Clinical Importance of CD7 Expression in Acute Myelocytic Leukemia

By Kenkichi Kita, Hiroshi Miwa, Kazunori Nakase, Keiki Kawakami, Tohru Kobayashi, Shigeru Shirakawa, Isao Tanaka, Chizuko Ohta, Hiroshi Tsutani, Shigeru Oguma, Taiichi Kyo, Hiroo Dohy, Nanao Kamada, Kaori Nasu, and Haruto Uchino
(The Japan Cooperative Group of Leukemia/Lymphoma)

Forty patients (9 females and 31 males; mean age 41.9 years) with CD7$^+$ acute myelocytic leukemia (AML) were investigated; they were classified into the following subgroups according to French-American-British classification: 15 M1, 18 M2, 3 M4, and 4 M5. Leukemic cells from all the patients were negative for T-cell–specific antigens, surface CD3, and T-cell–receptor molecules. The sex and age distributions were different from those of CD7$^-$ AML patients ($P < .01$). Hepatomegaly and central nervous system involvement were also frequent in the CD7$^+$ AML patients. The phenotype of and responsiveness to hematopoietic growth factors by the leukemic cells showed their immaturity, as evidenced by frequent expression of CD34, HLA-DR, and TdT, and the greatest growth response to interleukin-3. No particular karyotypic abnormality was shown. One hundred eighty AML patients were treated with a therapeutic regimen routinely used for AML. The CD7$^+$ AML patients showed a significantly lower response than CD7$^-$ AML patients ($P < .01$), and had a poorer prognosis ($P < .01$). CD7$^+$ AML patients with M1 or M5b had unfavorable responses to the therapeutic regimen in comparison with patients with M2, M4, or M5a. In addition, 3 of 4 CD7$^+$ CD2$^+$ AML patients, who did not respond to the therapy, were induced into complete remission with an acute lymphoblastic leukemia therapy. The results presented here indicate the diagnostic importance of CD7 positivity in AML, suggesting that the cellular and clinical characteristics of CD7$^+$ AML are sufficient for it to be recognized as a distinct category of AML.

HEMATOLOGIC investigations in the past decade show that, to make precise diagnoses of acute myelocytic leukemia (AML), it is necessary to consider information from the various fields.[1-4] Patients with several kinds of karyotypic abnormalities, such as t(8;21) and t(15;17), are well known to manifest unique cytologic and clinical features.[3-6] Immunophenotyping has made it possible to recognize AML cells according to appropriate stages of myeloid differentiation,[1,2] and, further, has enabled the identification of peroxidase (POX)-negative AML.[7] However, approaches involving myeloid antigen expression, as compared with the cytogenetical approach, have not always succeeded in aiding in the diagnosis of AML, because no distinct myeloid phenotype related to the clinical features of this disease has yet been identified except that of acute promyelocytic leukemia.

It has been shown that considerable numbers of AML patients had both myeloid and lymphoid antigens.[5,6,8-13] A pan T-cell antigen, CD7, is one of the lymphoid antigens often expressed on AML cells.[8,12] Several investigators have drawn attention to the immaturity of CD7$^+$ AML cells manifested by, for instance, their frequent expression of terminal deoxynucleotidyl transferase (TdT).[12,14] With regard to acute lymphoblastic leukemia (ALL), Kurtzberg et al[15] proposed CD7$^+$CD4$^-$CD8$^-$ ALL as "lymphohematopoietic stem cell leukemia" because of its multipotentiality for myeloid and lymphoid differentiation. CD7 expression in AML leads us to the issue of whether or not these two types of acute leukemia can be recognized as part of a continuous disease spectrum, disregarding the division of AML and ALL. Clinically, the expression of myeloid antigen in ALL is recognized as one of the prognostic risk factors.[16] CD7 expression in AML is also speculated to have some clinical importance, but little is known about it.

In the present study, a large number of AML patients were investigated to clarify the relationship between the cellular characteristics and clinical features. Our results show that CD7 expression on AML cells is indicative not only of phenotypic, but also of functional immaturity and can be regarded as a prognostic risk factor in AML.

MATERIALS AND METHODS

Patients. Two hundred ten Japanese adult patients (older than 15 years) with AML were enrolled in this study between January 1987 and June 1991. All patients gave their informed consent for the procedures involved. Bone marrow (BM) and peripheral blood (PB) smears were prepared for May-Giemsa, POX, naphthol AS-D chloroacetate esterase, and α-naphthyl butylate esterase staining. Leukemic cells from all patients fulfilled French-American-British (FAB) criteria for AML,[17] and the patients were classified as follows: 40 patients as M1, 72 as M2, 40 as M3, 32 as M4, and 26 as M5. In this study, we excluded M6 and M7 patients because it is usually difficult to determine the phenotype of their leukemic cells. We also excluded acute leukemia after myelodysplastic syndrome.

Immunophenotyping. Mononuclear cells (MNC) were separated from heparinized PB and BM by Ficoll-Hypaque centrifugation (Pharmacia LKB Biotechnology AB, Uppsala, Sweden). Stocked samples, which had been isolated and frozen at −196°C in RPMI 1640 medium with 20% heat-inactivated fetal calf serum (FCS) and 10% dimethyl-sulfoxide, were also used as needed.

