HIGH INCIDENCE OF CD56 EXPRESSION AND RELAPSE RATE IN ACUTE MYELOID LEUKEMIA PATIENTS WITH t(8;21) IN TAIWAN

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Background and Purpose: CD56 is a marker of natural killer cells, but can also be found on blast cells in acute myeloid leukemia (AML). The prognostic implications of CD56 expression in AML are not clear. In this study, we evaluated the correlation among CD56 expression, cytogenetic abnormality, and clinical outcome in AML. ***Methods:*** CD56 expression was analyzed in leukemic cells from 94 adults with primary AML in Taiwan and was correlated with clinical and hematologic features, cytogenetics, and immunophenotypes of the leukemia. ***Results:*** Thirty patients (32%) showed CD56 expression. CD56+ AML patients had a higher lactate dehydrogenase level than CD56- patients (1,136 *vs* 730 U/L, p = 0.048). Patients with t(8;21) had a significantly higher incidence (89%, 8/9) of CD56 positivity in leukemic cells than those with normal karyotype or other cytogenetic abnormalities (26%, 22/85, $p < 0.001$). In general, there was no difference in overall survival time in CD56+ and CD56- AML patients. However, three patients had central nervous system involvement at initial presentation; two of these had concomitant CD56 expression and t(8;21). In addition, five of the seven patients with t(8;21) and CD56 expression who achieved complete remission later relapsed. ***Conclusions:*** The incidence of CD56 expression in AML patients with t(8;21) is very high in Taiwan, and it may imply a poor prognosis in this subgroup of patients.

CD56 is a 200 to 220 kD glycoprotein expressed predominantly on human natural killer cells, a minor subset of T cells and neural/neuroendocrine tissues [1]. It has been recognized as a neural cell adhesion molecule (N-CAM) [2]. In acute myeloid leukemia (AML), CD56 expression has been demonstrated in leukemic cells from 20% to 40% of patients [3–5]; however, the exact role of CD56 in AML is largely unknown. Some investigators suggest that it may affect and modulate both cell–cell and cell–substrate interactions [6].

The prognostic implications of CD56 expression in AML are not clear. Controversial results have been reported [3, 4, 7, 8]. Recently, Baer et al reported that 55% of AML patients with t(8;21) showed CD56 expression, which was associated with shorter complete remission (CR) duration and survival [9]. It was also demonstrated

that t(15;17)-positive AML patients with CD56 expression had a lower CR rate and shorter survival than those without CD56 expression [10]. Most reports concerning CD56 expression in AML are from Western countries, and only few studies have involved Oriental people [11, 12]. In this study, we evaluated the correlation among CD56 expression, cytogenetic abnormality, and clinical outcome in AML patients.

Materials and Methods

Patients

From October 1996 to November 1999, immunophe-

notype including CD56 expression was determined in leukemic cells from 94 newly diagnosed patients with primary AML at National Taiwan University Hospital. None of the patients had a history of prior chemotherapy or radiotherapy, and none had a history of other hematologic disorders. The median age was 50 years, ranging from 16 to 84 years. There were 49 males and 45 females. The diagnosis and classification of AML were based on the criteria established by the French-American-British (FAB) Cooperative Study Group [13]. Seventy-five patients received standard 3 + 7 induction chemotherapy with doxorubicin 30 mg/m^2/day or idarubicin 12 mg/m^2 per day for 3 days plus cytosine arabinoside (ara-c) 100 mg/m^2 per day for 7 days (in non M3 patients) or all-trans retinoic acid with or without chemotherapy (in M3 patients). The remaining 19 patients did not receive any chemotherapy, or were only treated with low-dose ara-c. Fifty-eight

(77%) of the patients who received standard chemotherapy achieved CR. Among them, eight patients received hematopoietic stem cell transplantation (6 allogeneic and 2 autologous), 26 patients received consolidation chemotherapy with regimens similar to those in induction treatment, 16 received high-dose ara-c 2 to 3 g/m^2 twice per day for eight to 12 doses plus one of anthracyclines, two received low-dose ara-c 10 mg/m^2 per day for 14 to 21 days, and the remaining six patients did not receive further consolidation treatment (Table 1).

