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Colony Forming Abilities of Bone Marrow Cells in Patients with Hematological Malignancies

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Abstract Colony forming abilities of the bone marrow cells were studied in 36 patients with hematological malignancies. Leukemic cells showed poor colony formations in acute leukemias, except for acute myelomonocytic leukemia (M_4) which showed an increased colony formation. The colony formations of erythroid progenitor cells were preserved in some cases with acute leukemias. The patients with acute leukemias who formed few colonies or small clusters of leukemic cells tended to be induced to remission. In erythroleukemia (M_6) and myelodysplastic syndromes, both myeloid and erythroid colony formations were markedly suppressed, except for chronic myelomonocytic leukemia which showed an increased leukemic colony formation. In chronic myelocytic leukemia, both myeloid and erythroid colonies were subnormal, normal or increased. In polycythemia vera, abundant erythroid colonies were formed.

During remission induction chemotherapy for acute leukemias, normal hematopoiesis remained suppressed until the leukemic blasts decreased below 5% in the bone marrow, thereafter rapid increase of normal hematopoietic progenitors occurred. Granulocyte and macrophage colonies recovered simultaneously. After achieving remission, normal hematopoiesis were preserved as long as the remission was maintained. Decreased colony formations were often preceded the hematological relapse in acute leukemias and the blastic transformation in chronic myelocytic leukemias.

It is concluded that studies on colony forming abilities of the bone marrow cells provide an useful information on the treatment and management of patients with hematological malignancies.

Key Words: Leukemia, L-CFU, CFU-C, BFU-E, CFU-E

Introduction

In the past two decades, in vitro clonal assay systems for the hematopoietic stem cells have been established[1-3]. The committed stem cells for myeloid and macrophage series, i.e., colony forming unit in culture (CFU-C), are frequently examined for the evaluation of normal myeloid hematopoietic ability in hematological malignancies such as acute leukemias, myeloproliferative disorders and myelodysplastic syndromes (MDS). However, the committed erythroid stem cells, i.e., burst forming unit of erythroid (BFU-E) and colony forming unit of erythroid (CFU-E), have rarely been studied in these

Table 1 Pertinent clinical data of 36 patients with hematological malignancies.

CASE	DIAGNOSIS	AGE	SEX	BONE MARROW		THERAPY	OUTCOME
				% blasts	cellularity		
1. H.M.	AML(M_1)	32	M	70.0	Hyper	BH-AC DMP	C.R.
2. N.Y.	AML(M_1)	78	F	97.0	Hyper	none	Death
3. Y.K.	AML(M_1)	35	M	70.0	Hyper	BH-AC DMP	Death
4. Y.D.	AML(M_1)	66	M	86.0	Hyper	low dose Ara-C	Death
5. R.M.	AML(M_1)	15	F	42.6	Hyper	BH-AC DMP	C.R.
6. T.O.	AML(M_2)	48	F	79.5	Hyper	BH-AC DMP	C.R.
7. M.T.	AML(M_2)	27	F	79.5	Hyper	VP, BH-AC DMP	Death
8. M.M.	AML(M_3)	38	M	60.4	Normo	BH-AC DMP	C.R.
9. A.S.	AML(M_3)	60	F	75.4	Hyper	BH-AC AMP	C.R.
10. M.F.	AML(M_4)	62	F	95.0	Hyper	cyclophosphamide	Death
11. U.U.	AML(M_4)	54	F	38.0	Hypo	BH-AC AMP	C.R.
12. T.U.	AML(M_6)	69	F	68.8	Hyper	none	Alive
13. M.K.	AML*	60	M	35.0	Hypo	low dose Ara-C	Alive
14. S.H.	ALL(L_1)**	39	M	58.8	Hyper	VAP	Death
15. M.K.	ALL(L_1)	16	F	91.2	Hyper	VAP	C.R.
16. A.K.	ALL(L_2)	17	M	72.0	Hyper	VAP adriamycin	C.R.
17. K.M.	ALL(L_2)	16	F	94.2	Hyper	VAP	Death
18. Y.H.	ALL(L_2)	30	M	91.2	Hyper	VAP adriamycin	C.R.
19. R.O.	ALL(L_2)**	19	F	97.2	Hyper	BH-AC DMP	Death
20. M.Y.	CML***	33	M	86.8	Hyper	VP	C.P.****
21. H.I.	CML***	34	M	52.8	Hyper	VP	Death
22. K.S.	CML	19	M	–	Hyper	busulfan	Alive
23. K.O.	CML	19	M	–	Hyper	busulfan	Alive
24. N.Y.	CML	37	M	–	Hyper	busulfan	Alive
25. M.N.	CML	64	F	–	Hyper	busulfan	Alive
26. O.N.	PV	64	M	–	Hyper	none	Alive
27. T.O.	ET	87	F	–	Hyper	busulfan	Alive
28. S.H.	RAEB	58	M	17.0	Hypo	low dose Ara-C	C.R.
29. O.W.	RAEB	75	F	18.4	Hyper	none	Alive
30. H.M.	RAEB	29	M	22.6	Hypo	low dose Ara-C	Alive
31. K.Y.	CMML	41	M	32.4	Hyper	low dose Ara-C	Alive
32. M.H.	CMML	53	F	17.3	Hyper	anabolic steroid	Alive
33. K.N.	PARA	65	M	–	Hyper	none	Alive
34. K.K.	PARA	69	M	–	Hyper	none	Alive
35. N.S.	AISA	41	M	–	Hyper	anabolic steroid	Death
36. K.A.	CLL	58	M	51.6	Normo	none	Alive

