Journal of Supramolecular Structure 13:501–511 (1980)
Control of Cellular Division and Development: Part B: 55–65

Self-Renewal of Factor-Dependent Hemopoietic Progenitor Cell-Lines Derived From Long-Term Bone Marrow Cultures Demonstrates Significant Mouse Strain Genotypic Variation

Joel S. Greenberger

Joint Center for Radiation Therapy, Department of Radiation Therapy, and Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115

Long-term bone marrow cultures established from C57Ks/J mice have been shown to spontaneously release endogenous ecotropic RNA type-C virus (retrovirus). C57Ks/J marrow cultures produced granulocyte-macrophage progenitor cells (GM-CFUc) and immature and mature granulocytes for over 45 weeks. In contrast, NIH Swiss mouse marrow cultures failed to release detectable ecotropic virus and generated GM-CFUc and granulocytes for 25–35 weeks and established WEHI-3 conditioned medium (CM) dependent cell lines in vitro and did not establish permanent cell lines. To determine whether viral and/or cellular genes regulated the longevity of C57Ks/J marrow cultures, groups of cultures were established from the marrow of (NIH-Swiss X C57Ks/J) F1 hybrid, F2 hybrid, and (NIH Swiss X C57Ks/J) X NIH Swiss backcross generations. Release of endogenous ecotropic virus was measured weekly in each culture as was the duration of production of immature granulocytic cells and GM-CFUc over a 58-week period. The results demonstrated a complex pattern of inheritance of longevity of long-term in vitro hemopoiesis. Increased longevity did not absolutely correlate with detectable replication of the C57Ks/J N-tropic virus.

Key words: bone marrow cultures, hemopoiesis in vitro, mouse genotype, factor-dependent cell lines

Genetically inheritable characteristics have been shown to influence the duration and magnitude of expression of a variety of biologic markers of in vitro cellular proliferation [1]. These include: susceptibility to infection by a variety of bacterial, protozoan, and viral agents [1–6]; and the immune response to histocompatibility antigens and infectious agents [1]. Specific genetic markers for obesity, diabetes, neurological abnormalities, and abnormalities of growth and development have also been shown to be genetically determined in inbred mouse strains [1, 7–11]. The role of genetic variables in the self-renewal capacity of stem cells has only recently been investigated due to the recent availability of a long-term bone marrow culture system [12–13]. Studies with this culture system have indicated that at least two parameters influence the duration in vitro replication of hemopoietic stem cells: stability of the marrow stroma, and intrinsic self-renewal capacity of the stem cell population known as pluripotent hemopoietic stem cells [12–16].

Studies with C3H/HeJ mouse marrow cultures demonstrated that integrity of the marrow stromal component, including those cells which adhere to a plastic surface, determines the longevity of hemopoiesis as reflected in numbers and biologic activity

Received March 3, 1980; accepted July 18, 1980.

0091-7419/80/1304-0501$02.30

of weekly removed nonadherent cells [16]. However, C3H/HeJ marrow cultures and those of NIH Swiss mice [14] have been shown to be relatively short-lived, with regard to longevity of in vitro hemopoiesis, compared to identically established marrow cultures from other mouse strains including: Akr/J, C58/J, DBA2/J, and C57Ks/J [14]. Marrow cultures from each of these latter strains spontaneously released ecotropic endogenous retrovirus which replicated to levels detectable by an assay for reverse transcriptase activity in culture supernatants [14]. In previous studies C57Ks/J mouse marrow cultures produced granulocyte-macrophage colony-forming cells in excess of 40 weeks, while C3H/HeJ marrow cultures rarely if ever generated GM-CFUc past week 20 [14]. Marrow cultures from NIH Swiss mice, a strain which contains no endogenous ecotropic retrovirus, were similar to C3H/HeJ marrow cultures in short duration of in vitro marrow hemopoiesis [14]. Since addition of ecotropic murine leukemia viruses to NIH Swiss marrow cultures greatly increased the magnitude and longevity of in vitro hemopoiesis [17, 18], it was reasoned that the longevity of hemopoiesis in C57Ks/J marrow cultures might be attributable to the regularly detectable associated phenomenon of spontaneous induction of replication of endogenous virus [14].

