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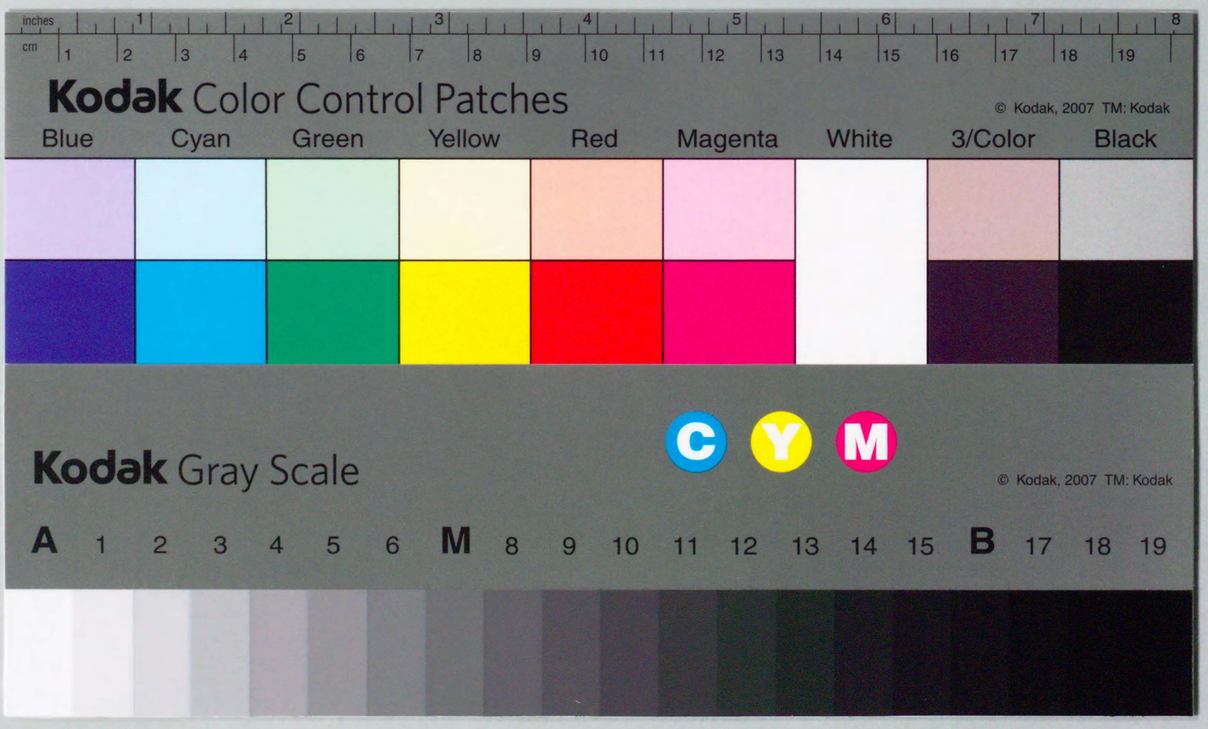
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Biological characteristics of CD7 positive acute myelogenous leukaemia

