GROWTH OF FACTOR-DEPENDENT
HEMOPOIETIC PRECURSOR CELL LINES*

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Hemopoiesis is regulated by the production of cell lineage-specific factors influencing stem-cell proliferation (1–3) and a variety of molecules controlling the production of granulocytes, macrophages, erythroid cells, megakaryocytes, and lymphocytes from their respective progenitor cells (4). Although the self-renewal potential of stem cells (spleen colony-forming cells [CFU-S])[1] has been amply documented in vivo (5, 6) and in vitro (7, 8), there is little indication that the committed myeloid progenitor cells such as granulocyte/macrophage (GM) colony-forming cells (CFC) or erythroid blast-forming units (BFU-E) are self-renewing populations. In fact, recloning analysis of in vitro progeny generated by GM-CFC and BFU-E, suggest that these progenitor cells are capable of generating only terminally differentiated progeny when stimulated to proliferate by their specific regulators (9).

Because of the transient nature of these progenitor cells (i.e., the inability to detect significant self-renewal potential) more emphasis has been placed upon the stem cells, rather than the committed progenitor cells, as probable targets in leukemogenesis. Recently, Greenberger et al. (10, 11) have reported that infection of long-term murine marrow cultures with certain murine type-C retroviruses will induce promyelocytic leukemia cell lines, provided that the cells are maintained in a conditioned medium obtained from WEHI-3 myelomonocyte leukemia cells. The ability to sustain the growth of these promyelocyte leukemia cells was ascribed to the presence of GM colony-stimulating factors (CSF) in the WEHI-3 conditioned medium (CM) (11). Whether these cell lines represent virus infection and subsequent transformation of a pluripotent stem cell or of a committed progenitor cell has not been determined. In attempting to confirm this work we have found that virus infection is not necessary for establishing the cell lines and, furthermore, that the cells appear to represent a population of committed granulocyte progenitor cells capable of extensive self-renewal.

Materials and Methods

CSF and CM. The WEHI-3 myelomonocytic leukemia cell line was obtained from Dr. M. A. S. Moore (Sloan-Kettering Institute, New York) and was maintained in McCoy's medium supplemented with 10% fetal calf serum (Grand Island Biological Co., Grand Island, N.Y.). The CM used in these experiments was pooled from WEHI-3 cells grown for 7 d in this medium, filtered through a 0.22-μm filter, and stored at $-20°C$. No loss of activity has occurred over a 12-mo period. CM obtained from pokeweed mitogen-stimulated mouse spleen cells (PWMSCM) (13, 14) and a highly purified preparation of GM-CSF from mouse lung cells (15) were gifts from Dr. A. W. Burgess (Cancer Research Unit, Melbourne, Australia). L-cell CM and a purified macrophage-type CSF (M-CSF) derived from this medium (16) were obtained from Dr. R. K. Shadduck (University of Pittsburgh, Pittsburgh, Pa.). Mouse heart CM that contained GM-CSF was prepared as described (12). In all experiments to be reported, these various stimulating activities were used at a dose that stimulated plateau numbers of GM-CFC in the soft-agar systems. For all activities, numbers of GM-CFC stimulated fell within the range of 80-150 GM-CFC/10^5 marrow cell plated.

Long-Term Marrow Cultures. Long-term marrow cultures were established as previously (7) by flushing the contents of a single femur into 25-cm^2 culture flasks (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) that contained Fischer's medium supplemented with 20% horse serum and maintained at 33°C in an atmosphere of air plus 5% CO_2. In some cultures, hydrocortisone sodium succinate was added to give a final concentration of 10^{-6} M— based upon the finding (17) confirmed by us, that hydrocortisone facilitates the maintenance of in vitro hemopoiesis. Several mouse strains were used, including Swiss, C57BL/6, DBA/2, and B6D2F$_1$ (C57BL/6 × DBA/2)F$_1$. Marrow donors were between 8 and 20 wk of age. The cultures were fed at weekly intervals by removal of one-half (5 ml) of the growth medium and addition of an equal volume of fresh medium (7). After 3 wk, the cultures were recharged with a further inoculation of 10^7 syngeneic marrow cells and fed as before by weekly demidepopulation. In cultures supplemented with hydrocortisone, this recharge was not necessary for the maintenance of hemopoiesis. In all cultures, stem cell (CFU-S) proliferation and production of committed progenitor cells (GM-CFC, BFU-E, and megakaryocyte CFC) occurred for periods up to 15-30 wk, as previously described (7, 18).