In the present study, we investigated whether genetic segregation of the C57Ks/J endogenous ecotropic virus in F1 and F2 hybrid and backcross mice derived from matings with the virus-negative NIH Swiss strain affected the longevity of hemopoiesis in a large number of marrow cultures. These studies were designed to determine whether longevity of hemopoiesis in C57Ks/J marrow cultures was attributable to spontaneous induction of the N-tropic virus of C57Ks/J mouse cells or to other nonviral genetic factors. The results indicate that factors other than replication of ecotropic virus are involved in regulation of hemopoiesis in long-term bone marrow cultures. Furthermore, permanent WEHI-3 growth factor dependent hemopoietic progenitor cell lines were derived from marrow cultures of several mouse strains in the absence of virus infection.

MATERIALS AND METHODS

Mice

NIH Swiss mice were obtained from the Animal Colonies of the National Institutes of Health. DBA/2J, C57BL/6J, and C57BL/KsJ mice were obtained from Jackson Laboratories, Bar Harbor, Maine. (NIH Swiss × C57Ks/J) F1, F2, and (C57Ks/J × NIH Swiss) × NIH Swiss backcross mice were bred in our animal quarters, housed 10 per cage, and fed standard laboratory chow.

Tissue Culture

Continuous embryo fibroblast cell lines including NIH/3T3 [19], Balb/3T3 [20], and NRK [21], have been reported. These were grown in Dulbecco's modified Eagles medium supplemented with 10% fetal calf serum (FCS) and antibiotics. A Kirsten sarcoma virus transformed nonproducer clone of each embryo fibroblast line designated KNIH, KBALB, and KNRK, respectively, has been reported and was passaged in DME medium [22].

Viruses and Virus Assays

A method for detection of virion-associated reverse transcriptase has been reported [23]. An assay for quantitation of virus titer by polymerase induction has been reported [23]. A focus-forming assay for detection of KiMSV rescued from transformed nonproducer cells by helper virus has been described [22]. Focus assays were performed according to published procedures by infecting exponential phase cultures of NIH/3T3, BALB/3T3, or NRK cells that had been incubated overnight in 2.0 μg/ml polybrene [24] with Millipore filtered (0.45 mμ) supernatant fluids from virus-producing cultures.

Long-Term Bone Marrow Cultures

A method for establishment of continuous bone marrow cultures from the contents of a single tibia and femur of each of a variety of mouse strains has been reported [13, 25]. The differences between this method and that reported by Dexter [12] have been outlined [25]. Briefly, the present method differs from the Dexter system in the following ways: 1) The absence in our technique of a second marrow inoculum (recharge) such that all nonadherent cells are produced from a single age inoculum. 2) The removal of all nonadherent cells and medium weekly rather than removal of a 50% volume [12]. The cells are removed by pouring the 10.0 ml of medium into a 10.0 ml conical centrifuge tube. Loosely adherent cells are not removed. Fresh medium is then added in a 10.0 ml volume. 3) The maintenance of cultures in 25% FCS supplemented with 10^{-7}M hydrocortisone sodium hemisuccinate rather than in special lots of horse serum [12].

The advantages of the present non-recharged system are as follows: The system allows study of stem cells of a single age and depopulation schedule. The maintenance of cultures in FCS rather than horse serum prevents excessive lipid accumulation in adherent cells. Lipid accumulation causes subsequent sloughing of the fat-containing cells and associated hemopoeitic cell islands from the culture flask surface. Further advantages of this system have been detailed previously [15]. The present conditions maintain hemopoiesis for longer periods than that initially reported by Dexter [12].

Assays for Hemopoietic Colony-Forming Cells

The methods for detection of granulocyte-macrophage progenitor cells (GM-CFUc), designating separately colonies and clusters, have been reported previously [26]. Cells were removed from long-term bone marrow cultures or fresh marrow and suspended at 5.0×10^4 or 2.0×10^5 cells per ml in 1.0 ml of McCoy's 5A medium supplemented with 10% FCS, 10% L929 cell colony stimulating factor (CSF), and 0.3% Difco Bactoagar [27]. Colonies were scored at day 7 as those of $\geqslant 50$ cells and as culsters of those of 10–49 cells. Individual colonies were removed and stained as reported [17]. Methods for detection of the erythroid colonies, burst-forming unit erythroid (BFUe), and colony-forming unit erythroid (CFUe) have been reported [28]. An assay for detection of B-lymphocyte colony-forming cells (CFU_L) has been reported [27]. CFU_L were scored as those colonies of $\geqslant 50$ cells forming at day 7 in 100 μg/ml LPS (salmonella typhosa, Difco) and which were inhibited in parallel assay plates incubated in addition in the presence of 0.05 ml of a 1:50 dilution of goat antimouse immunoglobulin antiserum (Litton Bionetics, Kensington, MD [27].