Cytogenetic study

Chromosome analysis was performed as described previously [14]. Briefly, bone marrow (BM) cells were harvested either directly or after 1 to 3 days of unstimulated culture. Metaphase chromosomes were banded by the conventional trypsin-Giemsa banding

Table 1. Correlation of CD56 expression with clinical and hematologic features in patients with acute myeloid leukemia

	CD56$^+$	CD56$^-$	Total	p
Number of patients	30	64	94	
Median age, yr (range)	49 (16–79)	50 (18–84)	50 (16–84)	0.370
Lymphadenopathy (%)*	6 (20)	12 (19)	18 (19)	0.886
Hepatomegaly (%) *	8 (27)	10 (16)	18 (19)	0.205
Splenomegaly (%)*	3 (10)	4 (6)	7 (7)	0.676
CNS involvement (%)*	2 (7)	1 (2)	3 (3)	0.238
Cytogenetics**				< 0.001
t(8;21)	8 (89%)	1 (11%)	9	
Others	22 (26%)	63 (74%)	85	
Hemoglobin (median, g/dL)	8.5	7.9	8.0	0.901
WBC (median, /μL)	18,845	13,920	15,550	0.408
Blast (median, %)	60	44	51	0.541
Platelet (median, x10^3/μL)	36	41	40	0.244
Lactate dehydrogenase (median, U/L)	1136	730	900	0.048
FAB subtype, n†				
M0	1 (20%)	4 (80%)	5	1.000
M1	8 (32%)	17 (68%)	25	0.991
M2	12 (40%)	18 (60%)	30	0.250
M3	3 (25%)	9 (75%)	12	0.746
M4	3 (19%)	14 (81%)	17	0.163
M5	3 (60%)	2 (40%)	5	0.323
CR rate (%)	90.5% (19/21)	72.2 %(39/54)	77.3 (58/75)	0.127
Consolidation C/T‡				0.366
Standard	10 (52%)	16 (41%)	26	
High dose	2 (11%)	14 (36%)	16	
Low dose	1 (5%)	1 (3%)	2	
No treatment	3 (16%)	3 (8%)	6	
Transplantation	3 (16%)	5 (13%)	8	
CR duration (median, mo)	13	12	12	0.9106
Survival (median, mo)	23	18	23	0.3362

CNS = central nervous system; WBC = white blood cell count; FAB = French-American-British; C/T = chemotherapy. *% patients with characters among those with or without CD56 expression; †numbers in parenthesis represent % patients with or without CD56 expression among subgroups; ‡consolidation chemotherapy in patients obtaining complete response (CR): standard = conventional dose of cytosine arabinoside (ara-c) plus anthracycline; high dose = high dose of ara-c plus anthracycline; low dose = low dose of ara-c.

technique and karyotyped according to the International System for Human Cytogenetic Nomenclature (ISCN) [15].

Cytochemical staining and immunophenotyping

Air-dried smears of BM aspirates, stained by Riu's method (Romanowsky system), were observed for morphologic abnormalities [16, 17]. The smears were also stained routinely for myeloperoxidase, chloroacetate esterase, and α-naphthyl butyrate esterase, as described previously [16]. A panel of monoclonal antibodies to myeloid-associated antigens including CD13, CD33, CD14, CD11b, CD15, CD41, and glycophorin A, as well as to lymphoid-associated antigens including CD2, CD5, CD7, CD10, CD19, and CD20, lineage-nonspecific antigens HLA-DR and CD34, and CD56, were selected to characterize immunologic phenotypes of leukemic cells. Expression of surface antigens on leukemic cells was shown by an indirect immunoalkaline phosphatase method [18]. Samples in which more than 20% of leukemic cells were positively stained with antibody were considered positive for that marker.

Statistical methods

Continuous variables were compared by Wilcoxon rank-sum test and discrete variables were compared by chi-square or Fisher's exact test. Curves of survival and CR duration were plotted using the Kaplan-Meier method; differences between curves were analyzed using the log-rank test.

Results

Correlation of CD56 expression with clinical and hematologic features

Thirty (32%) patients were found to have CD56 expression in leukemic cells. Comparison of clinical and hematologic features between the patients with and without CD56 expression are summarized in Table 1. There was no statistical difference in age distribution, incidence of lymphadenopathy, hepatomegaly or splenomegaly, white blood cell (WBC) count, percentage of blasts, hemoglobin, or platelet count between the two groups of patients. However, lactate dehydrogenase (LDH) was significantly higher in the patients with CD56 expression than in those without (1,136 *vs* 730 U/L, $p = 0.048$). Three patients were found to have central nervous system (CNS) involvement at presentation; two had t(8;21) and CD56 expression and the other was CD56-negative.

Correlation of CD56 expression with chromosomal change, FAB classification, and expression of other immunologic markers

There were adequate metaphase cells for analysis for all patients but one. Fifty-five patients (58%) showed clonal chromosomal abnormalities, including 11 with t(15;17), nine with t(8;21), four with t(7;11) [19], three with 11q23 abnormalities, one with t(3;3), and one with inv(16). Four patients had trisomy 8 (+8), and three had monosomy 7 (-7) or deletion of 7q. Interestingly, eight (89%) of the nine AML cases with t(8;21) showed CD56 positivity, compared with 22 (26%) patients with normal karyotype or other chromosomal abnormalities ($p < 0.001$). Patients with t(15; 17) showed a similar incidence of CD56 expression (3/ 11, 27%) to others. The percentage of CD56+ leukemic cells in BM from the eight patients with t(8;21) and CD56 expression ranged from 41% to 100% with a mean of 82% (Table 2 and Figure).