AML, acute myeloblastic leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myelocytic leukemia; PV, polycythemia vera; ET, essential thrombocythemia; RAEB, refractory anemia with excess of blasts; CMML, chronic myelomonocytic leukemia; PARA, primary acquired refractory anemia; CLL, chronic lymphocytic leukemia
DMP: daunorubicin, 6-mercaptopurine and prednisolone
AMP: aclacinomycin A, 6-mercaptopurine and prednisolone
VP : vincristine and prednisolone
VAP: vincristine, l-asparaginase and prednisolone
* low percentage leukemia
** relapse
*** blastic transformation
**** chronic phase

disorders. Recently, methods for in vitro cloning of leukemic cells have been developed [4-7], and many valuable informations on the pathogenesis, diagnosis, treatments and prognosis in patients with leukemias have been obtained from leukemic colonies (L-CFU). However, there are yet some disagreements about the clonological behavior of leukemic blasts, and the effects of leukemic blasts on normal hematopoiesis are still matters of speculation. Furthermore, colony forming ability in each type of leukemias classified according to French-American-British (FAB) classification [8,9] is not fully elucidated. This investigation was performed in order to know (1) the differences in clonological behavior of leukemic cells among each subtype, (2) the relationship and the interactions between leukemic cells and normal hematopoietic stem cells during the courses of disease, and (3) the state and the degree of normal hematopoietic restoration after antileukemic chemotherapy.

Materials and Methods

Patients (Table 1)

The diagnostic criteria for acute leukemia and MDS by the FAB classification were adopted in this study. Bone marrow cells were obtained from 36 patients suffering from hematological malignancies: acute myeloblastic leukemia (M_1-M_6), acute lymphoblastic leukemia (L_1, L_2), chronic lymphocytic leukemia (CLL), myeloproliferative disorders including chronic myelocytic leukemia (CML), polycythemia vera (PV) and essential thrombocythemia (ET), and MDS including refractory anemia with excess of blasts (RAEB), primary acquired refractory anemia (PARA), acquired idiopathic sideroblastic anemia (AISA) and chronic myelomonocytic leukemia (CMML).

Preparation of marrow cells

After an informed consent was obtained, about 5 ml of bone marrow aspirate was obtained from the sternum or iliac crest into a syringe rinsed with heparin. The marrow aspirate was diluted with the same volume of alpha-medium, and then layered on Ficoll-Hypaque with the density of 1.077. After centrifugation for 20 min. at 400×g, mononuclear cells (MNC) were collected from the interface, and washed twice with alpha-medium. In patients with acute myeloblastic leukemia, T-lymphocytes were removed by rosetting with sheep red blood cells.

Cell culture

All cultures were done in triplicate. CFU-C and L-CFU were cultured by the method of Pike and Robinson[10] with some modifications: mononuclear cells at a concentration of 2×10^5/ml were cultured in 0.3% soft agar monolayer containing 20% fetal calf serum (FCS), 10% human placental conditioned medium (HPCM) or leukocyte conditioned medium stimulated by phytohemagglutinin (PHA-LCM). Cultures were performed at 37°C in a humidified atmosphere of 5% CO_2 for 7 days. Cell aggregates consisting of 40 or more cells were counted as colony, and those consisting of less than 40 cells were counted as cluster. The macrophage colony and granulocyte colony in CFU-C were distinguished by double staining of esterase using alpha-naphthyl butyrate and naphtol AS-D chloracetate as substrates as reported by Yam et al[11].

CFU-E was cultured by the method of Terasawa et al[12] with some modifications: mononuclear cells at a concentration of 2×10^5/ml were cultured for 7 days in plasma clot containing 20% FCS, 10% bovine serum albumin (BSA) and 2 U/ml erythropoietin (Epo). Colonies were evaluated after fixation and staining with benzidine and hematoxylin. Cell aggregates consisting of 8 or more cells with positive benzidine reaction were counted as CFU-E colony.

BFU-E was cultured as described by Iscove et al[13]: mononuclear cells at a concentration of 2×10^5/ml were cultured for 14 days in 0.8% methylcellulose containing 30% FCS, 10% BSA and 2 U/ml Epo. Cell aggregates consisting of 100 or more cells, or of 3 or more subclusters were counted as BFU-E colony.

Results

Colony forming ability at the time of diagnosis (Table 2)