Hematologic Morphology and Histochemistry

Cells removed from marrow cultures were washed by centrifugation, resuspended in FCS, smeared on glass coverslips, and stained with Wright's/Giemsa or in histochemical methods for detection of lysozyme, myeloperoxidase, specific myeloid esterase, and leukocyte alkaline phosphatase [17].

Establishment of Secondary Suspension Culture Lines and Attempts to Produce Clonal Spontaneous Permanent Lines in WEHI-3 CM

Nonadherent cells from long-term marrow cultures were washed by centrifugation and resuspended at 10^6–10^7 cells in a 4.0 ml volume of McCoy's 5A medium supplemented with 10% FCS (Gibco) and 10% 5X-concentrated (by Amicon filtration) WEHI-3 cell-conditioned medium. Cells were then split 1–2 every third day for 6 weeks, then 1–10 for 6 weeks, then 1–100. Where possible, cultures passaged for 12 weeks were then cloned in 0.3% agar in 10% WEHI-3 CM and subclonal lines removed at day 7 and established in suspension culture [18, 33].

RESULTS

Longevity of Hemopoiesis in C57Ks/J, NIH Swiss, (C57Ks/J X NIH Swiss) F1, F2, and (Ks X N) X N Backcross Long-Term Bone Marrow Cultures

The contents of a femur and tibia from each mouse in the groups listed in Table I were established in long-term bone marrow cultures according to the Methods. For each individual animal one culture from the right hind limb including femur and tibia (2.0×10^7 cells $\pm$ 0.4×10^6 cells) was established in 25% FCS and 1 culture from the left hind limb including 1 femur and 1 tibia established in 25% horse serum (Flow Laboratories), both supplemented with 10^{-7}M hydrocortisone hemisuccinate. Thus, for 20 NIH Swiss mice, 40 cultures were established including a duplicate for each animal, one initiated in horse serum and one initiated in FCS. Similar cultures were prepared with each of the strains listed in Table I. All cultures were medium changed according to the Methods with removal of all nonadherent cells and medium each week with replacement of a fresh 10.0 ml volume of medium. At the fourth medium change, day 28, those cultures established in horse serum were refed with 25% calf serum and all cultures then maintained in 25% FCS [18].

As shown in Table I, cultures of NIH Swiss marrow generated GM-CFUc for 25–28 weeks. No culture produced detectable GM-CFUc past 28 weeks. Immature granulocytic cells (myeloblasts, promyelocytes, and myelocytes) were detected up to 42 weeks (range, 28–42 weeks). In marked contrast, cultures from C57Ks/J mice generated GM-CFUc for 40–45 weeks and immature granulocytic cells for 52–55 weeks (Fig. 1, Table I). These differences between strains were highly significant ($P < 0.001$) and could not be attributed to differences in lots of horse or fetal calf serum used or to other conditions of incubation. Marrow cultures from each strain were incubated at 33°C, 7% CO_2, and media changed on the same day each week [14]. There were fluctuations in the level of GM-CFUc detected each week with both strains (Figs. 1, 2). These could not be correlated to differences in the percent of immature granulocytes at that week.

TABLE I. Effect of Mouse Strain Genotype on the Longevity of Granulopoiesis in Long-Term Bone Marrow Cultures

Mouse strain[a]	Number of mice tested	Number of cultures	Duration of hemopoiesis[b] GM-CFUc (wks)	Duration of hemopoiesis[b] Immature granulocytes (wks)
NIH	20	40	25–28	28–42
C57Ks/J	20	40	40–45	52–55
(NIH Swiss X C57Ks)F1	35	70	23–45	28–55
(NIH Swiss X C57Ks)F2	37	74	24–43	24–53
(NIH Swiss X C57Ks) X NIH Swiss	28	56	25–46	21–51

[a]Duplicate marrow cultures were established from individual mice in each group as described in Materials and Methods.