At various time intervals after initiation of the cultures, cells present in the growth medium of at least six cultures (i.e., removed during feeding) were centrifuged (800 *g* for 10 min), and the cell pellet was resuspended in a small volume of Fischer's medium. Aliquots of this cell suspension were then plated in 10 ml of growth medium alone, in growth medium supplemented with 10% WEHI-3 CM, or in growth medium supplemented with 15% mouse heart CM. Occasionally, PWMSCM, lung GM-CSF, or lung-conditioned or L-cell CSF medium was used. The cell concentration was adjusted to 10^5 cells/ml, and the cultures were maintained either at 33° or 37°C in an atmosphere of air plus 5% CO_2. Cultures were examined twice weekly for cell growth, and were subcultured as required (by transfer of the nonattaching cells) to maintain the cell concentration at 10^5 cells/ml.

Agar Colony Assays. Cells were assayed for their ability to proliferate in soft agar by plating between 10^3 and 10^5 cells as 1-ml cultures (growth medium supplemented with agar to a final concentration of 0.3%) in 35-mm dishes. All assays were performed in triplicate. Various GM-CSF- and M-CSF-containing media (above) were used as stimulating factors. Plates were scored after 7 d of incubation at 37°C.

For recloning analysis, individual colonies were isolated with a Pasteur pipette and resuspended (by gentle aspiration) in 3 ml of growth medium to give a single-cell suspension. Duplicate 1-ml soft-agar cultures were then established as before. The plates were scored after 7 d, and the number of CFC in the original colony was then calculated. On some occasions, individual colonies were resuspended in 1 ml of growth medium that contained 10% WEHI-3 CM and incubated in Sterilin multiwell culture dishes (Sterling Ltd., Teddington, England). When growth was observed, the cells were further diluted.

Virus Assays. Infectivity assays for virus were performed as previously described (19, 20). Falcon 60-mm Petri dishes (Falcon Labware, Div. of Becton, Dickinson & Co.) were seeded with 1 × 10^5 SC-1 cells or 1 × 10^5 mink lung cells in 3.0 ml of Dulbecco's media that contained 10% fetal calf serum and 8 μg/ml polybrene. The next day, the supernates from the myeloid

cell lines were filtered through a 0.45-μm millipore filter and were inoculated at varying dilutions onto the cells: undiluted, and 10-fold dilutions through a 1,000-fold dilution. After 1 wk, the SC-1 cells were assayed for XC plaques as previously described (19). Parallel duplicate plates of SC-1 and plates of mink cells were assayed for reverse transcriptase as previously described (20). Other details are given in Results. Appropriate ecotropic viruses that grow on SC-1 cells and xenotropic viruses that grow on mink cells were used as positive controls. In addition, virus-positive samples were mixed with virus-negative samples to rule out an inhibitor of viral infectivity in the virus-negative culture fluids. No reduction in viral titer (<10%) of growth of ecotropic viruses on SC-1 cells or xenotropic viruses on mink cells was observed in such infectivity-mixing experiments.

Results

Generation of Cell Lines from Normal Bone Marrow Cells.   In initial experiments, freshly isolated normal mouse bone marrow cells were seeded at 10^5 or 10^6 cells/ml in Falcon TC flasks in 10 ml of Fischer's medium supplemented with 20% horse serum and 10% WEHI-3 CM. When normal marrow cells were grown in this medium, there was an initial cell growth (seen only in the presence of WEHI-3 CM), and the cultures became acidic 7 d later. The nonattaching cells were split 1:4 or 1:10 with fresh medium that contained WEHI-3 CM and transferred to new culture flasks. After four to five similar subcultures, at weekly intervals, extensive proliferation was still occurring (Table I). At this time, the majority of cells consisted of blasts and early granulocytes. When assayed for the presence of GM-CFC in soft agar it was observed that colonies formed in the presence of WEHI-3 CM but not in the presence of heart CM. Colonies produced consisted of multifoci of granulocytic cells (see below). However, within two to three further subcultures, cell proliferation had essentially ceased, and the cultures were discarded. Normal bone marrow from several strains tested have so far failed to produce continuously growing WEHI-3 CM-dependent cell lines.