From the Second Department of Internal Medicine, Mie University School of Medicine, Tsu; the Department of Internal Medicine, Shuzuka Kaisei Hospital, Suzuka; the Department of Internal Medicine, Matsusaka Chuo Hospital, Matsusaka; the First Department of Internal Medicine, Fukui Medical School, Fukui; the First Division of Internal Medicine, Faculty of Medicine, Kyoto University, Kyoto; the Department of Internal Medicine, Hiroshima Red Cross Hospital, Hiroshima; the Department of Hematology, Research Institute for Nuclear Medicine and Biology, Hiroshima University, Hiroshima; and the Department of Internal Medicine, Osaka Red Cross Hospital, Osaka, Japan.

Submitted July 13, 1992; accepted December 14, 1992.

Supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, and Grants-in-Aid for Cancer Research from the Ministry of Health and Welfare of Japan.

Address reprint requests to The Second Department of Internal Medicine, Mie University School of Medicine, 2-174 Edobashi, Tsu 514, Japan.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" *in accordance with 18 U.S.C. section 1734 solely to indicate this fact.*

0006-4971/93/8109-0019$3.00/0

Before immunostaining, MNC were treated with 5% heat-aggregated human AB serum for blocking of binding due to receptors for IgG Fc portion (FcγR).[6] CD11b (recognized by OKM1), CD13 (by MCS2), CD15 (by LeuM1), CD33 (by My9), and CD34 (by My10) were tested as myeloid cell markers: CD2 (by T11), CD5 (by Leu1), CD7 (by TP40), CD3 (by Leu4), CD4 (by Leu3a), CD8 (by Leu2), CD1 (by OKT6), T-cell receptor (TCR) α and β chain complex (by WT31), and TCR δ chain (TCRδ: by TCRδ1) as T-cell markers; and CD19 (by Leu12 and/or B4), CD20 (by Leu16), and CD22 (by Leu14) as B-cell markers. CD10 and HLA-DR were examined with J5 and OKIa1, respectively. Goat antimouse Ig fluorescein-conjugates (GAM-FITC) was used as the second reagent.

Cytoplasmic CD3 (cCD3) was stained by labeled avidin-biotin-alkaline phosphatase technique on aceton-fixed cytospin smears.[6] TdT was tested by indirect immunofluorescence with rabbit anti-TdT antibody or anti-TdT monoclonal antibody (MoAb) on methanol-fixed cytospin smears.

Samples were evaluated by flow cytometry (Cytoron; Ortho Diagnostic Systems, Tokyo, Japan), light microscopy (Olympus, Tokyo, Japan), or immunofluorescence microscopy (Olympus). At least 5,000 cells were examined for surface marker study, and 200 cells were examined for the cCD3 and TdT studies. Samples were considered as positive for an MoAb if more than 15% of cells showed specific labeling above that of controls.

The MoAb TP40 and MCS2 were kindly provided by Drs R. Ueda (Aichi Cancer Center, Nagoya, Japan), and E. Tatsumi (Kobe University, Kobe, Japan), respectively. The Leu series, My10, and WT31 MoAb were purchased from Becton Dickinson (Mountain View, CA); My4, My9, B4, J5, anti-TdT MoAb, and GAM-FITC were from Coulter Inc (Hialeah, FL); the OK series were from Ortho; TCRγ1 MoAb was from T-Cell Science Inc (Cambridge, MA); and rabbit anti-TdT antibody was from Bethesda Research Labs. (Gaithersburg, MD). Whole mouse Igs, IgG1, and IgG2 used as the controls were purchased from Chemicon Int Inc (Temecula, CA).

Southern blot study and DNA probes. Extraction of high molecular weight DNA from MNC, and Southern blot analysis were performed as described previously.[18] DNA probes for determining the gene structures of Ig heavy chain (IgH), TCRβ, TCRγ, and TCRδ were as follows: JH for IgH joining (J) region genes, Cβ1 for TCRβ constant (C) region genes, Jγ1 for TCRγ Jγ1 region genes, Jδ1 for TCRδ Jδ1 region genes, and Cδ1 for TCRδ C region genes. DNA probes, except for TCRδ genes, were kindly provided by Dr T. Honjo (Kyoto University, Kyoto, Japan). Probes for TCRδ genes were kindly provided by Dr Y. Takihara (Osaka University, Osaka, Japan).

Cell cultures. The T-cell– and adherent cell–eliminated MNC fraction was used as the leukemic cell-enriched fraction. Response of leukemic cells to various growth factors was tested by [^{3}H]TdR incorporation method in short-term liquid culture as described previously.[19]

The stimulating factors used were recombinant human interleukin-3 (IL-3) (10 ng/mL), granulocyte-macrophage colony-stimulating factor (GM-CSF; 10 ng/mL), and G-CSF (10 ng/mL). G-CSF was kindly provided by Chugai Pharmaceutical Co (Tokyo, Japan). IL-3 and GM-CSF were purchased from Genzyme Co (Boston, MA).

Karyotype analysis. Karyotype analysis was performed on aspirated BM cells, as described previously.[20]

Statistical analysis. Differences in cellular and clinical characteristics between CD7$^+$ and CD7$^-$ patients were evaluated by chi-squared statistics or Student's *t*-test. The survival of CD7$^+$ and CD7$^-$ AML patients was calculated by the Kaplan-Meyer method, and the difference was evaluated by generalized Wilcoxon's test. Cox's proportional hazard model for estimating prognostic significance for these AML patients was tested, and selection of the best-fit model in the multivariate analysis was based on Akaike's information criterion.[21,22]

RESULTS

Clinical manifestations of CD7$^+$ AML patients. CD7 expression was found in 15 M1, 18 M2, 3 M4, and 4 M5 patients (Table 1). The clinical manifestations in these CD7$^+$ AML patients are shown in Table 2. CD7$^+$ AML occurred in younger males than CD7$^-$ AML ($P < .01$). There were no differences of age and sex distribution among the FAB subtypes of CD7$^+$ AML. The incidence of hepatomegaly and percentage of blasts in PB were higher in CD7$^+$ AML than in CD7$^-$ AML. Central nervous system (CNS) involvement at initial diagnosis and relapse was also frequent in CD7$^+$ AML.