[b]Results are expressed as the range of weeks over which GM-CFUc colonies per 5×10^4 nonadherent cells removed that week were detected. Immature granulocytes accounting for $\geq 1\%$ of differential cell counts were considered positive for that mouse culture for that week. All cultures were carried for at least 6 weeks past the time when GM-CFUc or immature granulocytes were last detected.

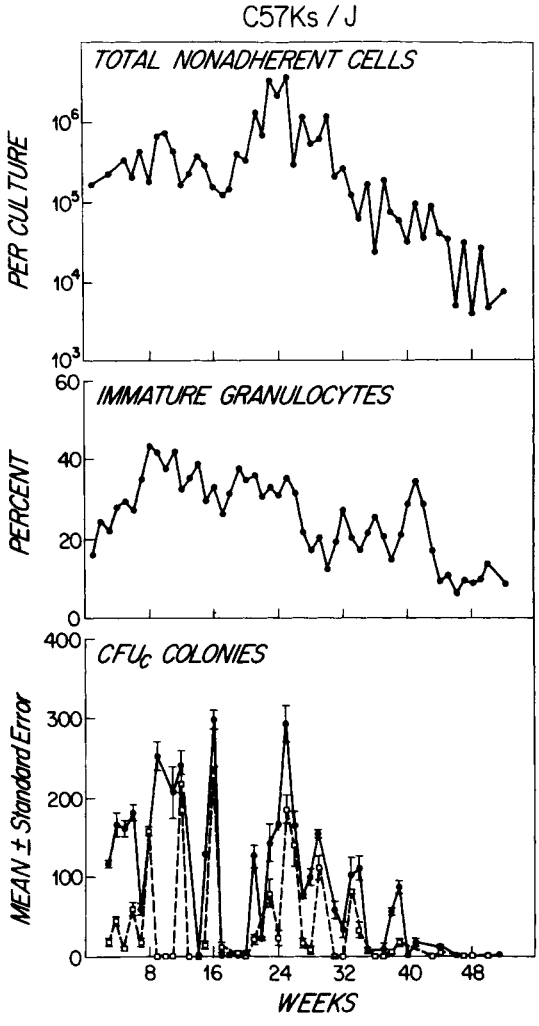

Fig. 1. Production of nonadherent GM-CFUc and granulocytes over 45 weeks in C57Ks/J mouse long-term marrow cultures. Results are presented as the mean of total nonadherent cells and % immature granulocytes (myeloblasts, promyelocytes, and myelocytes) removed per week for at least 8 cultures at each point. The standard error was < 10% of the mean for these determinations. GM-CFUc are presented as the mean ± SEM for total colonies scored at day 7 for duplicate assay plates for each of 8 cultures (total 16 for each time point). Cells were plated each week at two densities: •, 2×10^5; and ○, 5×10^4 per 1.0 ml volume in 0.3% agar (Bactoagar) containing 10% L929 cell CSF [27, 17]. There were < 0.1% toluidine blue positive mast cells after week 10 [34].

To determine whether longevity of marrow hemopoiesis in vitro segregated as a genetic characteristic, 8–10-week-old mice from F1, F2, and first generation backcross matings of (Ks × N) F1 mice on the NIH Swiss background were sacrificed and marrow cultures prepared in duplicate as described above. As shown in Table I, each of 35 F1 mice was studied. Of these 35, 21 demonstrated generation of GM-CFUc in marrow culture for less than 40 weeks, while 14 demonstrated generation of CFUc in excess of 40 weeks (Table II).