Generation of Cell Lines from Long-Term Marrow Cultures.   For the 1st 4–8 wk after establishing the long-term cultures and subculturing the cells weekly in WEHI-3 CM (Materials and Methods), cell lines were not generated under the experimental conditions. Cells subcultured in growth medium alone, or in the presence of mouse heart (H)CM, showed rapid decline in cell number within 1 wk and produced cultures that contained an adherent population of phagocytic cells. On the other hand, WEHI-

TABLE I

Culture of Normal Bone Marrow Cells in WEHI-3 CM

Bone marrow cells*	Cells/ml after four subcultures	Morphology		WEHI-3 CM‖	HCM‖
		B/EG‡	LG/Mono§		
				GM-CFC/10^4 cells	
BDF₁	4.0×10^5	62	38	79	0¶
DBA/2	7.3×10^5	92	8	15	0¶

* Initially seeded at 10^6 cells/ml.
‡ B/EG, blast cells, promyelocytes, and myelocytes.
§ LB/Mono, mature granulocytes and macrophages.
‖ WEHI-3 CM was used at a concentration of 10% and HCM at 15%.
¶ With freshly isolated, normal bone marrow cells, HCM and WEHI-3 CM stimulated equivalent numbers of colonies (~10/10^4 cells plated).

3 CM-supplemented cultures, after the 1st wk, consisted of both an adherent population of phagocytic cells and a population of nonattaching cells (predominantly immature granulocytes). These cells could be subcultured for 4–5 wk, but, subsequently, proliferation ceased, and the cultures declined. In this respect, they resembled the cultures derived from freshly isolated bone marrow cells described above.

Cells removed from long-term cultures grown for more than 8 wk, however, consistently produced permanently growing cell lines in the presence of WEHI-3 CM (Table II). When subcultured in growth medium alone, or in HCM-supplemented cultures, no cell proliferation was seen, and the cultures were discarded. In WEHI-3 CM-supplemented medium, such cultures consisted initially of an adherent phagocytic population and nonadherent (nonphagocytic) cells. Transfer and subculture of the nonadherent population selectively removed the adherent phagocytic cells and consistently generated cell lines. In at least 15 separate experiments, with several mouse strains (C57BL/6, DBA/2, Swiss, and BDF₁), all long-term cultures have eventually produced cells that will permanently grow in the presence of the WEHI-3 CM. Many of these lines have now been maintained for more than 6 mo. Because of the numbers of cell lines being produced, we propose the nomenclature FDC-P (*F*actor-*D*ependent *C*ontinuous cells lines, *P*aterson Laboratories).

Characterization of Factor-dependent Cell Lines. For the first two to three subcultures, the lines contained 70–80% myeloblasts/early promyelocytes; the remaining cells consisting of mature neutrophils and occasional monocytes. The percentage of immature cells then progressively increased to between 90 and 91%, although some mature neutrophils were still produced after several months. Large irregular granules were often present in the immature cells—the significance of which is not known at the present (Fig. 1 a). A preliminary surface-marker analysis of three cell lines is shown in Table III. The majority of cells show Fc receptors, and a minority show the presence of Thy-1.2 antigen. Whether these markers are present on the same, or discrete, cell population(s) has not been determined. However, even after single-cell cloning (see

TABLE II
Long-Term Marrow Cells Subcultured in Various Media

Bone marrow cells	Treatment	Cell count/ml × 10⁵ at each weekly subculture								
		1	2	3	4	5	6	7	20	30
BDF₁	—*	0.1	0‖							
	HCM‡	0.2	0‖							
	WEHI-3 CM§	3.0	3.5	3.0	4.0	4.0	5.0	4.5	5.0	5.5
DBA/2	—	0	0‖							
	HCM	0	0‖							
	WEHI-3 CM	8.0	4.8	3.8	4.0	4.5	5.1	4.5	4.8	5.0
Swiss	—	0.1	0‖							
	HCM	0	0‖							
	WEHI-3 CM	2.0	2.0	0.8	3.5	2.5	2.5	3.5	4.0	3.5

