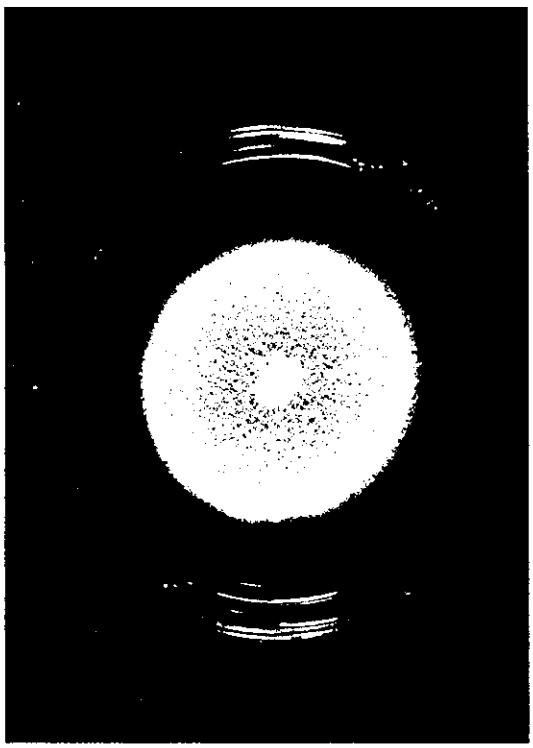
入院時検査所見：白血球数 12,300/μl と上昇しており, うち好酸球が 19.5% を占め末梢血好酸球増多を認めた. 赤沈 1 時間値 40mm, CRP 1.0mg/dl であった. 胃液の結核菌の塗抹・培養および PCR は陰性で, ツベルクリン反応は陽性(発赤 10mm×10mm) であった.

胸部単純 X 線：左中肺野に斑状の浸潤影を認め, 両側中下肺野に一部結節様にも見える小浸潤影が多発していた (Fig. 1).

胸部 CT：左肺舌区に濃度が高く辺縁が比較的明瞭な air space consolidation があり, 両側中下肺野に大小不同で辺縁の比較的不明瞭な結節性病変が散在していた (Fig. 2).

臨床経過：アリゾナ州への旅行歴, 肺炎様の急性の臨床経過および末梢血好酸球増多などより原発性肺コクシジオイデス症を疑い, 入院第 9 病日に TBLB を施行した. しかし, 確定診断には至ら

Fig. 4 A white giant colony which appearance was compatible with that of *C. immitis* was observed after the biopsied lung tissue of the patient had been inoculated onto a potato dextrose agar plate.



なかったため，第13病日に胸腔鏡下肺生検を施行した．

病理組織学的所見：著明な好酸球浸潤と多核巨細胞が混在した肉芽腫形成（Fig. 3A）を認め，多核巨細胞内にPAS染色およびGrocott染色に陽性の球状体（Fig. 3B）が確認された．また，生検組織の一部をポテトデキストロース寒天（PDA）培地に接種したところ，*C. immitis* として矛盾しない白色の巨大集落が発育した（Fig. 4）．以上より，肺コクシジオイデス症と診断した．

第14病日よりitraconazole 200mg/日を開始したところ，投与2週目より自覚症状，末梢血好酸球数および胸部X線所見の改善を認めた．8週間治療を継続し，胸部X線上では陰影はほぼ消失した．その後，3年間にわたり経過観察したが，再発はみられなかった．

考　案

コクシジオイデス症は，アメリカ大陸の乾燥地域の土壌中に存在する *C. immitis* の吸入によって発症する全身感染症である．本邦では稀な輸入感染症ではあるが，感染症新法では届け出を義務づけられた第4感染症に指定されている[3]．

本症の流行地は，アメリカ大陸のなかの高温，半乾燥地帯である．アメリカ合衆国では，カリフォルニア州，アリゾナ州，ネバダ州，ニューメキシコ州，ユタ州，テキサス州などで頻度が高く，とくにカリフォルニア州のサンホアキン渓谷（San Joaquin Valley）で患者が多発しており，別名，Valley Feverと呼ばれている[4]．また，メキシコの太平洋側半乾燥地帯の他に，南アメリカのベネズエラ，ペルー，ボリビア，アルゼンチンなどにおける流行が知られている[5]．流行地は年間降雨量が125～500ミリの乾燥地帯で，年間平均気温が26～32℃の熱帯～亜熱帯に多く分布している[5]．

本邦では，1937年榊原と水野[6]により第1例が報告されて以来，輸入感染症として約30例あまりが報告されている[2]．約85%がアメリカ合衆国での感染例で，近年増加が目立っており，毎年3～4名の発病が確認されている[2]．