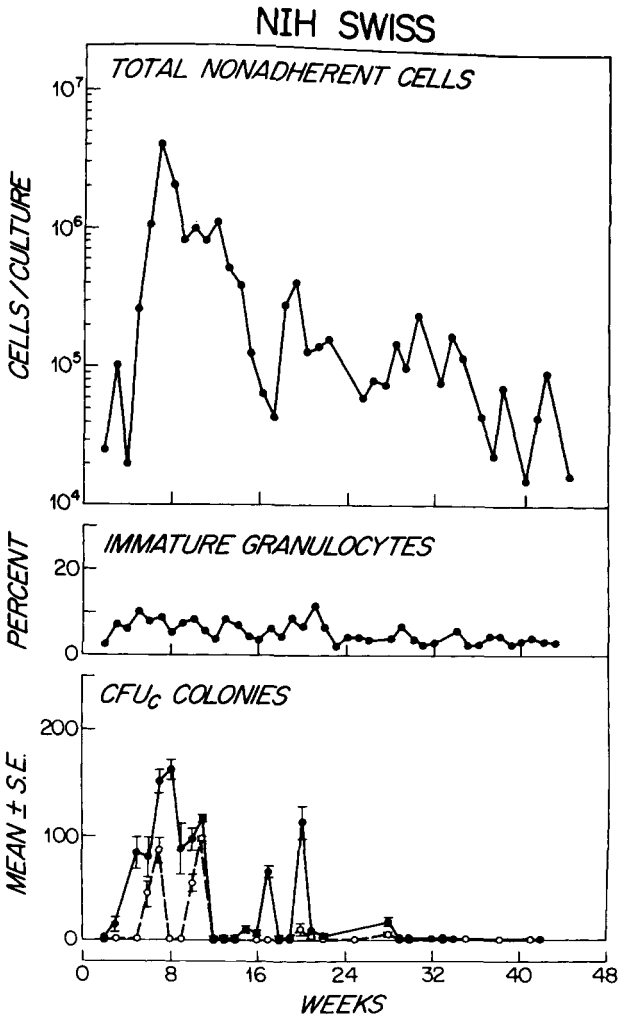

Fig. 2. Production of nonadherent GM-CFUc and granulocytes in NIH Swiss mouse long-term bone marrow cultures. Results are presented as described in legend to Figure 1 for total nonadherent cells, percent immature granulocytes, and GM-CFUc. For GM-CFUc: •, 2×10^5 cells plated per dish; ○, 5×10^4 cells plated per dish. Mean ± SEM for at least 10 cultures for each time point.

Thus, approximately 40% of F1 mouse marrow cultures demonstrated longevity less than that of C57Ks/J mice. There was no difference in the duration of generation of BFUe or CFUe between strains ($\leqslant 4$ weeks), and no cultures showed release of CFU_L at weeks 2, 8, 10, or 20.

An attempt was next made to correlate longevity of hemopoiesis (growth past 40 weeks) with expression of the C57Ks/J N-tropic endogenous virus. As shown in Table II, there was incomplete correlation between many of the 34/35 (97%) of F1 cultures re-

TABLE II. Absence of Concordance Between Release of C57Ks/J Endogenous N-Tropic Virus and Longevity of Granulopoiesis in (C57Ks/J × NIH Swiss) F1 Marrow Cultures

		Virus detected[b]					
Mouse strain[a]		wk 4	wk 8	wk 12	wk 20	wk 40	Longevity[c] (wks)
(NIH Swiss × C57KS)F1							
Culture No	1	N.T.	+	+	–	–	29
	2	N.T.	+	+	–	–	28
	3	+	N.T.	N.T.	N.T.	N.T.	29
	4	N.T.	+	N.T.	N.T.	N.T.	31
	5	N.T.	+	+	+	N.T.	37
	6	+	+	+	+	+	41
	7	+	+	+	+	+	46
	8	+	+	N.T.	+	+	49
	9	+	+	N.T.	+	+	41
	10	–	+	+	–	–	42
	11	+	–	+	–	–	41
	12	N.T.	+	+	–	–	28
	13	N.T.	+	+	+	N.T.	31
	14	N.T.	N.T.	+	–	–	35
	15	+	+	N.T.	N.T.	N.T.	39
	16	+	+	N.T.	N.T.	N.T.	39
	17	+	N.T.	N.T.	N.T.	N.T.	39
	18	+	+	+	N.T.	N.T.	41
	19	+	–	N.T.	+	N.T.	45
	20	+	–	–	N.T.	–	41
	21	+	N.T.	N.T.	N.T.	N.T.	43
	22	+	N.T.	N.T.	N.T.	N.T.	42
	23	+	N.T.	N.T.	N.T.	N.T.	41
	24	+	–	–	+	–	29
	25	+	+	–	–	–	27
	26	–	–	–	–	–	29
	27	–	+	–	–	–	29
	28	N.T.	+	–	–	–	33
	29	+	N.T.	N.T.	+	+	37
	30	+	N.T.	N.T.	+	–	36
	31	+	–	–	–	–	33
	32	+	N.T.	+	+	+	35
	33	+	N.T.	–	–	–	29
	34	N.T.	N.T.	+	+	+	42
	35	+	+	N.T.	N.T.	N.T.	48