* Growth medium alone.
‡ Growth medium supplemented with 15% HCM.
§ Growth medium supplemented with 10% WEHI-3 CM.
‖ Cultures discarded. All cultures were initiated from 8- to 10-wk-old cultures of uninfected mouse marrow.
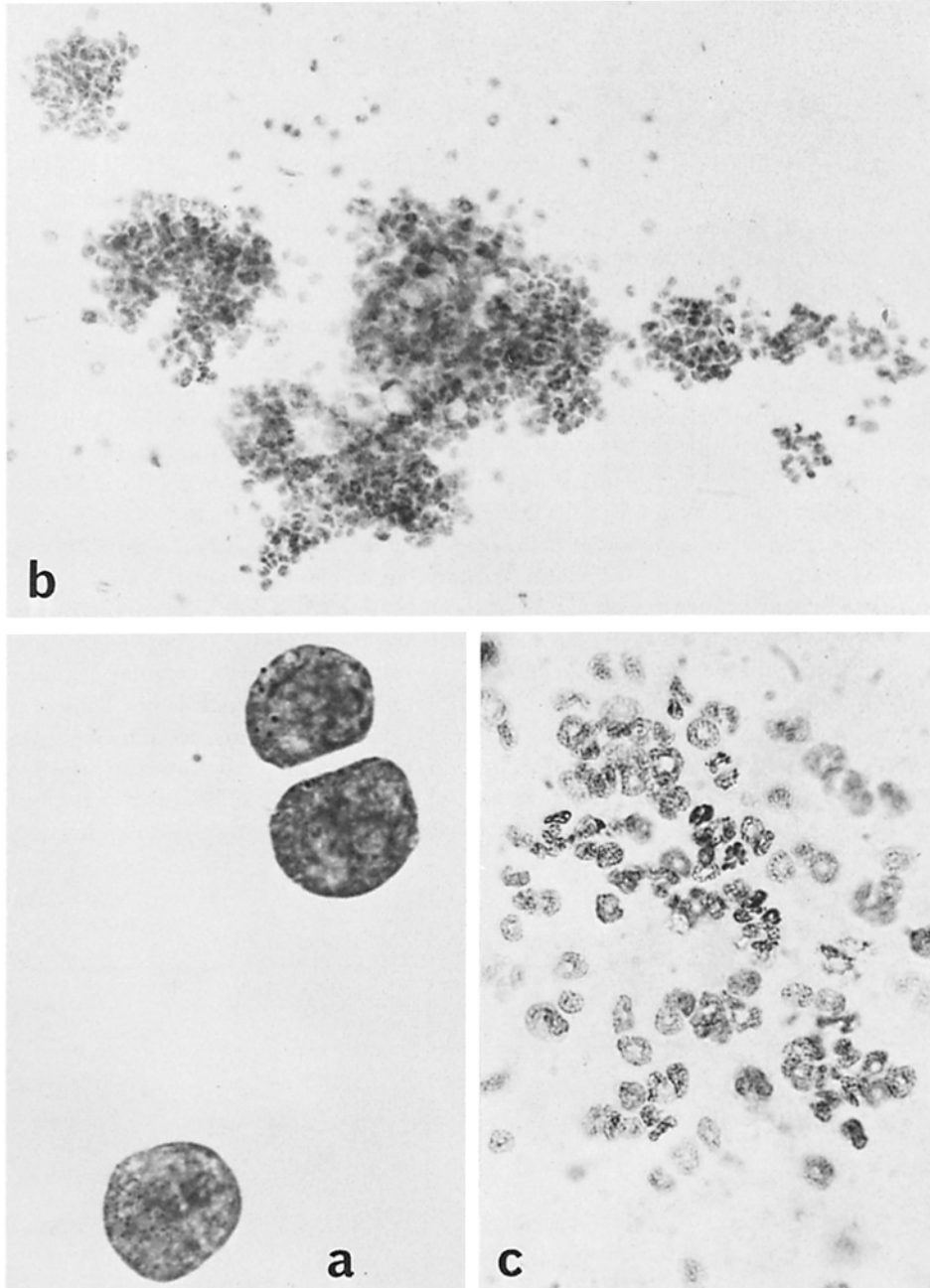
Fig. 1.   (a) FDC-P2 cells grown in suspension culture in WEHI-3 CM. The majority of cells resemble promyelocytes, often containing large, irregular granules. × 100. (b) FDC-P2 cells grown in sot agar in WEHI-3 CM. Note multicentric appearance of colonies. × 16. (c) Higher power of part of colony of FDC-P2 cells in soft agar that shows typical granulocyte morphology. × 40.