Cellular characteristics of CD7$^+$ AML cells. AML cells show morphologic heterogeneity in common, and they are widely spread on the cytogram in the flow cytometric study. In principle, to clarify the phenotypes of the leukemic stem cells, we focused on the blastic fraction of the cytogram, as reported previously.[13] Nonspecific binding caused by FcγR was avoided by sufficient pretreatment with human AB serum.

The lymphoid antigens, CD7, CD2, and CD19, were detected on AML cells of 40, 26, and 38 patients, respectively. No other T-cell and B-cell lineage-restricted antigens, surface CD3 (sCD3), TCR molecules, or CD20 were expressed on AML cells in any of the patients. Positivities of CD7, CD2, and/or CD19 on AML cells were judged to the population in which contaminating sCD3$^+$ cells (T cells) and CD20$^+$ cells (B cells) were excluded from the blastic fraction.

Leukemic blasts from all CD7$^+$ AML patients had more than two myeloid-associated antigens with various degrees of POX positivity (from 7% to 95%), as well as other myeloid cell-related enzymes. CD13 was detected in 35 patients, and CD33 in 36 (Table 3). Myeloid progenitor-associated antigens, CD34 and HLA-DR, were also detected in 35 and all patients, respectively. CD2 expression was found in 5 M1 patients and 1 M2 patients, and CD5 expression was found in 2 M1 patients. TdT expression was found in 10 of 26 patients examined. Leukemic cells from CD7$^+$ AML were more apt to display CD34, HLA-DR, or TdT than those from CD7$^-$ AML (Table 3; $P < .01$, $P < .05$, or $P < .01$, respectively). Coexpression of CD34 and HLA-DR on the CD7$^+$ leukemic cells was confirmed by the double staining or marked overlapping of percentage of cells expressing each antigen (data not shown). CD19 expression was found in five CD7$^+$ patients. There were no significant differences of CD2 and CD19 positivity between CD7$^+$ and CD7$^-$ AML (Table 2).

Table 1. Incidence of CD7$^+$ Patients in Each AML FAB Classification Category

FAB Category	No. of Patients Examined	No. of CD7$^+$ Patients	Incidence (%)
M1	40	15	37.5
M2	72	18	25.0
M3	40	0	0
M4	32	3	9.4
M5	26	4	15.4
Total	210	40	19.0

Table 2. Differences in Clinical Manifestations Between CD7+ and CD7− AML Patients

	All Patients	CD7+	CD7−	CD7+ v CD7−
No. of patients	210	40	170	
Age (yrs)				
Range	15-89	15-71	15-89	
Mean	49.5 ± 16.8	41.9 ± 13.8	51.2 ± 17.0	$P < .01$
Sex (M:F)	124:86	31:9	93:77	$P < .01$
Hematologic data				
Hb (g/dL)	8.82 ± 2.42	9.14 ± 2.2	8.75 ± 2.47	NS
Plt ($\times 10^3/\mu L$)	6.25 ± 6.13	6.26 ± 7.65	6.25 ± 5.74	NS
WBC ($\times 10^3/\mu L$)	44.5 ± 59.0	53.6 ± 64.7	42.4 ± 57.5	NS
Bl ($\times 10^3/\mu L$)	33.7 ± 32.8	46.0 ± 60.6	34.5 ± 54.4	NS
Bl (%)	61.3 ± 32.8	74.9 ± 24.0	58.1 ± 33.8	$P < .01$
Organomegaly				
Liver	20.5%	35.0%	17.1%	$P < .01$
Spleen	5.2%	10.0%	4.1%	NS
LN	13.3%	15.0%	12.9%	NS
MT	0.5%	0%	0.6%	NS
CNS involvement*	6.4%	22.9%	2.5%	$P < .01$
Therapeutic effect				
Numbers†	164	35	129	
CR ratio	78.0%	48.6%	86.0%	$P < .01$
M1	50.0% (n = 30)‡	25.0% (n = 12)	72.2% (n = 18)	$P < .05$
M2	83.9% (n = 62)	68.8% (n = 16)	87.0% (n = 46)	NS
M3	93.4% (n = 32)			
M4	86.4% (n = 22)	33.3% (n = 3)	89.5% (n = 19)	NS
M5	72.2% (n = 18)	50.0% (n = 4)	78.6% (n = 14)	NS

Values for age and hematologic data indicate mean ± 1 SD.

Abbreviations: Hb, hemoglobin; Plt, platelets; Bl, blast; LN, lymph node; MT, mediastinal tumor; CNS, central nervous system.

* 195 patients were evaluated; this included 5 CD7+ patients and 1 CD7− patient with CNS relapse.

† Number of evaluable patients treated with BHAC-DMP induction therapy.

‡ Number of evaluable patients.

Two CD7+ patients had sole rearrangement of TCRδ or TCRβ genes, respectively. IgH gene rearrangement was not found in any CD7+ samples examined. The incidence of rearranged immunogenotype in CD7+ AML did not differ from that in CD7− AML (Table 2).