1) *Leukemic colonies stimulated by HPCM (Fig. 1a)*

In acute leukemias, either myeloblastic or

Table 2 Colony forming abilities at the time of diagnosis

CASE	DIAGNOSIS	L-CFU (Cluster)		CFU-E	BFU-E
		HPCM	PHA-LCM		
1. H.M.	AML(M_1)	2.0± 0.8(0)	0 (0)	205.5±30.9	40.7± 2.5
2. N.Y.	AML(M_1)	0 (0)	3.3± 3.2(7.3±10.1)	10.3± 1.2	0
3. Y.K.	AML(M_1)	6.3± 2.5(ND)	ND	0	0
4. Y.D.	AML(M_1)	0 (0)	ND	0	0
5. R.M.	AML(M_1)	0 (0)	20.0±15.7(72.7±25.7)	0	0
6. T.O.	AML(M_2)	0 (0)	0 (0)	0	0
7. M.T.	AML(M_2)	0 (0)	ND	0	0
8. M.M.	AML(M_3)	4.0± 1.4(ND)	0 (0)	0	6.0± 0
9. A.S.	AML(M_3)	8.0± 1.0(ND)	ND	ND	ND
10. M.F.	AML(M_4)	101.0±12.0(ND)	ND	ND	ND
11. U.U.	AML(M_4)	0 (0)	ND	6.3± 4.6	2.5± 3.5
12. T.U.	AML(M_6)	2.0± 1.0(ND)	ND	0	0
13. M.K.	AML	1.3± 1.5(ND)	1.0± 1.7(ND)	0	0
14. S.H.	ALL(L_1)	3.5± 5.0(7.5±3.5)	48.0± 2.0(33.7±15.5)	0	0
15. M.K.	ALL(L_1)	0 (0)	0 (0)	0	0.5± 1.0
16. A.K.	ALL(L_2)	9.3± 6.7(ND)	0.7± 0.6(5.7± 1.5)	140.3±17.4	6.3± 1.5
17. K.M.	ALL(L_2)	0 (0)	27.0±26.9(44.0±26.3)	0	0
18. Y.H.	ALL(L_2)	1.0± 0.0(ND)	4.3± 0.6(ND)	2.5± 2.1	7.5± 2.1
19. R.O.	ALL(L_2)	0 (0)	ND	9.0± 1.7	2.0± 1.0
20. M.Y.	CML	6.0± 2.0	4.3± 1.5	28.0± 0.0	55.0±18.4
21. H.I.	CML	308.3±19.0	ND	154.3±20.4	21.7± 3.5
22. K.S.	CML	26.0± 6.5	ND	38.0± 5.0	24.0± 0.0
23. K.O.	CML	118.0±11.0	ND	0	14.9± 6.9
24. N.Y.	CML	32.3± 6.4	5.0± 1.0	4.3± 0.6	0
25. M.N.	CML	7.3± 2.1	0	ND	ND
26. O.N.	PV	12.7± 2.5	ND	374.5±12.0	68.5±10.3
27. T.O.	ET	0	18.0± 7.9	ND	ND
28. S.H.	RAEB	7.0± 1.8	3.0± 1.0	4.8± 2.8	6.0± 2.6
29. O.W.	RAEB	2.3± 0.6	1.0± 1.4	3.5± 2.1	0
30. H.M.	RAEB	1.7± 0.6	ND	0	0
31. K.Y.	CMML	7.7± 1.2	48.3± 4.0	31.7± 3.5	0
32. M.H.	CMML	26.8± 5.0	41.3±25.8	3.3± 2.5	0
33. K.N.	PARA	3.0± 1.7	ND	0	0
34. K.K.	PARA	3.3± 2.4	2.7± 2.1	1.7± 0.6	0
35. N.S.	AISA	18.0± 4.4	ND	19.5±10.5	0
36. K.A.	CLL	11.7± 2.3	75.3± 3.5	0.3± 0.6	0
Normal Range		121.0±58.0		155.0±61.0	54.0±21.0

lymphoblastic, the numbers of colonies and clusters were all markedly reduced, except in a case of myelomonocytic type (M_4) in which an increased number of colonies was formed. In erythroleukemia (M_6), myeloid colony formation was also reduced. In CML, the numbers of colonies formed were decreased, normal, or increased during chronic phase. In MDS, subnormal number of colonies was formed in a patient with CMML, and the patients with the other types showed very poor colony forming ability.

2) *Leukemic colonies stimulated by PHA-LCM* (Fig. 1a)

Good colony and cluster forming abilities were observed in the cases with M_1 (Case