TABLE III

Analysis of Properties of Three Cell Lines Grown in WEHI-3 CM-supplemented Medium

Marker	Percentage of positive cells		
	FDC-P1	FDC-P2	FDC-P3
		%	
Thy-1.2	24	5	24
Fc (IgG)	91	66	67
Alkaline P-diesterase	0	0	ND
Nonspecific esterase	0	0	ND

FDC-P1 and FDC-P2 were derived from DBA/2 cultures and FDC-P3 from BDF₁ culture. ND, not done.

TABLE IV

Chromosome Constitution of Five Factor-dependent Cell Lines

Cell line	Normal	Diploid with one metacentric marker chromosome	Tetraploid
		%	%
FDC-P3	94	2	4
FDC-P1	96	0	4
FDC-P4	90	0	10
FDC-P5	94	0	6
FDC-P6	67	0	33

50–100 cells analyzed per cell line. FDC-P4 was derived from C57BL/6 bone marrow cells, and FDC-P5 and FDC-P6 were derived from Swiss bone marrow cultures.

below) cell lines continue to illustrate this marker heterogeneity—maybe indicating a developmental sequence.

Five cell lines tested so far (including FDC-P1, -P2, and -P3) have a normal karyotype with varying proportions of tetraploid cells, even after continuous culture for 6 mo (Table IV). In one cell line (FDC-P3) a small proportion of cells had a metacentric marker.

Characterization of Cells for Type-C Virus.   Supernatant fluid from the cell lines FDC-P1, FDC-P2, and FDC-P5 and -P6 were inoculated into SC-1 mouse cells and mink lung fibroblasts as detailed in Materials and Methods. After 1 wk, the SC-1 cells were assayed by the XC plaque assay for ecotropic mouse type-C viruses. In addition, the supernate from the SC-1 mouse cells and the mink lung cells was tested for reverse transcriptase. 20% of the mouse and mink cells was subcultured at weekly intervals for 3 more wk and reassayed weekly as indicated above. No virus was detected on either cell line by either test, which indicates that no infectious type-C virus is being produced by these myeloid cell lines. We cannot exclude that the myeloid cells produce replication-defective type-C viruses or type-C viruses with a host range that would not be detected on either SC-1 or mink cells, or other classes of endogenous retroviruses. However, the results strongly suggest that production of infectious type-C virus is not required for the establishment or sustained growth of the myeloid cell lines.

To further exclude that a putative helper-independent type-C virus with a novel host range was being produced by the factor-dependent myeloid cells, we inoculated the cell-free supernates from FDC-P1, -P2, -P5, and -P6 onto the WEHI-3 myeloid cell line. WEHI-3, itself, does not produce type-C virus particles (E. Scolnick and T. M. Dexter. Unpublished data.). 3 wk after it was inoculated, the WEHI-3 cell still was not producing type-C virus particles as judged by supernatant viral reverse transcriptase assays.

Role of GM-CSF in the Growth of Factor-dependent Cell Lines. All lines described, amounting to >30 tested at present, exhibited an absolute requirement for the continued presence of a factor in WEHI-3 CM for sustained growth. In the presence of CM, the cells grew with a population doubling time ranging from 14 to 24 h. Because WEHI-3 CM is a potent source of GM-CSF, we investigated whether GM-CSF might be involved in the maintenance of proliferation of the cell lines by comparing the growth of cells in a variety of GM-CSF-containing preparations.