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Summary. We studied the biological characteristics of CD7$^+$ acute myelogenous leukaemia (AML). We diagnosed nine out of 88 consecutive AML cases as CD7$^+$ AML based on myeloperoxidase positivity and surface antigen expression. In eight of these nine cases more than 20% of leukaemic blasts were found to coexpress both CD7 and a myeloid-associated antigen, CD33, by a two-colour flowcytometric assay, while in the remaining case more than 90% of blasts were positive for CD7 and myeloperoxidase. CD7$^+$ AML was most frequently observed in M1 among AML subtypes according to the FAB classification. An early stage-specific antigen, CD34 was also expressed on leukaemic blasts from eight of these nine cases. Neither the T-cell receptor (TcR)-β nor the TcR-γ gene was clonally rearranged in any of the cases. We then studied the proliferative responses to stimulation by various growth factors. Among interleukin-3 (IL-3), granulocyte/macrophage colony-stimulating factor (GM-CSF), and granulocyte-CSF (G-CSF), IL-3 showed the strongest stimulatory effect on DNA synthesis and leukaemic blast colony formation in 8/9 and 6/8 CD7$^+$ AML cases examined, respectively. On the other hand, the strongest stimulatory effect exerted by IL-3 on blast colony formation was observed in only six out of the 33 CD7$^-$ AML cases examined. Furthermore, CD7$^+$ AML blasts could proliferate in response to stem cell factor (SCF); SCF alone showed stimulatory effects on blast colony formation (7/8 cases), and in 5/7 SCF-responding cases, stimulatory effects of SCF were more potent than those of IL-3. In addition, SCF enhanced blast colony formation synergistically with IL-3 in four of these seven cases. These data suggest that progenitor cells of CD7$^+$ AML may possess the biological properties characteristic of immature haematopoietic stem cells.

CD7 has been recognized as one of the early T cell antigens, and is usually expressed on immature T cells and prothymocytes (Palker *et al.* 1986; Sutherland *et al.* 1984). Recent reports have shown that leukaemic blasts in the minority of acute myelogenous leukaemia (AML) cases expressed CD7 (Lo Coco *et al.* 1989; Tien *et al.* 1990). One possible explanation for this observation is that the coexpression of CD7 and myeloid antigens in AML reflects the involvement of a minor normal counterpart of early haematopoietic progenitor cells. Alternatively, CD7 is aberrantly expressed in some cases of AML probably due to the leukaemic transformation.

In normal haematopoiesis it has been demonstrated that primary targets of interleukin-3 (IL-3) are more primitive multipotent progenitors than those of granulocyte/macrophage colony-stimulating factor (GM-CSF). Granulocyte-CSF (G-CSF) supports proliferation and differentiation of granulocytes in the terminal process of maturation (Leary *et al.* 1987; Lopez *et al.* 1987; Messner *et al.* 1987; Sieff *et al.* 1987; Sonoda *et al.* 1988). Recently, the ligand for c-kit proto-oncogene product, stem cell factor (SCF) (Martin *et al.* 1990; Zsebo *et al.* 1990a, b) or mast cell growth factor (Williams *et al.* 1990), has been reported to preferentially enhance *in vitro* colony growth of CD34$^+$ bone marrow cells (Bernstein *et al.* 1991). In the present study we characterized CD7$^+$ AML and investigated the proliferative potentials of leukaemic blasts from CD7$^+$ AML in response to these haematopoietic growth factors in order to clarify the differentiation stage of leukaemic progenitors in CD7$^+$ AML.

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MATERIALS AND METHODS

AML blast cells. Between 1986 and 1991, 88 consecutive *de novo* AML patients were admitted to our institution. The diagnosis was made according to the revised criteria of the French–American–British group (Bennett *et al.* 1985) based on the morphological and cytochemical evaluation. Peripheral blood mononuclear cells (PBMNC) were obtained by Ficoll-Hypaque density-gradient centrifugation. Adherent cells were removed by incubating the cell suspension (2×10^6/ml) for 1 h in a plastic tissue culture flask. All samples contained more than 85% blasts. These fresh non-adherent AML blasts were used for the following experiments. The leukaemic blasts were cryopreserved in Iscove's modified Dulbecco's medium (IMDM) containing 10% dimethyl sulfoxide (DMSO) and 40% fetal calf serum (FCS) until use for experiments with SCF.