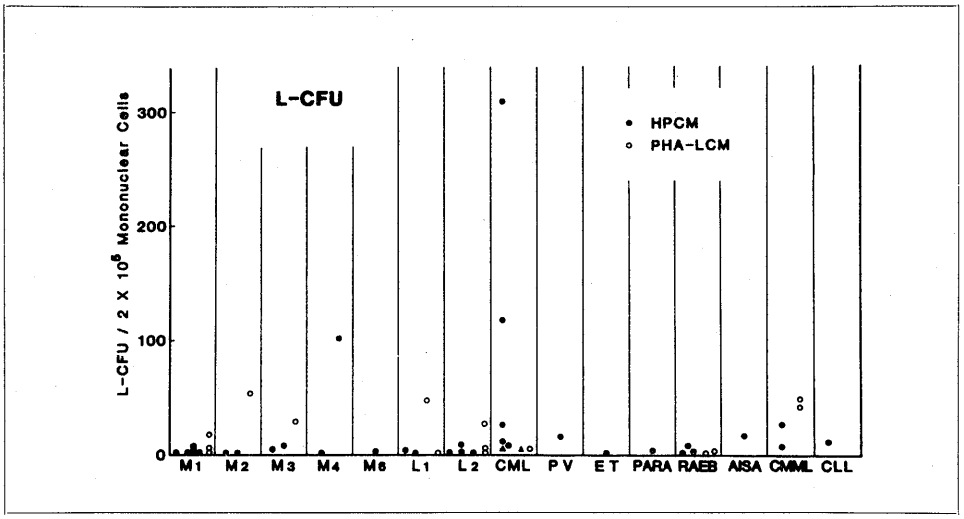

Fig. 1 a

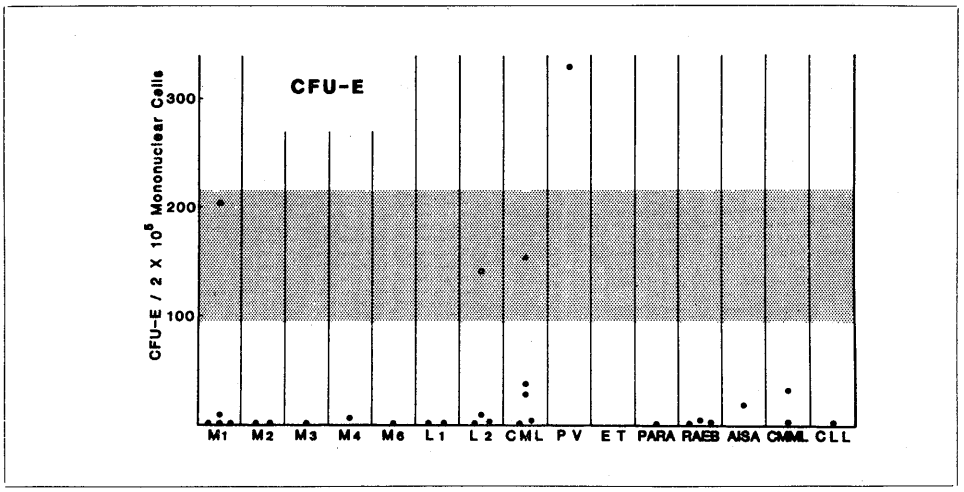

Fig. 1 b

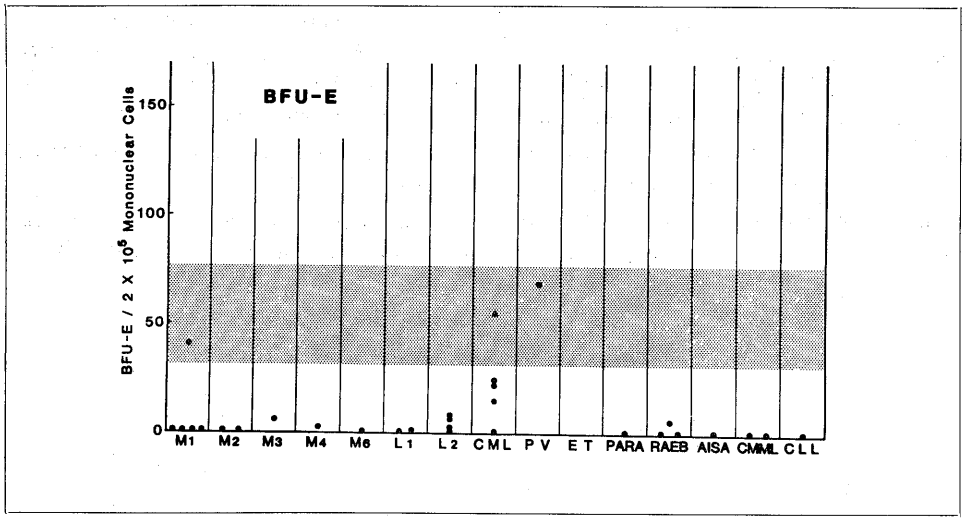

Fig.1 c

Fig. 1a–1c: The numebrs of L-CFU(1a), CFU-E(1b) and BFU-E (1c) in various types of leukemias. The normal range is shown by the shaded area.

No. 5), M_2 (Case No. 6), M_3 (Case No. 8) and L_2 (Case No. 17). CMML (Case No. 31 and 32) also showed good colony formations. However, the numbers of colonies or clusters in the other patients were decreased.

3) *CFU-E and BFU-E* (Figs. 1b and 1c)

In acute leukemias, erythroid colony forming abilities were preserved in some patients especially in cases of H.M. (Case No. 1) and A.K. (Case No. 16) who revealed normal colony formations, in contrast with the poor CFU-C colony formations. In erythroleukemia, the number of colonies was markedly decreased. MDS including RAEB, AISA and CMML also had reduced colony forming abilities. CML in chronic phase showed mild colony forming abilities. Poor colony formation was noted in CLL. In contrast, abundant colonies were formed in a patient with PV.

The relationship between the percentage of the marrow leukemic blasts and normal colony forming ability (Figs. 2a–2c)

Ten patients with acute leukemia who achieved complete remission (CR) were studied serially during the courses of remission induction chemotherapy. The relationship between colony formation (CFU-C, CFU-E and BFU-E) and the percentages of leukemic blasts in the bone marrow are shown in Figs. 2a–2c. As to CFU-C, colony formation was markedly suppressed when the leukemic blasts exceeded 50%. When the leukemic blasts were 10–50%, three of 11 marrow specimens formed normal or subnormal numbers of CFU-C. When the blasts were decreased less than 5%, most patients achieved normal or supranormal growth of CFU-C, although four of the 16 marrow specimens had still suppressed colony formation.

Such invert relationship was not noted in erythroid colony formations (Figs. 2b and 2c). Supranormal, normal or subnormal colony forming abilities were observed during the course of remission induction chemotherapy. Thus the erythroid colony forming abilities remained intact in some cases.