Among FAB subtypes, M5 had the highest incidence of CD56 expression (60%), followed by M2 and M1 (Table 1). The correlation of CD56 positivity with expression of myeloid- and lymphoid-associated surface markers was also analyzed. There was no difference between these two groups. However, when patients with specific chromosomal abnormalities t(8;21) and t(15;17) were excluded, those with CD56 expression showed a significantly lower incidence of CD34 expression than others (37% *vs* 69%, $p = 0.028$). The former group of patients also had a trend of lower frequency of HLA-DR expression than the latter (72% *vs* 89%, $p = 0.125$).

Correlation of CD56 expression with outcome

Among the 75 patients who received standard induction chemotherapy, CR rate, CR duration, and overall survival were not significantly different between patients with and without CD56 expression (Table 1). However, although all seven patients who had t(8;21) and CD56 expression and were treated achieved CR, five (71%) relapsed (Table 2). This incidence was higher than that in other CD56+ patients without t(8; 21) (42%) or in CD56− patients (46%), but the difference was not statistically significant ($p = 0.350$ and $p = 0.414$, respectively), probably because of the small number of patients. The same was also true if only patients receiving standard or more intensive postinduction treatment were included in the analysis (60% *vs* 40% *vs* 43%). Because the number of patients was limited, no statistical difference could be found in the outcome between t(15;17) patients with or without CD56 expression. The two CD56+ APL patients who were treated in this study remained in the first CR for 2 and 24 months, respectively.

Table 2. Immunophenotype of leukemic cells and outcome in acute myeloid leukemia patients with t(8;21) and CD56 expression

UPN	Markers (% cells with positive staining)*									CR	Post-induction treatment	Relapse	CR duration (mo)	Survival (mo)
	HLA-DR	CD13	CD33	CD11b	CD14	CD19	CD15	CD34	CD56					
436	97	48	10	0	0	29	19	85	95	Yes	No	Yes	4	+14
428	100	57	75	0	0	16	53	58	41	NT				
460	95	42	66	0	0	20	40	89	95	Yes	high dose	No	+36	+37
471	ND	60	54	18	10	15	80	42	85	Yes	auto-BMT	Yes	12	13
478	100	42	88	1	0	81	75	90	48	Yes	No	Yes	4	10
508	100	56	85	15	0	50	92	89	100	Yes	allo-BMT	No	+8	+9
514	100	99	90	ND	2	0	48	58	100	Yes	high dose	Yes	2	4
474	81	42	7	18	ND	52	ND	84	94	Yes	allo-BMT	Yes	21	25

*Positivity ≥ 20% leukemic cells react with antibody. CD2, CD5, CD7 and CD10 negative. UPN = unique patient number; allo-BMT = allogeneic bone marrow transplantation; auto-BMT = autologous bone marrow transplantation; CR = complete remission; high dose = high-dose cytosine arabinoside (ara-c) plus anthracycline; ND = not done; NT = no induction chemotherapy.

Discussion

As demonstrated in this study, AML patients with t(8;21) had a significantly higher frequency of CD56 expression in leukemic cells than others. Patients with normal karyotype, t(15;17), and other chromosomal abnormalities had similar incidences of CD56 expression. Furthermore, with a similar incidence of CD56 expression in total AML to that reported by other centers [4, 5, 7, 12], the frequency of CD56 expression in patients with t(8;21) in this area was truly very high,