These included: L-cell CM and a purified M-CSF derived from these cells; HCM and mouse lung (ML) CM, both good sources of GM-CSF; a purified GM-CSF from mouse lung; and a CM obtained from PWMSCM. Both WEHI-3 CM and PWMSCM supported the growth of these cells, with overall similar morphological and biological characteristics being maintained in both media. However, HCM, impure and purified L-cell M-CSF, MLCM, and purified lung GM-CSF, did not support the proliferation of these cells. In fact, when cultured in media that contained these activities, most of the cells had died within 48 h. The inability of these GM-stimulating factors to sustain growth was not a result of inhibitory activity, because cell suspensions could be cultured for an indefinite period in medium that contained both WEHI-3 CM and HCM. Because these media are all potent sources either of granulocyte CSF or M-CSF, it is reasonable to conclude that neither GM-CSF nor M-CSF is involved in growth maintenance of these cell lines.

Growth of Cells in Semisolid Cultures. Suspensions of cells from the above cell lines regularly formed colonies when plated in soft agar in the presence of WEHI-3 CM or PWMSCM. No colony or cluster formation was observed in culture with other GM-CSF- or M-CSF-containing materials (Table V). In this respect, therefore, growth in suspension cultures and in soft agar showed a similar pattern.

TABLE V
Colony Formation in Soft Agar by Factor-dependent Cell Lines

Stimulus	Cell line			
	FDC-P1	FDC-P2	FDC-P3	FDC-P4
None	0	0	0	0
HCM	0	0	0	0
L-cell CM	0	0	0	0
Pure L-cell CSF	0	0	0	0
Lung CM	0	0	0	0
Pure lung GM-CSF	0	0	0	0
WEHI-3 CM	80	90	76	137
PWMSCM	96	120	ND	260

Results are expressed as colonies/10^4 cells plated. FDC-P1 and FDC-P2 are DBA/2 derived, FDC-P3 is BDF$_1$, and FDC-P4 is C57BL/6 derived. ND, not done.

Colony formation was not linearly related to cell concentration when $<10^3$ cells/ml were plated. However, linearity could be achieved by plating at a high cell density (10^4-10^5 cells/ml) or by adding an excess of lethally irradiated cells of the respective cell line. This suggests that the growth of these cell lines may be, in part, a result of autostimulation. Nonetheless, colony formation did not occur even at high cell densities when cells were plated in the absence of WEHI-3 CM or PWMSCM.

10 of the cell lines have been assayed for their capacity to produce GM-CSF. After culturing in WEHI-3 CM, cells were centrifuged, washed, and resuspended in normal growth medium. After incubation for 48 h, the growth medium was collected and assayed for GM-CSF activity with normal bone marrow cells as targets. No colony formation occurred, even when 70% of the CM was used, i.e., GM-CSF activity could not be detected.

Colony Morphology in Semisolid Cultures and Recloning Analysis. In cultures of FDC-P1, the majority of colonies formed were of a diffuse nature; in FDC-P4 cultures, the colonies were mixed (diffuse and compact), whereas in cultures of FDC-P2 and FDC-P3, >85% of the colonies were compact, often containing multiple foci resembling the granulocytic bursts described by Metcalf (4) (Fig. 1b). Irrespective of the gross morphology, the majority of colonies contained cells showing granulocyte maturation (Table VI; Fig. 1c). A variable, but low, percentage of colonies appeared to contain only maturing granulocytic cells, but the majority consisted of a mixture of undifferentiated cells and maturing granulocytes. Invariably, the maturing granulocytes constituted between 20 and 50% of the colony cells, and, often, mature neutrophils were present (Fig. 1c). Some colonies were observed that were composed wholly of undifferentiated cells. It was quite obvious that more extensive maturation occurred in soft-agar cultures (in contrast with the same cells maintained in suspension cultures), although the same stimulus had been used in both cases.