Immunophenotypic analysis. Leukaemic blasts were phenotyped with a direct immunofluorescence technique. For immunofluorescence assays, mouse IgG1 conjugated to fluorescein isothiocyanate (FITC) or mouse IgG conjugated to phycoerythrin (PE) was used as a negative control. The FITC- or PE-conjugated antibodies used were as follows: CD2 (T11), CD3 (Leu4), CD4 (Leu3a), CD7 (Leu9), CD8 (Leu2a), anti-HLA-DR and CD34 (HPCA-1) were obtained from Becton Dickinson Monoclonal Center (Mountain View, Calif., U.S.A.); CD10 (J5), CD13 (My7), CD19 (B4), CD20 (B1) and CD33 (My9) were obtained from Coulter Immunology (Hialeah, Fla., U.S.A.). The immunofluorescence positivity was determined on a FACScan flowcytometer (Becton Dickinson); leukaemic blasts were judged as positive for each marker when more than 20% of the blasts showed fluorescent staining.

Growth factors. Recombinant human (rh) interleukin (IL)-3 and rh stem cell factor (SCF) were obtained from Genzyme (Boston, Mass.). Rh granulocyte/macrophage colony-stimulating factor (GM-CSF) was kindly provided by Sumitomo Pharmaceutical Co. (Tokyo, Japan) (Specific activity, 10^9 U/mg protein). Rh granulocyte-CSF (G-CSF) was a gift from Chugai Pharmaceutical Co. (Tokyo, Japan) (specific activity, 10^8 U/mg protein).

Molecular analysis. High molecular weight DNA was extracted by the method of Wigler *et al* (1979). 10 μg of DNA were digested with one of the following restriction endonucleases: EcoR I, Hind III or BamH I plus Hind III, according to the suppliers' recommendations. The cut DNA was separated by a 0·8% agarose gel, transferred to a nitrocellulose membrane, and hybridized to a ^{32}P-labelled cDNA. The cDNA probes used were as follows: Cβ and Cγ fragments of the T-cell receptor (TcR) gene for TcR-β and TcR-γ respectively, and a JH probe for the Ig-heavy (IgH) chain gene (Kitazato Bio-Chemical Laboratories, Tokyo, Japan).

Leukaemic blast colony assay. To evaluate proliferative responses of leukaemic progenitor cells to growth factors, clonogenic assays for leukaemic blasts were performed as previously described (Akashi *et al.* 1991). Blast cells were cultured in IMDM containing 0·88% methylcellulose and 20% FCS, in a plastic 96-microwell plate (Falcon No. 3072). Each well contained 2×10^4 cells in 100 μl medium either with or without different growth factors. After 7 d incubation under 100% humidity with 5% CO_2 in air at 37°C, compact colonies consisting of 20 or more cells were counted as leukaemic blast colonies under an inverted microscope. According to our previous report (Akashi *et al.* 1991), IL-3, GM-CSF and G-CSF were added at the doses of maximum stimulation, which were 500 U/ml, 1000 U/ml and 100 ng/ml, respectively. As for SCF, the maximum stimulation was observed at a concentration of 50 ng/ml (data not shown). These concentrations were employed throughout this study.

Proliferation of leukaemic blasts. Proliferation of leukaemic blasts stimulated by growth factors was evaluated by DNA synthesis, which was measured with ^{3}H-thymidine (^{3}H-TdR) incorporation. 4×10^4 blast cells were incubated in triplicate in 100 μl of IMDM supplemented with 10% FCS in flat-bottomed 96-well microtitre plates (Falcon No. 3072) either with or without the addition of different growth factors: IL-3 (500 U/ml), GM-CSF (1000 U/ml), G-CSF (100 ng/ml) or SCF (50 ng/ml). After 36 h of incubation, blast cells were pulsed with 0·25 μCi of ^{3}H-TdR for 8 h, and ^{3}H-TdR uptakes were determined by liquid scintillation counting.

Table I. CD7$^+$ AML cases according to FAB classification.