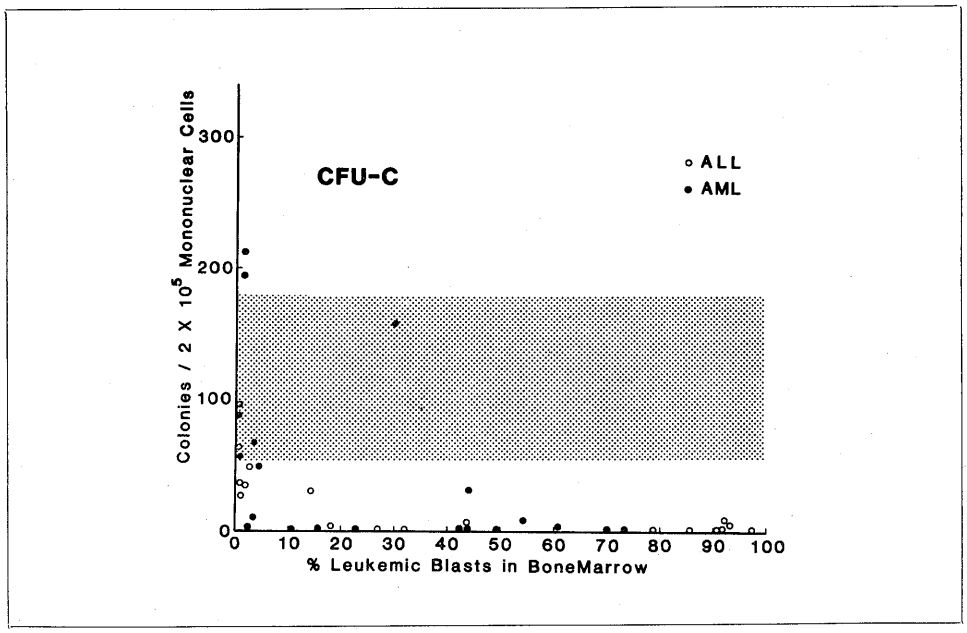
CFU-C
o ALL
• AML
Colonies / 2 X 10⁵ Mononuclear Cells
300
200
100
0
0 10 20 30 40 50 60 70 80 90 100
% Leukemic Blasts in BoneMarrow

Fig. 2a

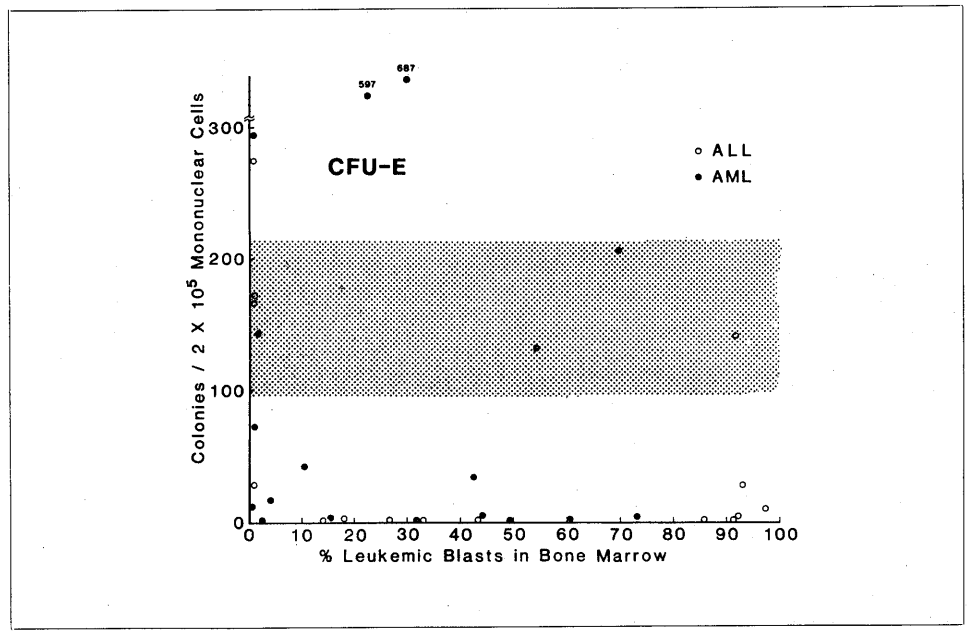
687
597
CFU-E
o ALL
• AML
Colonies / 2 X 10⁵ Mononuclear Cells
300
200
100
0
0 10 20 30 40 50 60 70 80 90 100
% Leukemic Blasts in Bone Marrow