Many colonies contained >1% mitotic figures, which indicated that extensive proliferation was occurring even after 7 d in soft agar. To check whether such colonies represented terminally differentiating cells, or whether they contained additional clonogenic cell populations, individual colonies were resuspended and subcultured in agar (in WEHI-3 CM) to determine their content of CFC. It was found that almost all colonies contained CFC (Table VII), and that, as before, colony formation occurred only in the presence of WEHI-3 CM or PWMSCM. No obvious differences were

TABLE VI

Morphological Analysis of 7-d Colonies Developing in Soft Agar

Cell line	Gross morphology	Microscopic analysis*			Percentage of colonies with mitotic figures
		G	G/U	U	
			%		%
FDC-P1	Diffuse	5	90	5	40
FDC-P2	Compact	12	65	33	75
FDC-P3	Compact	10	82	8	49
FDC-P4	Mixed	28	52	20	25

* Individual colonies were picked from the agar cultures, placed on a glass slide, and allowed to air dry. Colonies were then stained with aceto-orcein and examined at a magnification of 600. At least 40 colonies were scored. G, neutrophil colonies; G/U, mixed neutrophil/undifferentiated cells; and U, undifferentiated cells.

TABLE VII

Self-Renewal Potential of Cells Forming Colonies in Agar

Cell line	CFC content of individual colonies						
FDC-P1	1	23	0	140	50	12	12
FDC-P2	86	400	400	0	18	0	34
FDC-P3	0	23	9	136	16	4	1
FDC-P4	10	15	250	400	1	0	0

observed between the primary and secondary colonies either in size range or gross morphology, which indicated that the original CFC were capable of extensive self-renewal. With one cell line tested (FDC-P2), a further recloning of the secondary colonies again resulted in further colony formation.

When individual colonies were gently resuspended in fresh growth medium that contained WEHI-3 CM, it was found that 60–70% of the isolates would readily grow and establish permanently growing, cloned cell lines, with characteristics similar to the parent cell population.

Developmental Potential of Cell Lines. At different times after isolation, the cell lines and the cell-free growth media have been injected into syngeneic adult or neonatal recipients (up to 5×10^6 cells/recipient). No leukemias have developed over a period of 6 mo. Furthermore, injection of up to 10^6 cells into potentially lethally irradiated mice did not result in the development of spleen colonies (CFU-S). In repeated experiments, we have been unable to induce erythroid maturation in these cells by using the appropriate growth conditions and additions such as erythropoietin, burst-promoting activity, or dimethylsulfoxide. Finally, in the soft-agar cultures we have seen no evidence for the formation of megakaryocytes or eosinophils. It seems, then, that under the present condition the cell lines are restricted to granulocyte development.

Discussion

We have found that precursor cells, apparently committed specifically to the granulocyte lineage, can be readily immortalized in vitro when normal marrow cells are cultured in the presence of CM produced by a mouse myelomonocytic leukemia cell line (WEHI-3 CM) or by PWMSCM. The active principle involved is not equivalent to GM-CSF or M-CSF. Although such cell lines appear to comprise mixtures of undifferentiated blast cells and early promyelocytes, all appear to be nonleukemic, have a normal karyotype (at least up to 6 mo), and are able to generate cells with the morphology and properties of differentiated granulocytic cells. Although we have been unable to induce maturation of the cells into other hemopoietic lineages (megakaryocytes, erythroid cells), and despite the fact that the cells do not form spleen colonies in unirradiated mice, we cannot exclude the possibility that multipotential cells may be present in the blast cell population, which may begin to express their potential when appropriate inducing agents are available. In this context it may be significant that although most of the cells are Fc positive, but negative for macrophage and granulocyte cytochemical markers, a variable proportion of the cells are Thy-1 positive. It will be interesting to separate these two cell populations and investigate their biological properties in vitro and developmental potential after in vivo injection.

Our findings raise two important questions: first, the nature of the proliferating cells and their role in hemopoiesis in vivo, and second, the nature of the factor(s) required for their sustained growth.