FAB classification	No. of cases	No. of CD7$^+$ AML
M0	1	0
M1	24	5
M2	20	2
M3	13	0
M4	19	1
M5	4	1
M6	3	0
M7	4	0
Total	88	9

RESULTS

CD7$^+$ AML cases

The incidence of CD7$^+$ AML is shown in Table I. Nine cases were diagnosed as CD7$^+$ AML among the 88 consecutive cases studied; these nine cases included five M1, two M2, one M4 and one M5a according to the FAB classification. CD7$^+$ AML was observed most frequently in M1 (55·6%) among FAB subtypes of AML. Clinical characteristics of CD7$^+$ AML cases are shown in Table II. Six of the nine cases were 40 years old or under, and there was no difference between the two sexes. Cytochemical analysis indicated that all cases were committed to a myeloid lineage. Table III shows the results of immuno-fluorescence analysis. In eight cases more than 20% of the blasts expressed CD33 and HLA-DR in addition to CD7 (cases 1, 3, 4, 5, 6, 7, 8 and 9). In the same eight cases the coexpression of CD33 and CD7 was detected in more than 20% of leukaemic blasts by a two-colour flowcytometric assay (data not shown). In the remaining case (no. 2), myeloid-associated antigens were negative, although more than 90% of the leukaemic blasts were positive for myeloperoxidase. Interestingly, in eight cases (nos. 1–8), leukaemic blasts were positive for CD34, which has been demonstrated to be preferentially expressed on immature haematopoietic stem cells (Watt *et al.* 1987). Although the coexpression of CD7 and CD34 was not analysed, leukaemic blasts were considered to be CD7$^+$/CD34$^+$ from the high proportions of CD7$^+$ and CD34$^+$ cells in most of the cases. Neither B

Table II. Patient characteristics and cytochemical features of CD7$^+$ AML.

			Peripheral blood				Cytochemistry				
Patient	Age/sex	FAB	Hb	WBC	% Blast	Plt	MPO	PAS	NSE	CE	AP
1	27/F	M1	5·4	4·5	64	19	+	−	−	+	−
2	24/F	M1	7·4	3·7	28	09	+	−	−	+	−
3	59/F	M1	9·8	23·3	30	21	+	−	−	nd	nd
4	16/M	M1	11·6	58·9	96	32	+	−	−	+	−
5	56/M	M1	11·3	172·5	92	105	+	−	+	nd	nd
6	40/F	M2	8·1	7·2	42	30	+	−	−	+	nd
7	38/M	M2	6·9	111·5	30	140	+	−	−	nd	−
8	17/M	M4	6·7	21·0	85	102	+	−	+	+	nd
9	48/M	M5a	6·4	33·4	97	49	−	−	+	−	+

Abbreviations: Hb, haemoglobin (g/dl); WBC, white blood cell ($\times 10^9$/l); Plt, platelet ($\times 10^9$/l); MPO, myeloperoxidase; PAS, periodic acid Schiff; NSE, non-specific esterase; CE, chloroacetate esterase; AP, acid phosphatase; nd, not determined.

lymphoid (CD10, CD19 or CD20) nor mature T lymphoid (CD3, CD4 or CD8) antigens were detected in any of the nine cases. With respect to CD7$^-$ AML, 33 cases were diagnosed as CD7$^-$/CD34$^+$ AML, and 46 cases were diagnosed at CD7$^-$/CD34$^-$ AML.

Molecular analysis

Neither clonally rearranged bands of the TcR-β, the TcR-γ nor the IgH gene were observed except for case 8 in which the clonal rearrangement of the IgH was detected (Table III).