Fig. 2b

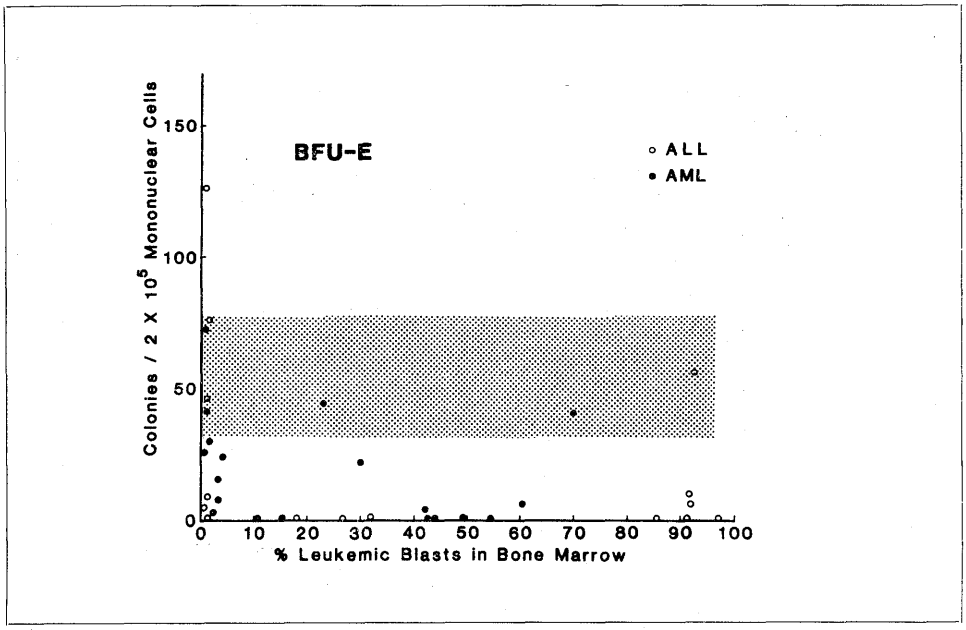

Fig. 2c

Fig. 2a–2c The relationship between the percentage of the marrow leukemic blasts and normal colony forming ability. The normal range is shown by the shaded area.

Colony formations after CR (Fig. 3)

Twenty-four patients with acute leukemia were studied with this respect. After achieving CR, most of the patients showed a prompt recovery of colony formations of normal marrow progenitors. Erythroid colony formation, however, still remained subnormal in contrast with myeloid colony formation.

Granulocyte and macrophage colony subpopulations during the courses of normal hematopoietic recovery after remission induction therapy

These were serially evaluated in 5 patients with acute leukemia. When stimulated by HPCM, almost all of the colonies were consisted of granulocytic series throughout the course. On the other hand, when stimulated by PHA-LCM, about 50% of colonies were always occupied by macrophage colonies throughout the course.

A typical pattern of disappearance of leukemic blasts and appearance of normal hematopoietic progenitor cells during the remission induction chemotherapy (Fig. 4)

In a case of L_2(Case No. 16), leukemic blasts occupied 92% of bone marrow cells, and numbers of CFU-C, CFU-E and BFU-E were 9, 0, and $6/2\times10^5$ MNC, respectively, at the time of diagnosis. The leukemic blasts rapidly decreased both in the bone marrow and in peripheral blood by chemotherapy. On day 9, the blasts still occupied 17%, and the numbers of CFU-C, CFU-E and BFU-E were 4, 0, and $3/2\times10^5$ MNC, respectively. Nineteen days after the chemotherapy, the blasts in bone marrow had decreased to 0.3 %, and the numbers of CFU-C, CFU-E and BFU-E recovered to 96, 172, and $74/2\times10^5$ MNC, respectively.

Decreased colony formation before relapse

In this study, decreased colony formations

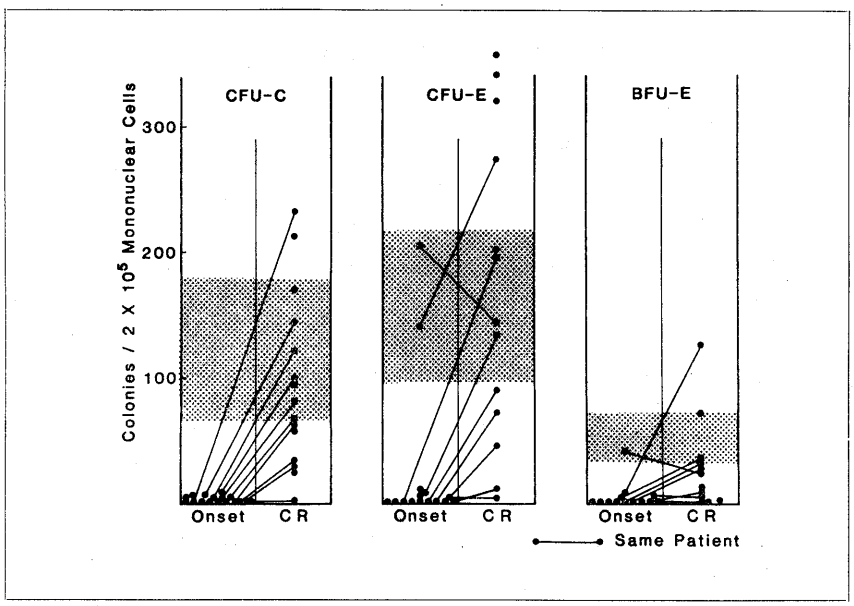

Fig. 3 Colony formations before treatment and after complete remission.