Effects of exogenous hematopoietic growth factors on CD7+ AML cells. The effects of exogenous growth factors on leukemic cells were evaluated by determining [^{3}H]TdR incorporation in 23 CD7+ and 53 CD7− AML samples. The response of leukemic cells to any factor was considered to be positive when the difference between [^{3}H]TdR uptake counts in the cells cultured with the factor and the control was more than 2,000 cpm and over twice the standard deviation (SD) of the control value.

Leukemic cells from 15 CD7+ and 37 CD7− AML patients were stimulated by one or more of the growth factors. Prominent spontaneous growth or no response was recorded from the remaining 7 CD7+ and 9 CD7− samples. IL-3 was the most powerful stimulator of DNA synthesis in leukemic cells from 15 of the CD7+ responders, while IL-3 exerted such effects on only 8 of the CD7− responders. The differences between CD7+ and CD7− AML samples in responsiveness to IL-3 were significant ($P < .01$; Table 4). The responsive behavior of CD7+ M2 samples to IL-3 was not different from that of CD7+ M1 samples.

Karyotypes of CD7+ AML patients. A normal karyotype was found in 20 of 31 CD7+ AML patients examined. Abnormal karyotypes were found in 11 patients as follows: +8

Table 3. Phenotype and Genotype of CD7+ AML in Comparison With CD7− AML

										Genotype	
	CD13	CD33	CD34	HLA-DR	CD2	CD5	TCR*	CD19	TdT	TCR	IgH
CD7+	35/40	36/40	35/40	40/40	6/40	2/40	0/33†	5/40	10/26	2/18	0/18
CD7−	152/170	154/170	73/157	109/170	20/170	1/152	0/62†	33/170	11/99	3/36	3/36
	NS	NS	$P < .01$	$P < .01$	NS	NS	NS	NS	$P < .01$	NS	NS

Number of positive cases/number of cases examined.

Abbreviations: TCR, T-cell receptor; NS, not significant.

* TCR α β and γ δ complex examined by WT31 and TCRδ1 antibodies.

† All samples were negative for surface CD3.

Table 4. Response Pattern of CD7$^+$ and CD7$^-$ AML Cells to Hematopoietic Growth Factors

	N	IL-3 Responder: IL-3 > GM-CSF/G-CSF*	IL-3 Responder: Others†		IL-3 Nonresponder
CD7$^+$	16	15	0	$P < .01$	1
CD7$^-$	44	8	27		9

Abbreviation: N, evaluable case number.

* Case showing the greatest response to IL-3.

† Case showing the greatest response to GM-CSF and/or G-CSF.

in 1, +13 in 1, +21 in 2, Yq+, Yq+ in 1, −7 in 1, −X, −11, −12, −13 in 1, −15, −18, +del(15)(15;?) in 1, t(11;17) in 1, t(8;21) in 1, and −13, −14, t(5;16), t(6;11), +del(14)t(14;?), +mir in 1 patient. Thus, no common form of karyotypic abnormality was identified in CD7$^+$ AML.

On the other hand, 10 of 14 CD7$^-$CD2$^+$ AML patients had t(15;17), and 20 of 26 CD19$^+$ AML patients had t(8;21). The incidence of CD2 expression in t(15;17) and CD19 expression in t(8;21) was significantly higher than that in patients with normal karyotype or other karyotypic abnormalities.

Therapeutic outcome of CD7$^+$ AML patients. In this study, 180 patients were treated with BHAC-DMP chemotherapy (behenoyl cytosine arabinoside [BH-AC], daunorubicin [DNR], 6-mercaptopurine [6-MP], and prednisolone [PDN], which is a standard regimen for AML in Japan. Chemotherapeutic effects could be evaluated in 164 of those patients; the remaining 16 patients were excluded because of early death, ie, within 2 weeks (14 patients) and nonpathologic death (2 patients). The survival of these patients was evaluated on December 31, 1991. Two CD7$^+$ and 6 CD7$^-$ AML patients were treated by bone marrow transplantation (BMT) in complete remission (CR), and they were regarded as censored cases at the time of BMT. Scheduled intensification chemotherapies, which consisted of three to six drugs selected from DNR (or acracinomycin or mitoxantron), BHAC (or cytosine arabinoside), 6-MP, etoposide, vincristine (or vindesine), and PDN were administered every month, at least for 12 months, to all CR patients.

The CR rate for this regimen was lower in CD7$^+$ AML than in CD7$^-$ AML (Table 2). For seven CD7$^+$ patients who failed to achieve CR with BHAC-DMP regimen, several kinds of ALL chemotherapies were tried, and thereby five of them achieved CR. Leukemic cells from four of the patients who responded to the ALL regimens expressed CD2 and/or TdT (Table 5). In addition, the therapeutic response of CD2$^+$ and TdT$^+$ AML patients to BHAC-DMP therapy is shown in Table 6. Neither CD2 nor TdT positivity in AML patients was related to the induction of CR by this therapy. CD7$^+$ patients with both CD2$^+$ and TdT$^+$ AML had a lower CR rate than CD7$^-$ patients.