Proliferation of leukaemic blasts

Table IV shows the results of blast colony formation in the presence of IL-3, GM-CSF or G-CSF in 42 AML cases examined. Blast colonies were formed in 8/9 CD7$^+$ AML cases, in 15/18 CD7$^-$/CD34$^+$ AML cases and in 18/28 CD7$^-$/CD34$^-$ AML cases examined. In 6/8 CD7$^+$ AML cases (75%) which formed blast colonies, IL-3 showed the strongest stimulatory effect on blast colony formation compared to GM-CSF and G-CSF (cases 1, 2, 3, 4, 5 and 8) ($P<0{\cdot}05$). In case 9, IL-3 showed a significantly stronger stimulatory effect than G-CSF, but the effect was almost the same as that of GM-CSF. On the other hand, the strongest stimulatory effect of IL-3 on blast colony formation was observed in only 3/15 CD7$^-$/CD34$^+$ AML cases (20%) (cases 18, 21 and 22) and in only 3/18 CD7$^-$/CD34$^-$ AML cases (18%) (cases 47, 57 and 58). Table V shows the effects of growth factors on DNA synthesis. Interestingly, IL-3 showed a vigorous stimulatory effect on DNA synthesis (more than three by stimulation index) compared to the effects of GM-CSF and G-CSF in 8/9 CD7$^+$ AML cases (nos. 1, 2, 3, 4, 5, 6, 7 and 8) ($P<0{\cdot}05$). In case 9, IL-3 and GM-CSF showed almost similar stimulatory effects on DNA synthesis, which were significantly stronger than the effect of G-CSF ($P<0{\cdot}05$) (Table V). Fig 1(a) shows the

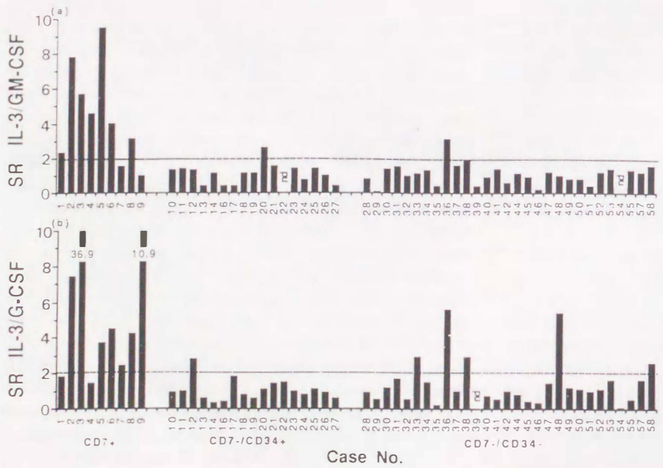

Fig 1. Effects of growth factors on DNA synthesis of leukaemic blasts determined by a ^{3}H-TdR incorporation assay. Results are shown as the stimulation ratio of IL-3/GM-CSF (SR IL-3/GM-CSF: mean cpm in triplicate culture in the presence of IL-3/GM-CSF) (a), and the stimulation ratio of IL-3/G-CSF (SR IL-3/G-CSF: mean cpm in the presence of IL-3/G-CSF) (b). Cases 1–9 are CD7$^+$ AML, cases 10–27 are CD7$^-$/CD34$^+$ AML and cases 28–58 are CD7$^-$/CD34$^-$ AML.

Table III. Results of immunophenotypic and molecular analysis of CD7$^+$ AML.

Patient	% Blast	CD2	CD3	CD4	CD5	CD7	CD8	CD10	CD13	CD14	CD19	CD20	CD33	CD34	HLA-DR	TdT	JH	Tβ	Tγ
1	96	77	4	2	12	79	3	3	5	4	2	3	70	83	80	−	GL	GL	GL
2	98	96	0	0	nd	94	0	1	1	5	0	0	2	95	95	−	GL	GL	GL
3	92	0	6	4	nd	65	1	nd	63	nd	0	nd	23	85	84	−	GL	GL	GL
4	99	51	12	3	5	69	4	2	27	7	5	2	60	56	55	−	GL	GL	GL
5	97	3	2	6	2	86	2	0	80	12	3	3	93	68	95	−	GL	GL	GL
6	98	1	1	1	nd	40	1	0	2	2	0	0	62	49	30	+	GL	GL	GL
7	88	6	5	3	4	27	3	0	3	6	0	nd	36	22	25	−	GL	GL	GL
8	96	9	7	5	7	52	5	3	16	11	5	3	27	75	91	−	GL	GL	GL
9	98	1	0	0	2	23	1	7	2	6	1	1	91	14	58	−	RA	GL	GL