With regard to the first point, the present work has shown that both freshly isolated and long-term cultured marrow cells possess a granulocyte CFC population that undergoes proliferation in response to a factor in WEHI-3 CM or PWMSCM—with the former cells, the cultures eventually decline; but with the latter cells, permanently growing cell lines can be established. In both cases, the colony-forming capacity (or proliferative ability in suspension) is not expressed with conventional GM-CSF-containing media. This is interesting in view of the finding that in standard GM-CFC assays roughly equivalent numbers of normal marrow GM-CFC are induced irrespective of the GM-CSF source used (i.e., WEHI-3 CM, PWMSCM, HCM, or MLCM), and additive effects are normally not observed. Therefore, the vast majority of normal marrow GM-CFC do not show a colony-forming response specific to WEHI-3 CM or PWMSCM. This leads to the conclusion that WEHI-3 CM-specific GM-CFC are a minority population of normal marrow GM-CFC—a population that would normally not be detected in standard soft-gel assay systems where they are masked by the presence of GM-CSF-responding GM-CFC. If this premise is correct, the implications are far reaching in normal hemopoiesis. It has been assumed so far (based upon lack of evidence that indicates self-renewal potential of erythroid progenitors and GM-CFC [4, 9]) that mylopoiesis in vivo requires a continued output (differentiation) from a pluripotential stem-cell population (the CFU-S?). In fact, most present day mathematical and biological model systems in hemopoiesis are based upon this concept. However, such an output from an earlier stem-cell compartment may not be necessary in a situation where a restricted cell (i.e., restricted to one lineage) has extensive self-renewal potential. The cell lines described herein may represent such a cell population. Indeed, evidence from other systems indicates that B lymphocyte generation (21) and T lymphocyte production (22) may occur over long periods in vivo from a committed (restricted) progenitor cell and that specific growth factors can maintain the proliferation of T lymphocytes in vitro (23). In the case of the cell lines reported here, it is important to establish whether the self-renewal ability is an intrinsic property of the cells or whether it is a reflection of culture history, maybe representing an early stage in leukemic transformation.

It seems clear that the factor necessary for growth of these granulocytic cell lines in vitro, is not GM-CSF, because GM-CSF-containing media derived from sources other than WEHI-3 cells or mitogen-stimulated spleen cells do not support growth or clonal expansion of the cell lines. WEHI-3 CM contains GM-CSF, megakaryocyte CSF, granulocyte CSF (24, 25), and factors stimulating proliferation of multipotential cells. Similarly, mitogen-stimulated spleen CM contains factors stimulating granulocytic, megakaryocyte, erythroid, and multipotential cells (9. 13, 14). So far, it has not been possible to separate these activities using biochemical techniques—thus raising the possibility that one molecule may be responsible for stimulation. In preliminary experiments, it has been shown that the active factor involved in maintenance of proliferation of the cell lines cosegregates (on fractionation) with the factor that promotes multipotential cell development in soft agar. This raises the possibility that proliferation of the cell line may be maintained by a more ancestral cell than GM-CFC. If this is true, the absence of macrophage differentiation in the cell lines, and

lack of responsiveness to GM-CSF, suggests that a further differentiation step is required before the cells acquire GM-CSF responsiveness and potential for macrophage development, i.e., the true developmental sequence in granulopoiesis and macrophage development is granulocyte CFC → GM-CFC → M-CFC, with only the latter two populations responding to GM-CSF.

Contrary to the observation by Greenberger et al. (11) these WEHI-3 CM-dependent cell lines can regularly be established in the absence of exogenous murine leukemia virus infection. Furthermore, experiments indicate that proliferation of the cell lines can occur in the absence of endogenous virus expression—a finding that makes them particularly useful for examining the leukemia-inducing potential of a range of type-C viruses, chemicals, and irradiation.

Summary

Cell lines have been produced from long-term cultures of mouse bone marrow that require a factor, present in WEHI-3 conditioned medium (CM) or in spleen CM, for their sustained growth. The cell lines were obtained from nonvirus-treated cultures, are nonleukemic, maintain a normal karyotype, and form colonies showing granulocyte maturation when plated in soft agar. Granulocyte/macrophage (GM) colony-stimulating factor is not the inductive moiety involved in the maintenance of proliferation of these cells. It is suggested that the cell lines represent a self-renewing population of cells ancestral to GM colony-forming cells, which may be responding to a hitherto unrecognized regulator.

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