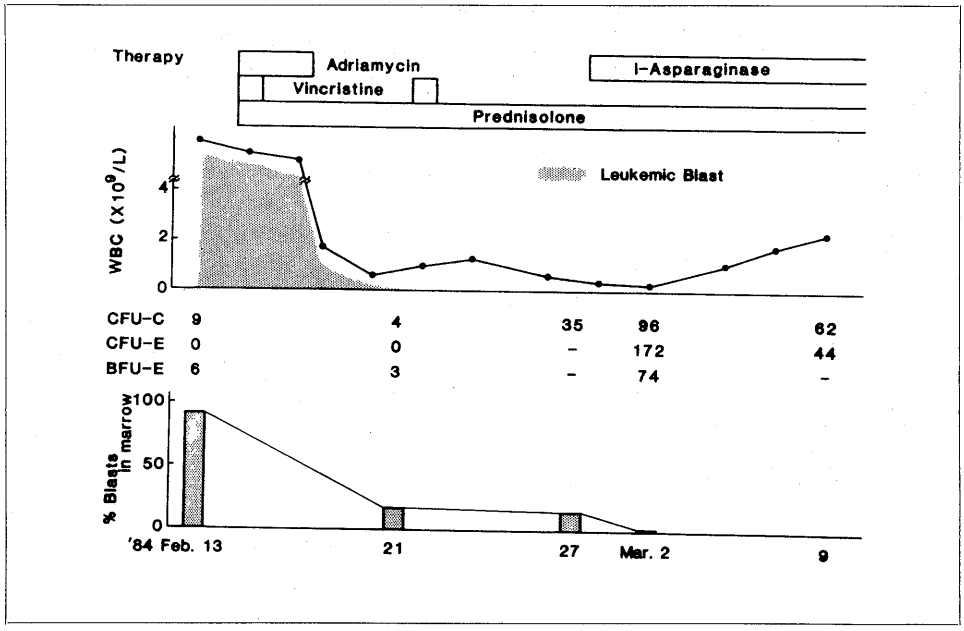

Fig. 4 Time-course studies of CFU-C, CFU-E and BFU-E in a case with L_2 (Case No. 16) who was induced to remission.

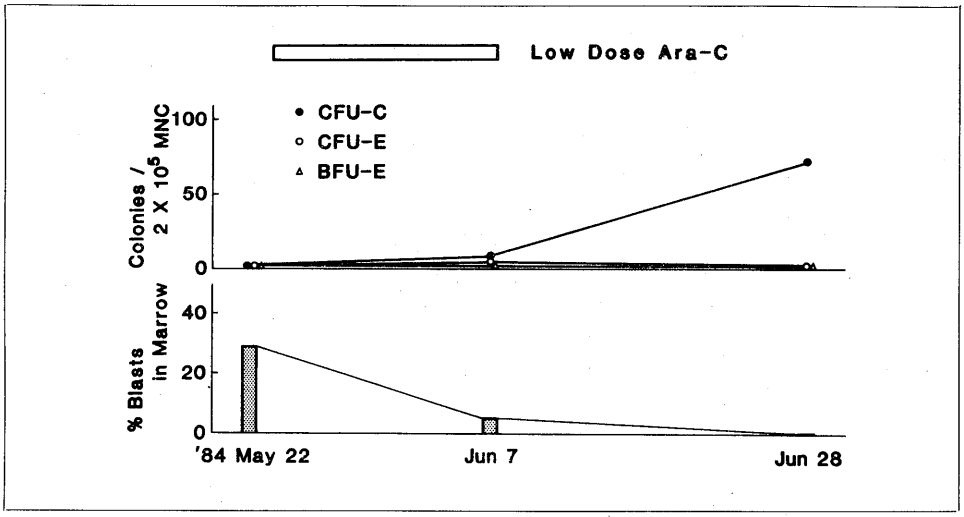

Fig. 5 Change in colony formation in a case with RAEB (Case No. 28) successfully treated with low dose Ara-C.

before hematological relapse were observed in two cases. In a case with L_2 (Case No. 19), subnormal CFU-C colony formation and normal CFU-E and BFU-E colony formations were observed when hematological relapse was apparent with 26% of blasts in the bone marrow. In a case with CML (Case No. 20), the number of CFU-C markedly decreased about 40 days prior to the blastic transformation. Erythroid series, however, were relatively preserved even after the blastic transformation.

Effect of low dose Ara-C on colony formation in MDS

Two cases with RAEB were serially studied. In S.H. (Case No. 28), 14 days after the initiation of administration of low dose of Ara-C, the number of blasts in the bone marrow was decreased, and the number of CFU-C was increased up to 72. However, erythroid series showed no recovery (Fig. 5). On the contrary, in H.M. (Case No. 30), low dose Ara-C therapy was not effective and the numbers of both leukemic blasts and colonies formed remained unchanged.

Discussion

Leukemic colony formations

It has been proved that acute leukemias were clonal disorders involving myeloid or lymphoid series but not erythroid series except for some elderly patients with AML who are often preceded by preleukemic syndrome[14-16]. These leukemic blasts form leukemic colonies in the presence of appropriate colony stimulating factor (CSF). Up to the present, many CSFs have been obtained from various sources such as placenta, leukocytes, lung and urine. PHA-LCM, which stimulates macrophage eosinophil, T-lymphocyte, erythroid and megakaryocytic colonies[17-19], is usually used as CSF for L-CFU in AML after T-lymphocytes depletion. Clonologic assay system for ALL is still incomplete comparing that for AML, and several methods using PHA-LCM[20,21] or without CSF[7] have been reported. On the other hand, HPCM, which is a stimulator for normal granulocyte colony[22], also has a leukemic colony stimulating activity. The colonies formed in leukemic patients at the

time of diagnosis were thought to be mostly leukemic colonies since almost all cells cultured were leukemic blasts and, in some cases, the colony forming cells were certified as leukemic origin by cytogenetic marker or by ultrastructural study[23].