Results are shown as positive % of cells.
Abbreviations: JH, immunoglobulin heavy chain gene; Tβ, T-cell receptor β chain gene; Tγ, T-cell receptor γ chain gene; GL, germline; RA, rearrangement; nd, not determined.

ratios of IL-3 to GM-CSF in their stimulatory abilities on DNA synthesis, which we herein refer to as 'stimulation ratio (SR) of IL-3/GM-CSF' (SR IL-3/GM-CSF: mean cpm in the presence of IL-3/mean cpm in the presence of GM-CSF). Fig 1(b) shows the 'stimulation ratio of IL-3/G-CSF' (SR IL-3/G-CSF: mean cpm in the presence of IL-3/mean cpm in the presence of G-CSF) of all the AML cases examined. In the CD7$^+$ AML cases, 7/9 cases (78%) showed SR IL-3/GM-CSF >2 (cases 1, 2, 3, 4, 5, 6 and 8), and 7/9 cases (78%) showed SR IL-3/G-CSF >2 (cases 2, 3, 5, 6, 7, 8 and 9). However, in the CD7$^-$ AML cases, only 2/45 cases (4%) showed SR IL-3/GM-CSF >2 (cases 20 and 36), and 6/46 cases (13%) showed SR IL-3/G-CSF >2 (cases 12, 33, 36, 38, 48 and 58).

We next investigated the responsiveness of leukaemic blasts to SCF in CD7$^+$ AML. The results are shown in Table VI. SCF alone showed potent stimulatory effects on the *in vitro* growth of leukaemic progenitor cells in 7/8 cases examined. Furthermore, SCF showed more potent effects than IL-3 did (cases 3, 4, 5, 7 and 8), and enhanced blast colony formation synergistically in combination with IL-3 (cases 3, 4, 7 and 9).

DISCUSSION

In the present study, leukaemic blasts from CD7$^+$ AML responded preferentially to IL-3 and SCF in leukaemic blast colony formation and DNA synthesis in comparison with GM-CSF or G-CSF. In particular, six cases (nos. 1, 2, 3, 4, 5 and 8) in which more than 50% of leukaemic blasts were positive for CD7 showed biological characteristics such as those described above without exception. In addition, we purified CD7$^+$ leukaemic blasts in three cases (nos. 5, 8 and 9) with CD7 antibody (Leu9) and antimouse goat IgG-conjugated immunomagnetic beads. Their responsiveness to growth factors in blast colony assay was almost similar to that of unfractionated blast populations, indicating that CD7$^+$ leukaemic progenitors preferentially proliferated in response to IL-3 and SCF (data not shown). According to previous reports, there has been no correlation between response patterns of AML blasts to these growth factors and morphocytochemical characteristics such as the FAB classification (Miyauchi *et al*, 1987; Delwel *et al*, 1988; Lemoli *et al*, 1991). Similarly, in our data, the response patterns of leukaemic blasts from CD7$^+$ AML to growth factors were variable according to individual patients. Consequently, it is suggested that CD7$^+$ AML may be a biologically distinct subgroup of AML.

Previous reports indicated that IL-3 was more potent in its stimulatory effect on the *in vitro* growth of mixed colonies than GM-CSF or G-CSF (Messner *et al*, 1987), and that IL-3 showed higher replating efficiency of blast cell colonies than GM-CSF did (Leary *et al*, 1987). Recently, it has been reported that SCF supported the colony formation of CD34-positive primitive haematopoietic stem cells, which were negative for lineage-associated antigens (Bernstein *et al*, 1991). Therefore the potent responses to IL-3 and/or SCF preferentially observed in CD7$^+$ AML suggests the maturation arrest of leukaemic blasts at an early stage of haematopoietic differentiation process.