The survival rate of the CD7$^+$ AML patients calculated by the Kaplan-Meyer method and generalized Wilcoxon's test was lower than that of the CD7$^-$ patients ($P < .01$; Fig 1A), but there was no difference in disease-free survival between these two groups. Multivariate life-table analysis was performed on 156 patients with AML on whom 10 variables including age, sex, hematomegaly, CD7, CD2, CD19, HLA-DR, CD13, CD33, and CD34 were all available, and all of the possible combinations were estimated. The best model included age, CD7, and CD33 as predictors, and next model included age and CD7. Because CD33$^-$ patients were less than 10%, the model of age and CD7 was considered to be suitable for estimated relative hazard model; P value for global null hypothesis was .004655 ($\chi^2 = 10.74$, degree of freedom: 2), P value for CD7 was .003354, and P value for age was .023088.

DISCUSSION

In this study, we describe the clinical importance of CD7 positivity in AML fulfilling the FAB criteria. Patients with CD7$^+$ AML were younger males who had a higher incidence of hepatomegaly and CNS involvement than CD7$^-$ AML patients. They responded poorly to standard chemotherapy for AML, and had an unfavorable outcome. Thus, the CD7$^+$ AML patients showed unique and characteristic clinical manifestations.

CD7$^+$ AML cells showed a common form of phenotypic and proliferative properties. The incidence of positivity for the myeloid progenitor-associated antigens, CD34 and HLA-DR,[23] and TdT was significantly higher in CD7$^+$ AML than in CD7$^-$ AML. Recombinant hematopoietic growth factors have been used in hematologic investigations,[24] and this has made it possible to examine the proliferative and differentiational behaviors of AML cells along the developmental stages of hematopoietic differentiation.[25,26] However, the prolifer-

Table 5. Favorable Effects of Secondary Therapeutic Regimens for CD7$^+$ AML Patients After Failure of Remission Induction With BHAC-DMP

Patients	FAB	2nd Tx.	Effect	CR Duration	Phenotype (positive %)* POX	CD13	CD34	CD2	CD5	cCD3	TdT
H.S.	M1	AdVP-L	CR	8 M	15	54	82	96	0	39	84
N.A.	M1	AdVP-L	CR	14 M	82	7	73	80	0	0	0
T.M.	M2	AdVP-L	CR	>29 M	42	70	50	82	30*	2	30
I.M.	M1	AcrMVV	CR	13 M	7	96	90	0	0	4	89
K.H.	M2	MAP	CR	3 M	80	36	46	3	0	0	0

Abbreviations: Tx, therapy; POX, peroxidase; CR, complete remission; NT, not tested; AdVP-L, adriamycin, vincristine, prednisolone, and l-asparaginase; AcrMVP, acracinomycin, mitoxantron, etoposide, and vincristine; MAP, mitoxantron, cytosine arabinoside, and prednisolone.

* CD5 was expressed on leukemic cells.

Table 6. Differences Among Subgroups in Therapeutic Effects of AML Therapy

Phenotype	N	CR	PR/NR	CR Ratio (%)	
$CD2^+$	21	15	6	71.4	NS
$CD2^-$	143	114	29	79.7	
$CD2^+CD7^+$	6	1	5	20.0	$P < .005$
$CD2^+CD7^-$	15	14	1	93.3	
$CD2^+CD7^+$	6	1	5	20.0	NS
$CD2^-CD7^+$	29	16	13	55.2	
$CD2^-CD7^+$	29	16	13	55.2	$P < .01$
$CD2^-CD7^-$	114	97	17	85.1	
TdT^+	29	13	7	65.0	NS
TdT^-	77	64	13	83.1	
TdT^+CD7^+	10	4	6	40.0	$P < .05$
TdT^+CD7^-	10	9	1	90.0	
TdT^+CD7^+	10	4	6	40.0	NS
TdT^-CD7^+	13	6	7	46.2	
TdT^-CD7^+	13	6	7	46.2	$P < .01$
TdT^-CD7^-	64	58	6	90.6	

Abbreviations: N, number of patients evaluated; CR, complete remission; PR, partial remission; NR, no response.

ative behavior of $CD7^+$ AML cells is still uncertain. In this study, we found that IL-3, which acts on pluripotential stem cells,[23] promoted the greatest increase in DNA synthesis in leukemic cells in $CD7^+$ AML, including M2 and M4. This finding supports the phenotypic immaturity of the $CD7^+$ AML cells.

The incidence of TCR gene rearrangements in $CD7^+$ AML remains controversial.[27-29] In this study, TCR genes were found in germline configurations in most of the cases examined, as Cuneo et al[29] reported previously. In T-cell ontogeny, CD7 is expressed on most immature T cells, and TCR gene rearrangements are considered to occur during intrathymic development.[30-32] Therefore, the presence of TCR gene rearrangements is not regarded as a decisive indicator of $CD7^+$ AML cell immaturity. Thus, $CD7^+$ AML cells are more apt to preserve nature of myeloid progenitor cells or more immature hematopoietic stem cells.

It is well known that several kinds of karyotypic abnormalities are related to the cellular and clinical manifestations of AML,[3-6] and Koeffler[3] has proposed each of such variations in AML to be a syndrome. However, it remains unclear whether distinct clinical categories of AML are represented by AML with normal karyotype or sporadic karyotypic abnormalities. As shown in our results, no common karyotypic abnormality including any of ALL-associated karyotypes[33] was identified in $CD7^+$ AML, whereas the cellular and clinical characteristics of $CD7^+$ AML were sufficiently consist to allow us to recognize this type of AML as a distinct clinical category.