[a]Each of 35 F1 mice was sacrificed and duplicate cultures established as described in Methods, medium changed, and depopulated weekly.

[b]Tissue culture medium was assayed for virion-associated reverse transcriptase activity and pooled fluids from 5 negative cultures assayed for rescue of MSV from KNIH cells as described in Methods. Positives in the more sensitive assay were then identified by separate assay of each culture fluid at rescuing MSV from KNIH cells. N.T. = not tested.

[c]Longevity is defined as time in weeks when > 1 GM-CFUc per 5.0×10^4 cells was detected. Growth past 40 weeks is underlined as C57Ks/J-like.

leasing detectable N-tropic virus and the 21/35 F1 cultures failing to generate GM-CFUc past 40 weeks. Thus, many virus-releasing F1 cultures failed to demonstrate longevity similar to the C57Ks/J parent. The failure to detect virus in some cultures at some time points (example: culture #11 at weeks 8 and 20) did not correlate to a decreased number of cells or of GM-CFUc at that time compared to the same culture at weeks 4 and 12 when virus was detected.

As shown in Table I, marrow cultures from F2 and (Ks × N) × N backcross generations generated GM-CFUc and immature granulocytic cells for between 23–46 weeks. There was also incomplete correlation between those cultures generating GM-CFUc for over 40 weeks and those releasing detectable ecotropic endogenous virus (data not shown).

Correlation of Cultures Showing Morphologic Alteration of Immature Granulocytes and Cultures Expressing N-Tropic Virus

Infection of NIH Swiss marrow cultures with a variety of ecotropic viruses resulted in increased duration of hemopoiesis and dysplastic granulocyte morphology [14, 17, 18]. To determine whether those F1, F2, and backcross cultures generating granulocytes for over 40 weeks were those which demonstrated dysplasia of granulocytes, nonadherent cells from cultures in each of the groups listed in Table I were removed at weeks 4, 8, 12, 20, 35, and 40, stained with Wright's/Giemsa, and examined for evidence of dysplastic morphology. As shown in Table III, there was incomplete concordance between those cultures generating GM-CFUc past 40 weeks and those showing dysplasia defined as: in creased numbers of immature granulocytes to a level of $\geqslant 20\%$, aberrant nuclear-to-cytoplasmic ratio in $> 25\%$ of cells, and $> 10\%$ giant metamyelocytes [17, 18]. There was not absolute concordance between cultures showing dysplastic morphology and those releasing ecotropic virus. However, those cultures producing cells with aberrant nuclear morphology including folding, giant nucleoli, and polylobulation most closely correlated with a > 40 week duration of hemopoiesis (Table III).

TABLE III. Correlation of Cultures Showing Generation of Dysplastic Granulocytes and Those With Granulopoiesis Past 50 Weeks

	Dysplastic morphology		
	Number cultures with dysplastic granulocytes (subgroup that also showed growth > 40 wks)[b]		
Mouse strain[a]	> 20% Immature cells	> 10% Giant metamyelocytes	> 25% Atypical or aberrant nuclei
NIH Swiss	0 (0)	0 (0)	0 (0)
C57Ks/J	20 (20	20 (20)	20 (20)
(NIH Swiss × C57Ks/J)F1	17 (12)	31 (10)	14 (14)
(NIH Swiss × C57Ks/J)F2	18 (5)	33 (6)	8 (8)
(NIH Swiss × C57Ks/J) × NIH Swiss	21 (17)	20 (8)	10 (8)

[a]Marrow cultures were harvested weekly and nonadherent cells stained with Wright's Giemsa and scored for each of the criteria listed in our previous publications [13, 14, 17].