In this study, the plating efficiencies of leukemic colony in acute leukemia when stimulated by HPCM were markedly low, but those of colony and cluster stimulated by PHA-LCM were slightly enhanced in some cases. An exception was a case of myelomonocytic type (M_4) which formed many colonies. The blasts of monocytic origin were reported to have an increased proliferative activity[24].

The low plating efficiency in acute leukemias might be due to the original nature of leukemic blasts, or alternatively it suggests that leukemic blasts cannot grow easily in conventional assay systems. Recently, more devised cloning methods for L-CFU have been reported[7,20,21,25]. Leukemic and erythroid colony formations were both suppressed in a case with erythroleukemia (M_6). This result also suggests that erythroleukemia may be a clonal disorder originated from pluripotential stem cells.

The relations between leukemic colony and/or cluster formation and prognosis in acute leukemias have been investigated but still remain inconclusive. Although Mertelsmann et al[26] reported poor prognosis in leukemic patients with no colony formation, Spitzer et al[27] reported good prognosis with no colonies or many small clusters. Furthermore, Gustavsson et al[29] have reported that many colony and cluster formation predict good prognosis. In this study, similar result as that of Spitzer et al was observed in AMLs although only 5 patients were evaluated. Four patients who showed no colony and cluster formations could attain remission, while a patient who showed scattered colony and cluster formations failed to attain remission. In ALL, however, L-CFUs showed no relations with prognoses. It was suggested that colonies in ALL stimulated by PHA-LCM had contained some T-cell colonies because T-cells had not been removed from the ALL marrows before culture.

Myeloproliferative disorders including CML, PV and ET are proved to be the clonal disorders at the level of pluripotential stem cells[29-31]. The numbers of CFU-C in CML were reported to be increased[32,33], decreased[34,35], or either increased or decreased[36-38], and those of CFU-E were normal[39]. In PV, the erythroid progenitor cells are known to be hypersensitive to erythropoietin and form abundant erythroid colonies[40]. In ET, increased, norma lor decreased CFU-C colony formation were reported[36]. The results of this investigation on myeloproliferative disorders were almost the same as those of previous reports, that is, either increased or decreased CFU-C, CFU-E and BFU-E colony formations in CMLs; increased erythroid colony formations in PV; and decreased CFU-C colony formation in ET.

In general, colony formations of myeloid and erythroid progenitor cells were suppressed in MDS[41-44] except that either increased or decreased myeloid colony formations in CMML have been observed[24]. The present cases with MDS also showed increased myeloid colony formations in CMMLs and decreased in other types. Reduced colony formations in both myeloid and erythroid progenitors suggest that MDS originate at the level of pluripotential stem cells. In a successfully treated case (Case No. 28), the disappearance of blasts and reappearance of normal hematopoiesis were noted.

Normal hematopoiesis in acute leukemia

It is well known that normal hematopoiesis in leukemic patients is greatly suppressed[41,45-47]. The present studies are in agreement with these results, even if the small numbers of L-CFU stimulated by HPCM were counted as normal CFU-C. These poor colony formations were gradually recovered somewhat inversely with the reduced per-

centage of blasts in the marrow following chemotherapy. Normal hematopoiesis returned when the numbers of blasts were reduced less than 5%. Similar results that normal hematopoiesis had recovered after the leukemic blasts had decreased less than 10-20% had been reported[46,47]. These suggest that the effect of leukemic blasts on normal hematopoiesis is not simply due the replacement of normal hematopoietic cells by leukemic blasts. As a possible mechanism, Chiyoda et al reported the suppressive effects of leukemic blasts or their extracts on normal CFU-C[48,49].

The recovery of monocyte usually precedes that of granulocyte in the peripheral blood during chemotherapy for acute leukemia. This phenomenon is thought to be due to the production of CSF by monocyte and/or macrophage[50]. During the remission induction chemotherapy, it seems that macrophages regenerate first, then followed by granulocyte recovery in response to the stimulation by CSF. However, in this study, the recovery of the macrophage precursors and that of granulocyte occurred simultaneously.

As was reported previously[46,47,51], prompt recoveries of granulopoiesis and erythropoiesis were observed in almost all patients after attaining CR, and normal hematopoiesis were maintained during remission state.

Clinically, it is very important to detect the relapse of leukemia as early as possible and to start the re-induction chemotherapy. From this point of view, colony forming ability was examined during hematological remission. The early relapse of acute leukemia (Case No. 19) and blastic transformation of CML (Case No. 21) were highly suspected from the results of reduced colony formations. It is suggested that decreased colony forming ability in patients with hematological remission is one of predictive signs of forthcoming relapse of leukemias.

It is interesting that erythroid colony formations were preserved in a certain number of cases before and during the chemotherapy in contrast with the uniformly suppressed myeloid colony formations. Similar tendency was reported by Eridani et al[52]. These findings suggested that leukemic blasts had no toxic effects on either erythroid series or pluripotent stem cells in some instances. These patients tended to have good remission rates.

From the present studies, it is concluded that in vitro assay of hematopoietic stem cells provides much valuable information about (1) pathogenesis and pathophysiology of leukemias from the standpoint of hematopoiesis, (2) effects of leukemic blasts on the normal hematopoiesis, (3) dose and/or duration of chemotherapy to gain CR, and (4) the patients with poor risk to gain CR or with early relapse of leukemia.

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