Lo Coco *et al* (1989) and Tien *et al* (1990) speculated that

Table IV. Effects of IL-3, GM-CSF and G-CSF on CD7$^+$ and CD7$^-$ AML blast colony formation.

				L-CFU/5 × 10^4 cells*			
Patient	FAB	CD7$^+$	CD34$^+$	Control	IL-3	GM-CSF	G-CSF
1	M1	++	++	8±3	186±25[a]	24±2	38±13
2	M1	++	++	16±2	184±24[a]	132±21	39±1
3	M1	++	++	0	896±56[a]	128±19	0
4	M1	++	++	25±5	109±7[a]	28±9	30±12
5	M1	++	++	50±10	187±16[a]	70±5	110±13
7	M2	+	+	0	15±4	18±6	147±20
8	M4	++	++	0	489±36[a]	221±28	12±4
9	M5a	+	−	0	95±4	83±6	3±2
10	M1	−	+	0	8±2	22±6	673±45
11	M1	−	++	47±6	138±6	78±5	163±10
12	M1	−	++	0	51±6	38±6	50±4
13	M1	−	+	3±1	54±2	88±4	26±4
14	M1	−	++	2±3	132±13	115±10	645±28
15	M1	−	++	0	0	0	237±24
16	M2	−	++	0	0	26±2	143±9
17	M2	−	++	0	2±1	0	141±23
18	M2	−	++	25±4	122±6[a]	47±10	88±6
19	M2	−	++	0	0	0	5±1
20	M2	−	++	0	5±1	0	35±5
21	M4	−	+	22±2	270±26[a]	150±11	172±13
22	M4	−	+	0	88±8[a]	40±6	28±3
24	M4E	−	++	25±2	70±13	78±6	90±6
25	M4E	−	++	0	5±1	0	10±2
29	M1	−	−	0	0	298±29	16±4
33	M2	−	−	0	0	21±6	3±1
34	M2	−	−	7±2	37±2	43±2	47±6
35	M2	−	−	0	4±1	41±8	78±5
36	M2	−	−	0	23±4	17±1	116±5
37	M2	−	−	2±1	61±6	29±8	246±12
40	M3	−	−	2±2	8±2	13±2	18±2
41	M3	−	−	0	135±15	95±11	525±48
42	M3	−	−	0	73±4	60±4	53±8
43	M3	−	−	0	6±1	48±4	125±2
46	M4	−	−	0	0	5±1	3±1
47	M4	−	−	18±2	160±18[a]	92±9	42±2
48	M4	−	−	20±4	48±5	79±10	61±4
49	M4	−	−	16±3	25±2	24±2	25±1
55	M5a	−	−	0	0	0	15±4
56	M6	−	−	5±1	20±4	27±3	25±4
57	M7	−	−	26±4	790±55[a]	625±19	266±32
58	M7	−	−	0	112±10[a]	17±5	32±6

Blast colony assays were not performed in cases 45, 53, 54 and 59–88.

Colonies were not formed in the presence of any of these growth factors in cases 6, 23, 26, 27, 28, 30, 31, 32, 38, 39, 44, 50, 51 and 52.

* Data are shown as the mean ±SD in triplicate culture.

† Positivity of CD7 and CD34 was indicated as follows: −: <20%, +: 20%~50%, ++: >50%.

[a] Significant enhancement of blast colony formation compared to the cultures in the presence of GM-CSF and G-CSF ($P<0.05$).

the CD7 expression on myeloid leukaemic blasts might reflect the cell immaturity: leukaemic transformation would occur in a subset of CD7$^+$ myeloid progenitors because of the high incidence of CD7$^+$ AML in less differentiated subgroup of AML such as M0 or M1, and because of the rare occurrence of clonal rearrangement of the TcR-β or the TcR-γ gene. Similarly, in our study, the incidence of CD7$^+$ AML was also high in M1 and neither the TcR-β gene nor the TcR-γ gene

Table V. Effects of IL-3, GM-CSF and G-CSF on DNA synthesis of CD7$^+$ AML blasts.