The clinical outcome finding indicates that CD7 positivity can be considered to be a phenotypical risk factor for AML. Several investigators have documented phenotypical prognostic factors for AML.[11,13,19,34,35] Cross et al[11] reported that $CD2^+$ childhood AML had a poor prognosis. We should note that all of the $CD2^+$ childhood AML in that study were positive for CD7. The $CD7^+CD2^+$ patients in this study had a poorer response to the AML therapy, as did as the childhood AML patients. Nevertheless, when we evaluated all AML populations, we found no significant difference of prognosis between $CD2^+$ and $CD2^-$ patients. Patients with $CD2^+$ AML in this study were frequently M3 which was negative for CD7.

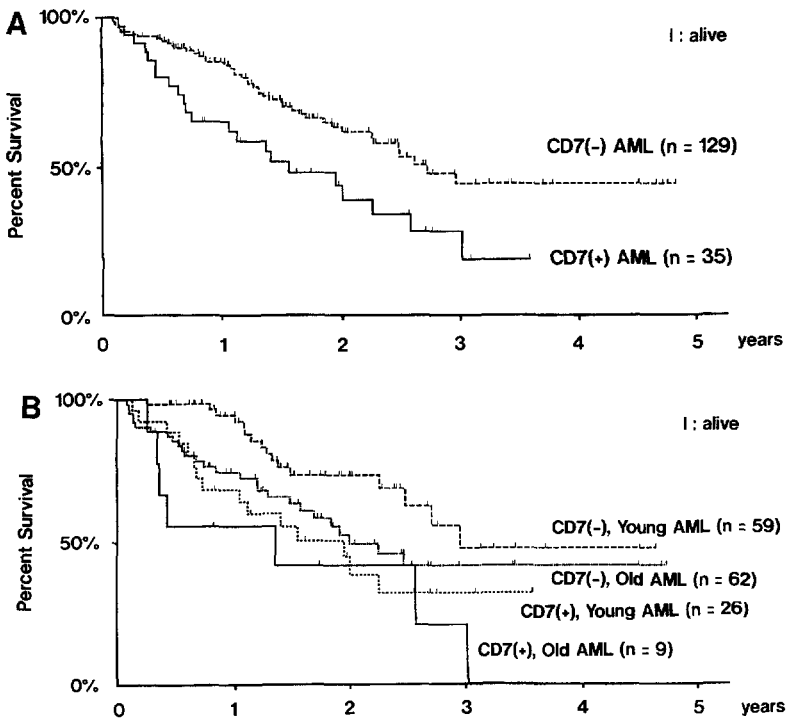

Fig 1. Survival probability of $CD7^+$ AML patients. The survival curve was calculated by the Kaplan-Meyer method. (A) Difference between $CD7^+$ and $CD7^-$ groups was statistically significant by generalized Wilcoxon's test ($P < .01$). (B) Differences of survivals among subgroups defined by two significant prognostic factors, CD7 ($P = .003354$) and age ($P = .023088$), because of multivariate analysis are presented. The relative hazard of $CD7^-$ Young, $CD7^-$ Old, $CD7^+$ Young, and $CD7^+$ Old were 0.6424, 1.1455, 1.4284, and 2.5470, respectively. This result was obtained from 156 AML patients who were tested for CD34. Young, < 50 years old; Old, ≥50 years old.

M3 has a favorable outcome, if the patient escapes early death due to disseminating intravascular coagulation. Indeed, Ball et al[13] described CD2 expression as a favorable prognostic factor for AML, although in that study the details of the $CD2^+$ AML cells were not always clear. Besides, in our study there was no difference in clinical outcome between TdT^+ and TdT^- patients. Thus, the diagnostic value of CD7 positivity for overall AML patients was superior to either CD2 or TdT positivity.

Patients with $CD7^+$ AML showed heterogeneous responses to chemotherapy in relation to morphology and phenotype of the leukemic cells. Patients with morphologically immature blasts (M1 and M5a) had a poorer response to the BHAC-DMP therapy than others. In particular, $CD7^+CD2^-$ M1 and M5a patients did not achieve CR with any of the therapeutic regimens used. It is worth noting that $CD7^+CD2^+$ AML patients who failed to respond to the BHAC-DMP therapy achieved CR with ALL therapy. Therefore, since, at present, an effective regimen for $CD7^+$ AML has not been established, we should choose an appropriate therapeutic regimen for these patients according to the morphology and phenotype of their leukemic cells.

Therapeutic response of $CD7^+$ AML in our study was different from that in Schwarzinger et al's[35] report. Different anti-CD7 antibody isotypes and immunodetection methods between theirs (WT1, mouse IgG2a, and fluorescence microscopy) and ours (TP40, mouse IgG1, and flow cytometry) might cause different patient populations: mouse IgG2a binds with high affinity to FcγR,[36] and intensity of lymphoid antigen expression on AML cells varies among samples.[6] Also, we could not rule out a possibility according to different therapeutic regimens.

Recently, $CD13/CD33^+POX^-$ acute leukemia, which is unrelated to CD7 positivity, has been proposed as a new category, "M0," by the FAB cooperative group.[7] However, the morphologic, biologic, and clinical features of "M0" are not always clearly defined, as mentioned in the report. Kurtzberg et al[15] proposed $CD7^+CD4^-CD8^-$ ALL as a syndrome "lymphohematopoietic stem cell leukemia." This type of ALL cell frequently expresses myeloid antigens[37,38] and shows responsiveness to several growth factors with myeloid differentiation. The clinical manifestation of this type of ALL were similar to those in $CD7^+$ AML; high incidence in males, frequent CNS involvement, and poor response to conventional therapies for acute leukemias. These similarities suggest a fascinating idea: $CD7^+$ AML can be recognized as part of a continuous disease spectrum that includes $CD7^+CD4^-CD8^-$ ALL, at least with germline TCR genes and myeloid antigens, and $CD7^+$ "M0." This may require us to develop a new diagnostic formulation that would be applicable to both AML and ALL.