[b]At least 1,000 cells were analyzed under oil-emersion and classified as normal or dysplastic [13, 14, 17]. The percent of immature granulocytes (myeloblasts, promyelocytes, and myelocytes) was not observed to exceed 20% for NIH Swiss cultures. Thus, a value > 20% was an indication of a "left-shift" similar to that found in C57Ks/J marrow cultures which generate 28–41% immature granulocytes [14].

Establishment of Secondary Suspension Cultures of WEHI-3 Factor Dependent Progenitor Cell Lines

Nonadherent cells removed from each of the groups of cultures listed in Table I at weeks 8, 12, 20, and 40 were transferred to 4.0-ml cultures containing McCoy's 5A medium supplemented with 10% FCS and 10% WEHI-3 CM (5X Amicon concentrated). Cultures were then passaged by a 1:10 split each 4 days for 70 days and clones of secondarily derived tissue culture lines established by removal of 50 cell colonies from agar at limiting dilution, according to published methods [18].

As shown in Table IV, cloned permanent cell lines derived from secondary suspension culture lines were established from 16 out of 20 C57Ks/J cultures tested. These cell lines were passaged weekly for over 18 months and were absolutely dependent upon WEHI-3 cell CM [18]. Six of 35 F1 cultures harvested and 1 of 37 F2 cultures harvested generated suspension culture lines. None of the 38 backcross cultures generated cell lines. Continuous growth of secondary suspension culture cells of NIH Swiss mouse marrow was not detected in the present studies nor in any of over 400 cultures harvested in our studies over the last two years. Ten of 10 DBA/2J and 8 of 8 C57BL/6J cultures generated permanent WEHI-3 CM dependent lines forming GM-CFUC. These data establish that virus infection is not required for establishment of hemopoietic progenitor cell lines.

DISCUSSION

The present data demonstrate a significant effect of mouse strain genotype on longevity of hemopoiesis in long-term bone marrow cultures. The methods designed for establishment of the cultures were extremely important and incorporated several changes in the original Dexter method [12] which we have previously delineated [13, 25]. In particular, cultures kept for over 35 days in 25% horse serum led to a significantly shorter longevity of hemopoiesis compared to cultures initiated in or switched at day 28 to growth in 25% FCS [25]. Our present report of hemopoiesis in C57Ks/J marrow for over 44 weeks is similar to that detected with DBA/2, AKr/J, and C58/J marrow cultures [14]. In contrast, marrow cultures identically established from C3H/HeJ or NIH Swiss mouse marrow cultures identically established from C3H/HeJ or NIH Swiss mouse marrow maintained hemopoiesis for a significantly shorter period in all cases less than 35 weeks

Table IV. Duration of Growth of Nonadherent Cells From Bone Marrow Cultures in Secondary Suspension Culture Supplemented With WEHI-3 CM

	Duration of growth of cells of week 8 harvest from primary culture in secondary suspension culture (wks) (number cultures growing/number tested)[b]			
Mouse strain[a]	10	25	30	>50
NIH	5/20	2/20	0/20	N.T.
C57Ks/J	20/20	20/20	16/20	16/20*
(NIH Swiss X C57Ks/J)F1	8/35	8/35	8/35	6/35*
(NIH Swiss X C57Ks/J)F2	20/37	18/37	6/37	1/37*
(NIH Swiss X C57Ks/J) X NIH Swiss	17/28	6/38	3/38	0/38

[a]Nonadherent cells were removed from each mouse marrow culture at week 8 and centrifuged at 1000g for 10 minutes and resuspended in 4.0 ml of McCoy's 5A medium supplemented with 10% WEHI-3 cell CM. Cultures were split as described in Materials and Methods.

[b]The results are presented as the number of culture harvests which grew in suspension for the indicated time over the number tested. *Sixteen of 20 C57Ks/J, one of 37 (Ks X N) F2, and 6 of 35 (Ks X N) F1 cultures generated cells which could be cloned in agar and established permanent subclonal lines. Harvests of nonadherent cells from late primary cultures did not establish. Each line produced GM-CFUc in WEHI-3 but not L929 CM, and 10^7 cells were not detectably leukenogenic in newborn mice after 12 months.