compared with 55% reported by Baer et al [9] and 54% reported by Seymour et al [4]. The percentage of CD56+ leukemic cells in these patients was also high (mean, 82%; range, 41–100%) (Table 2 and Figure). Both of the two additional cases with t(8;21) referred from other hospitals, who were not included in this study, also showed CD56 expression in leukemic blasts (data not shown). Uneven geographic distribution of nonrandom chromosome aberrations in malignant disorders has been reported [20]. The incidence of t(8; 21) among AML M2 patients with abnormal karyotype is higher in Japan and Taiwan than in Western countries [14, 20]. Whether the distribution of CD56 ex-
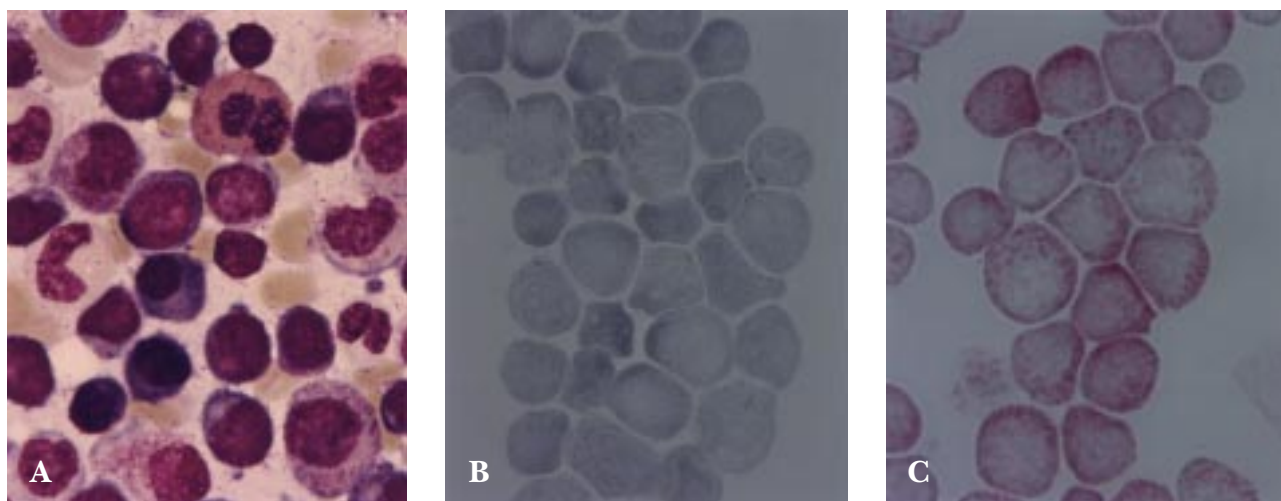
Figure. *A) Smear of bone marrow aspirate from one acute myeloid leukemia (AML) patient with t(8;21) (Liu stain). B) Immunoalkaline phosphatase (IAP) staining using normal mouse ascites as negative control. C) IAP staining using anti-CD56 antibody showing positive staining on leukemic cells (red).*

pression in AML with t(8;21) is also different in various geographic areas merits further study. Other antigen expression, including positivity for CD19 and CD34 (Table 2), in CD56+ AML with t(8;21) was similar to that of total t(8;21) AML [5, 21].

An association of CD56 expression in AML and extramedullary disease has been demonstrated in some reports [12, 22] but not others [4, 9]. In this study, there were no differences in the incidence of lym-phadenopathy, hepatomegaly, or splenomegaly between patients with CD56 expression and those without. Two of the 30 (6.7%) CD56+ patients showed CNS leukemia at presentation, compared with one of the 64 (1.6%) CD56−, but the difference was not statistically significant. Baer et al demonstrated that granulocytic sarcomas were present exclusively in t(8;21) cases with CD56 expression [9], and Seymour et al also found that CNS leukemia was detected only in CD56+, but not in CD56−, AML patients [4], though the differences were not statistically significant in both reports. The incidence of leukemic infiltration of lymph nodes, liver, or spleen was similar between CD56+ and CD56− patients in the latter study [4], as in this series. It is possible that the occurrence of extramedullary leukemia involving lymph nodes, liver, or spleen is similar between AML patients with and without CD56 expression, but that involving CNS or soft tissue is more frequent in the CD56+ AML patients than CD56− patients. Studies on larger groups of patients are needed to clarify this point.

Conflicting results regarding the prognostic implications of CD56 expression in AML have been reported [3, 4, 7–10]. In this study, CR rate, CR duration, and survival were not significantly different between AML patients with CD56 expression and those without. These results were similar to those reported by Thomas et al [8] and Seymour et al [4]. However, Lauria et al reported a shorter survival in CD56+ patients than in others [7], while Vidriales et al found a trend toward better outcome in these patients [3]. It may be more meaningful to perform analysis in subgroups of this heterogeneous disease separately than to do it in all patients. According to a recent study [9], CD56 expression is associated with a short remission duration and survival in AML patients with t(8;21)(q22;q22). Because all but one patient with t(8;21) in this study showed CD56 expression, a comparison of outcome between CD56+ and CD56− cases with t(8;21) was impossible. However, patients with t(8;21) and CD56 expression had a high relapse rate (71%), compared with the good prognosis in total t(8;21) cases reported in the literature [23, 24]. In a previous study, we found that 16 of 17 patients with t(8;21) who were treated obtained a CR; excluding the two patients who did not receive post-induction chemotherapy, seven of the nine patients treated with

standard-dose consolidation chemotherapy relapsed, while only one of the five patients who received allogeneic BM transplantation did so [14]. It seems obvious that patients with t(8;21) in Taiwan have a high relapse rate if they are only treated with conventional consolidation chemotherapy. We have noticed this condition for some time, but could not find any cause to explain it in the past. The high incidence of CD56 expression in leukemic cells from patients with t(8;21) shown in this study may explain the high relapse rate in these patients in Taiwan. Whether high-dose chemotherapy or hematopoietic stem cell transplantation may prolong CR duration and prevent relapse in CD56+ t(8;21) patients needs to be determined.

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