ACKNOWLEDGMENT

Thanks are due to Drs T. Honjo, Y. Takihara, R. Ueda, and E. Tatsumi for supplying DNA probes and monoclonal antibodies. We also thank Drs S. Kageyama, A. Otsuji, T. Sekine, K. Nishii, N. Morita, and N. Shimizu (Mie University, Tsu, Japan), H. Anazawa (Matsusaka Chuo Hospital, Matsusaka, Japan), T. Ueda (Fukui Medical School, Fukui, Japan), and S. Edamura (Osaka Red Cross Hospital, Osaka, Japan) for important information, and K. Hirose and J. Saitoh for technical assistance.

REFERENCES

1. Griffin DJ, Ritz J, Nadler LM, Schlossman SF: Expression of myeloid differentiation antigens on normal and myeloid cells. J Clin Invest 68:932, 1981

2. Foon KA, Todd RF III: Immunologic classification of leukemia and lymphoma. Blood 68:1, 1986

3. Koeffler HP: A syndrome of acute nonlymphocytic leukemia. Ann Intern Med 107:748, 1987

4. Second MIC Cooperative Study Group: Morphologic, immunologic, and cytogenetic (MIC) working classification of the acute myeloid leukemias. Cancer Genet Cytogenet 30:1, 1988

5. Claxton DF, Reading CL, Nagarajan L, Tsujimoto Y, Andersson BS, Estey E, Cork A, Huh YO, Trujillo J, Deisseroth AB: Correlation of CD2 expression with PML gene breakpoints in patients with acute promyelocytic leukemia. Blood 80:582, 1992

6. Kita K, Nakase K, Miwa H, Masuya M, Nishii K, Morita N, Takakura N, Otsuji A, Shirakawa S, Ueda T, Nasu K, Kyo T, Dohy H, Kamada N: Phenotypic characteristics of acute myelocytic leukemia associated with the t(8;21)(q22;q22) chromosomal abnormality: Frequent expression of immature B-cell antigen CD19 together with stem cell antigen CD34. Blood 80:470, 1992

7. Bennet JM, Catovsky D, Daniel MT, Flandrin G, Galton DAG, Gralnik HR, Sultan C: Proposal for the recognition of minimally differentiated acute myeloid leukaemia (AML-M0). Br J Haematol 78:325, 1991

8. Vodinelich L, Tax LV, Bai Y, Pegram S, Capel P, Greaves MF: A monoclonal antibody (WT1) for detecting leukemias of T-cell precursors (T-ALL). Blood 62:1108, 1983

9. Greaves MF, Chan LC, Furley AJW, Watt SM, Molgaard HV: Line age promiscuity in hematopoietic differentiation and leukemia. Blood 67:1, 1986

10. Gale RP, Bassat IB: Hybrid acute leukemia. Br J Haematol 65:261, 1987

11. Cross AH, Goorha RM, Nuss R, Behm F, Murphy SB, Kalwinsky DK, Raimondi S, Kitchingman GR, Mirro JJ: Acute myeloid leukemia with T-lymphoid features: A distinct biologic and clinical entity. Blood 72:579, 1988

12. Bradstock KF, Kirk J, Grimsley PG, Karbral A, Hughes WG: Unusual immunophenotypes in acute leukemias: Incidence and clinical correlations. Br J Haematol 72:512, 1989

13. Ball ED, Davis RB, Griffin JD, Mayer RJ, Davey FR, Arthur DC, Wurster-Hill D, Noll W, Elghetany T, Allen SL, Rai K, Lee EJ, Schiffer CA, Bloomfield CD: Prognostic value of lymphocyte surface markers in acute myeloid leukemia. Blood 77:2242, 1991

14. Lo Coco FL, De Rossi G, Pasqualetti D, Lopez M, Diverio D, Latagliata R, Fenu S, Mandelli F: CD7 positive acute myeloid leukemia: A subtype associated with cell immaturity. Br J Haematol 73:480, 1989

15. Kurtzberg J, Waldmann TA, Davey MP, Binger SH, Moore JO, Hersfield MS, Haynes BF: CD7+, CD4−, CD8− acute leukemia: A syndrome of malignant pluripotent lymphohematopoietic cells. Blood 73:381, 1989

16. Wiersma SR, Ostega J, Sobel E, Weinberg KI: Clinical importance of myeloid-antigen expression in acute lymphoblastic leukemia of childhood. N Engl J Med 324:800, 1991

17. Bennet JM, Catovsky D, Daniel MT, Flandrin G, Galton DAG, Gralnick HR, Sultan C (FAB Cooperative Group): Proposed revised criteria for the classification of acute myeloblastic leukemia. Ann Intern Med 103:620, 1985

18. Nosaka T, Kita K, Miwa H, Kawakami K, Ikeda T, Ohno T, Matsuoka N, Arita Y, Doi S, Nishikori M, Matsuda F, Lee KH, Shirakawa S, Honjo T, Hatanaka M: Cross-lineage gene rearrange-

ments in human leukemic B-precursor cells occur frequently with V-DJ rearrangements of IgH genes. Blood 74:361, 1989