[14]. Other methods of long-term marrow culture have yielded very different results. For example, "recharged" AKr/J marrow cultures established in horse serum without added hydrocortisone generated GM-CFUc for 6–8 weeks [29] as compared to over 25 weeks by the present method [14]. It is concluded that culture conditions are therefore extremely important for comparative studies of the longevity of hemopoiesis in vitro.

The present report demonstrates that marrow cultures from C57Ks/J, (NIH Swiss × C57Ks/J) F1, F2, and the backcross generation of (NIH Swiss × C57Ks/J) F1 on NIH Swiss mice show a duration of hemopoiesis which is on average longer than that detected with marrow cultures from NIH Swiss mice. However, individual cultures from individual mice in the F1, F2, and backcross generations were biologically similar in duration of growth to each parent marrow culture, some growing 45 weeks (C57Ks/J-like) and some failing to generate GM-CFUs past week 35 (NIH Swiss-like). However, spontaneously inducible C57Ks/J ecotropic endogenous virus was detectable in cultures from 34 of 35 (C57Ks/J × NIH Swiss)F1 mice and in 50–67% of (C57Ks × NIH Swiss) F2, and (C57Ks/J × NIH Swiss) × NIH Swiss backcross mouse marrow cultures. The high frequency of detection of virus replication was not concordant with increased longevity of hemopoiesis, since only 14 of 35 (C57Ks × NIH Swiss) F1 mouse marrow cultures generated GM-CFUc past 40 weeks. Thus, other nonviral factor(s) are associated with longevity of hemopoiesis in vitro, in addition to replication of endogenous or added exogenous virus [14]. The genetic pattern of spontaneous induction of the endogenous C57Ks/J virus in marrow cultures was detected at a frequency comparable to the detection of virus following Iudr treatment of mouse embryo fibroblast cells from C57B6/J, (C57B6/J × NIH Swiss) F1, (C57B6/J × NIH Swiss) F2, and (C57B6/J × NIH Swiss) × NIH Swiss backcross generations [30, 31]. In other studies we detected Iudr-inducible N-tropic virus in each of 9 (NIH Swiss × C57Ks/J) F1 embryo fibroblast cell lines (unpublished observations). These data suggest that the locus for the N-tropic endogenous virus in C57Ks/J marrow stromal and/or hemopoietic cells is also inherited as an autosomal dominant [30–32].

The establishment of permanent cell lines from nonadherent cells removed from primary marrow cultures also showed a significant effect of mouse strain genotype. Secondary suspension cultures from C57Ks/J mouse marrow, but not NIH Swiss marrow, grew as permanent cell lines and could be cloned in 0.3% agar with subclonal lines sustaining continuous passage in vitro for periods in excess of 18 months. However, permanent acute myelogneous leukemia cell lines have been induced in NIH Swiss marrow cultures by addition of Friend or Abelson murine leukemia virus [18, 25, 28]. Both virus infected leukenogenic [35] and the present nonleukenogenic cell lines differentiated to neutrophillic granulocytes, distinct from permanent mast cell/basophil lines also dependent on a factor(s) produced by WEHI-3 cells [34].

Of particular interest in the present study was the effect of mouse strain genotype on the kinetics of in vitro generation of hemopoietic stem cells. Significant numbers of GM-CFUc were detected in some C57Ks/J cultures for over 40 weeks. These GM-CFUc recovered from cultures of C57Ks/J mouse marrow at 40 weeks were morphologically indistinguishable in colony size and cell number from those found in early cultures at weeks 4 or 5. This data suggests that hemopoietic stem cells giving rise to the GM-CFUc were phenotypically indistinguishable between late and early cultures. These results confirm and extend our previous studies of CFUs in C3H/HeJ mouse marrow cultures, showing that the self-renewal properties of pluripotent hemopoietic stem cells from late cultures were not detectably different from those removed from early cultures once the steady state of hemopoiesis was reached [16]. Further studies are indicated to determine how endogenous retroviruses and other mouse strain genetically regulated functions affect hemopoiesis and leukemogenesis in vitro.

ACKNOWLEDGMENTS

Supported by NIH-NCI VCP Contract N01CP71051 and grants CA25412-02 and CA26785-01. I thank M.A. Sakakeeny and K. Berndtson for technical assistance.

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