C. immitis は，自然界ないし培地では菌糸の形態をとり糸状菌集落を形成する[1]．その後，菌糸の先端に近い部分の太さが増し，間欠的に分節型分生子（arthroconidia）が形成され，これが空中へ飛散し，再び土壌で菌糸を形成するという生活環を示す．この飛沫した分節型分生子は感染力が強く，これを吸入して経気道的に感染が成立する．しかし，生体内・組織内に吸入された *C. immitis* の分節型分生子は，菌糸を形成することなく，腫大しながらその細胞質は分割されていき，内部が直径2～5μmの内生胞子（endospore）で充満された直径40～200μmの球状体（spherule）が形成される．その後，球状体の壁の一部が破れ，内生胞子は組織内に放出され，この内生胞子が再び球状体を形成するというサイクルを繰り返す．この内生胞子は感染力が弱いため，人から人への直接的感染はないと考えられている．限局性の激しい化膿性炎症は，球状体の壁が破れて内生胞子が放出されるときに起こり，内生胞子が球状体へと成長するに従って肉芽腫形成を来たす．以上のように *C. im-*

mitis は，自然界では菌糸形，生体内では球状体という二つの発育形態をもつため，二形性真菌といわれる[7]．

菌糸より発生した分節型分生子の感染力は極めて高く，流行地域内の通過のみで発症した症例や，流行地から輸入された綿花からの感染の報告もみられる[8]．国立予防衛生研究所などの真菌の危険度分類ではclass 3にランクされている[9]．通常の細菌検査室では検査室内感染を来す恐れがあり，P3レベルの研究室でなければ培養できない．安全キャビネットの普及する以前は，アメリカでは過去に約200例の実験室感染があり死亡例も報告されている[10]．

C. immitis に感染した場合，その約60%は全く無症状であるが，皮内テストが陽性となることで検出が可能である[11]．約40%が有症状であり，7～28日の潜伏期を経て，発熱，胸痛，咳嗽，全身倦怠感，咽頭痛，頭痛，関節痛などのインフルエンザ様症状を来す[7]．皮膚病変として結節性紅斑，多形性滲出性紅斑を伴うことが多い[12]．有症状の例でも適切な治療により治癒することが多いが，約0.5%の症例では髄膜，骨関節，皮膚，軟部組織など肺外への播種を来たし，致死率が高い．HIV感染や臓器移植後などの免疫不全患者，有色人種，妊娠後期では重症化しやすいことが知られている[1]．

胸部単純X線所見に特徴的なものはなく，様々な程度の浸潤影，粒状影，網状影の他，肺門・縦隔リンパ節腫脹，結節影，空洞を認める[13]．わが国における報告例は，比較的時間が経過した後に発見された症例が多いためか，胸部X線では結節影や空洞を認めるものが多く，本症例のような肺炎像を呈する早期の症例は比較的稀である[14]．

一般的な血液検査では，白血球増多，赤沈の亢進などの炎症所見に加え，好酸球増多が特徴的である．急性肺炎の臨床像を呈する他の疾患で好酸球増多は稀であるため，診断的価値が高い[15]．本症例においても，末梢血好酸球増多の所見が本症を疑う所見の一つで診断の糸口となった．

肺コクシジオイデス症の確定診断には，*C. immitis* が培養で証明できれば確実であるが，バイオハザードの問題から一般的には困難である[9]．このため，生検組織，喀痰，気管支肺胞洗浄，肺穿刺材料などを用いて，病巣から球状体の証明することで確定診断が得られる．また，補助的診断として，血清学的検査や皮内テスト[16]が挙げられる．現在，皮内テストの試薬は製造中止となったため施行できないが，抗体測定は商業ベースになっていないものの千葉大学真菌医学研究センターに依頼すれば施行可能である[2]．

呼吸器系臓器のみに限局した急性感染症では，合併症や重症化しやすいリスクファクターがなければ，治療せずに自然寛解まで経過観察してもよいという意見もある[17]．しかしながら，全身播種型の病像を呈するものや，本症例のような両側性陰影を呈する重症肺病変の場合は，抗真菌薬の投与が必要である[11]．治療にはamphotericin Bが用られるが副作用が問題となるため，最近は毒性が少なく経口投与も可能なアゾール系抗真菌薬も第一選択とされることも多く，その効果はほぼ同等といわれる[17]．本症例でも，itraconazoleの投与で速やかな臨床所見の改善が得られた．

航空機旅行の一般化に伴う渡航者の増加や物資の国際的な流通化とともに，本症のような本来日本には存在しない輸入真菌感染症に遭遇する機会も今後増加することが予想される．このため，輸入感染症に対しても認識をさらに深め，同感染症患者に対して速やかに適切な診療を行いうる体制を整える必要があるものと考えられる．

謝辞：*C. immitis* の培養およびコクシジオイデス症の診断につき御教示戴いた西村和子教授（千葉大学真菌医学研究センター），胸腔鏡下肺生検を施行して戴いた谷村繁雄先生（虎の門病院呼吸器外科）に深謝する．

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A Case of Pulmonary Coccidioidomycosis Presented with Bilateral Infiltrative Opacities and Eosinophilia

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A 53-year-old male was admitted to our hospital complaining of high fever with chillness, cough and dyspnea after traveling to Arizona in the United States. The chest X-ray films taken on admission showed consolidation in the right middle lung field and bilateral nodular shadows. The laboratory data revealed an increase in white blood cell counts with eosinophilia, and a rise in erythrocyte sediment rate and serum C-reactive protein. The biopsied lung specimen by video-assisted thoracoscopic surgery showed granulomatous inflammation consisting of eosinophils and giant cells. In addition, typical spherules filled with endopores were detected in the specimen. The diagnosis of primary pulmonary coccidioidomycosis was made. After the treatment of a three months' regimen with itraconazole at the daily dosage of 200mg, the patient's symptoms, laboratory data and radiological findings markedly improved.

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