19. Nakase K, Kita K, Otsuji A, Anazawa H, Hoshino K, Sekine T, Shirakawa S, Tanaka I, Nasu K, Tsutani H, Dohy H, Tsudo M: Diagnostic and clinical importance of interleukin-2 receptor alpha chain expression on non–T-cell acute leukaemia cell. Br J Haematol 80:317, 1992

20. Asou H, Tashiro S, Hamamoto K, Otsuji A, Kita K, Kamada N: Establishment of a human acute myeloid leukemia cell line (Kasumi-1) with 8;21 chromosomal translocation. Blood 77:2031, 1991

21. Oguma S, Yoshida Y, Uchino H, Maekawa T: Factors influencing nonleukemic death in refractory anemia, refractory anemia with ring sideroblasts, and refractory anemia with excess of blasts. Cancer Res 47:3599, 1987

22. Akaike H: What is the information criterion "AIC?". Math Sci 153:5, 1976

23. Caux C, Favre C, Saeland S, Duvert V, Monnoni P, Durand I, Aurbry JP, deVries JE: Sequential loss of CD34 and class II MHC antigen on purified cord blood hematopoietic progenitors cultured with IL-3: Characterization of CD34−, HLA-DR+ cells. Blood 74: 1287, 1989

24. Metcalf D: The molecular control of cell division, differentiation commitment and maturation in hematopoietic cells. Nature 339: 27, 1990

25. Griffin JD, Lowenberg B: Clonogenic cells in acute myeloblastic leukemia. Blood 68:1185, 1986

26. Miyauchi J, Kelleher C, Young YC, Wong GG, Clark SC, Minden MD, Minkin S, MucCulloch EA: The effects of three recombinant growth factors, IL-3, GM-CSF and G-CSF, on the blast cells of acute myeloblastic leukemia mainteined in short term suspension culture. Blood 70:657, 1987

27. Zutter MM, Martin PJ, Hanke D, Kidd P: CD7+ acute nonlymphocytic leukemia: Evidence for an early multipotential progenitor. Leuk Res 14:23, 1990

28. Williere G, Stockinger H, Koller U, Majdic O, Schnabl E, Lutz D, Bettleheim P, Knapp W: Terminal deoxynucleotidyl transferase and CD7 expression in acute myeloid leukemias are not associated with a high frequency of immunoglobulin and/or T cell receptor gene rearrangement. Leukemia 4:278, 1990

29. Cueno A, Michaux JL, Ferrant A, Van Hove L, Bosly A, Stul M, Dal Cin P, Vandenberghe E, Cassiman JJ, Negrini M, Piva N, Castoldi G, Van den Berghe H: Correlation of cytogenetic patterns and clinicobiological features in adult acute myeloid leukemia expressing lymphoid markers. Blood 79:720, 1992

30. Haynes BE, Martin ME, Kay HH, Kurtzberg J: Early events in human T cell ontogeny. Phenotypic characterization and immunohistologic locarization of T cell precursors in early human fetal tissues. J Exp Med 168:1061, 1988

31. Furley AJ, Mizutani K, Weilbaecher K, Dhaliwal HS, Ford AM, Chan LC, Molgaad HV, Toyonaga B, Mak T, Van den Eisen P, Gold D, Terhorst C, Greaves MF: Developementally regurated rearrangement and expression genes encoding the T-cell receptor T3 complex. Cell 46:75, 1986

32. Lafaille JJ, DeCloux A, Bonneville M, Takagaki Y, Tonegawa S: Junctional sequences of T cell receptor $\gamma\delta$ genes: Implications for $\gamma\delta$ T cell lineages and for a novel intermediate of V-(D)-J joining. Cell 59:859, 1989

33. Pui CH, Crist WM, Look T: Biology and clinical significance of cytogenetic abnormalities in childhood acute lymphoblastic leukemia. Blood 76:1149, 1990

34. Benedetto P, Mertelsman R, Szatrowski TH, Andreef M, Gee T, Arlin Z, Kempin S, Clarkson B: Prognostic significance of terminal deoxynucleotidyl transferase activity in acute non-lymphocytic leukaemia. J Clin Oncol 4:489, 1986

35. Schwarzinger I, Valent P, Koller U, Marosi C, Schneider B, Haars O, Knapp W, Lechner K, Bettleheim P: Prognostic significance of surface marker expression on blasts of patients with de novo acute myeloblastic leukemia. J Clin Oncol 8:423, 1990

36. Osada H, Emi N, Ueda R, Seto M, Koike K, Suchi T, Kojima S, Obata Y, Takahashi T: Genuine CD7 expression in acute leukemia and lymphoblastic lymphoma. Leuk Res 14:869, 1990

37. Yumura-Yagi K, Hara J, Kurahashi H, Okamura J, Koizumi S, Toyoda Y, Murayama N, Inoue M, Ishihara S, Tawa A, Nishiura T, Kaneyama Y, Okada S, Kawa-Ha K: Clinical significance of CD7-positive stem cell leukemia. Cancer 68:2273, 1991

38. Nishii K, Kita K, Miwa H, Kawakami K, Nakase K, Masuya M, Morita N, Omaya SB, Otsuji N, Fukumoto M, Shirakawa S: c-kit gene expression in CD7-positive acute lymphoblastic leukemia: Close correlation with expression of myeloid-associated antigen CD13. Leukemia 6:662, 1992