	^{3}H-TdR Uptake (cpm) (S.I.)			
Patient	Control	IL-3	GM-CSF	G-CSF
1	12240±489	42385 (3·5)±976[ab]	18368 (1·5)±1035	24137 (2·0)±2629
2	1085±132	15170 (14·0)±697[ab]	1938 (1·8)±113	2045 (1·9)±116
3	2748±232	125759 (45·8)±3350[ab]	22121 (8·0)±752	3407 (1·2)±103
4	6828±912	38285 (5·6)±893[ab]	8339 (1·2)±835	27187 (4·0)±1372
5	9483±135	67706 (7·1)±2293[ab]	7111 (0·7)±288	18410 (1·9)±300
6	3288±488	19666 (6·0)±266[ab]	4968 (1·5)±29	4386 (1·3)±129
7	5850±769	21310 (3·6)±1278[ab]	14242 (2·4)±820	8761 (1·5)±664
8	2221±163	10184 (4·6)±180[ab]	3269 (1·5)±86	2428 (1·1)±168
9	4092±486	68042 (16·6)±392[b]	65566 (16·0)±349	6215 (1·5)±968

* Data are shown as the mean cpm (S.I.)±SD in triplicate culture. Abbreviations: S.I., stimulation index = mean cpm in the presence of growth factors/control cpm.

[a] Significant enhancement of ^{3}H-TdR incorporation of leukaemic blasts compared to the cultures with GM-CSF ($P<0.05$).

[b] Significant enhancement of ^{3}H-TdR incorporation of leukaemic blasts compared to the cultures with G-CSF ($P<0.05$).

was clonally rearranged in any of the CD7$^+$ AML cases examined.

Recently, multipotent progenitors expressing both CD7 and CD34 have been identified, which can give rise to clonal T-cell progenitors as well as myeloerythroid progenitors (Haynes *et al.* 1989; Kurtzberg *et al.* 1989; Chabannon *et al.* 1990). Our immunophenotypic analysis revealed the high incidence of CD34 expression in CD7$^+$ AML and the tendency of high proportion of CD7 positivity associated with high percentages of CD34$^+$ blasts. These data suggest the possibility that the leukaemic transformation may occur at a stage of CD7$^+$/CD34$^+$ or more immature haematopoietic progenitors.

Based on these biological characteristics of CD7$^+$ AML blasts, we deduce that the CD7 expression in AML could be ascribed to the involvement of leukaemic progenitors at an early stage of myeloid or myeloid/lymphoid differentiation rather than to the 'aberrant' expression of leukaemic progenitors due to the leukaemic transformation.

Table VI. Effects of SCF and IL-3 on CD7$^+$ AML blast colony formation.

	No. of blast colonies/5 × 10^4 cells*			
Patient	Control	SCF	IL-3	SCF + IL-3
1	0	199±16[a]	240±15	255±18
3	0	48±4[a]	9±2	102±8[b]
4	0	19±6[a]	0	422±13[b]
5	50±9	270±30[a]	187±16	257±16
7	0	79±5[a]	46±4	173±14[b]
8	4±3	84±11[a]	34±6	95±9
9	2±3	174±20[a]	270±23	709±38[b]

This experiment was independently carried out from those in Tables IV or V, or Fig 1.

Blast colony assay was not performed in case 2, and colonies were not formed in the presence of any of these growth factors in case 6.

* Data are shown as the mean ±SD in triplicate culture.

[a] Significant enhancement of blast colony formation compared to control cultures ($P<0.05$).

[b] Stands for synergistic effects of SCF defined according to the formula described below: (No. of colonies with SCF and IL-3 − No. of control)/{(No. of colonies with SCF alone − No. of control) + (No. of colonies with IL-3 alone − No. of control)} >130%.

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