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CHARACTERISTICS OF THE IN VITRO MONOCYTE-MACROPHAGE COLONY-FORM--ETC(U)
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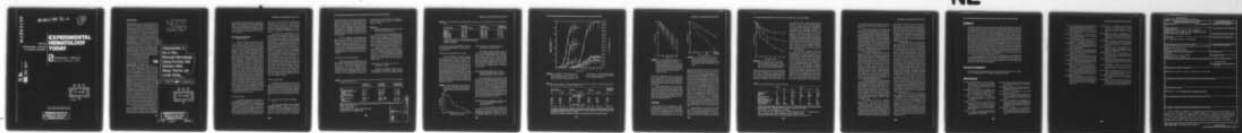
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INTRODUCTION

The population of *in vitro* colony-forming cells (CFU-c) has been found to be heterogeneous with respect to several characteristic parameters such as buoyant density (10, 21, 32), sedimentation rate (29), adherence to glass beads (21), cell cycle (19, 20), and response to various colony-stimulating activities (CSA) as well as colony-enhancing factors (28, 29, 32). In addition, the degree of heterogeneity has varied with the species and age of animal (20, 23) as well as the tissue source from which the CFU-c are derived. Recently, several subpopulations or different classes of colony-forming cells have been described (5, 13, 14, 16, 32). Lin and coworkers (5, 12-15) have reported on a class of monocyte-macrophage colony-forming cell (CFC) derived from thioglycollate-induced peritoneal exudate (PE-CFC) and pleural effusion (PL-CFC) as well as from free alveolar cells. van den Engh and Bol (29) and Williams and van den Engh (32) have also reported the detection of an additional subpopulation(s) of bone marrow-derived CFU-c that responded in the presence of CSA to the addition of specific enhancing factors. These subpopulations were identified by their differential buoyant density (32) and response to the enhancing factor (28, 29). In addition, we have recently reported the detection of monocyte-macrophage CFC within the thymus (T-CFC) and lymph nodes (LN-CFC) of the mouse (16). It was found that the T-derived and LN-derived CFC shared two characteristics with those CFC derived from the peritoneal exudate, pleural effusion, and alveolar space which differed significantly from the same parameters measured for bone marrow-derived CFU-c. They were: the markedly long lag period (6-10 days) prior to initiation of colony formation, and the apparent unidirectional monocyte-macrophage line of differentiation. Two additional parameters characteristic for T-derived and LN-derived CFC were at variance with those reported for BM-derived CFU-c as well as PE-derived, PL-derived, and alveolar cell-derived CFC. Colonies derived from thymus and LN-CFC exhibited a markedly slower rate of appearance once colony formation was initiated, and colony formation was apparently dependent on the presence of specific factor(s) in pregnant mouse uterus extract (PMUE) alone (16).

The present experiments were designed to determine additional properties of this class of CFC derived from thymus (T) and lymph node (LN) in an effort to examine their relationship to those CFC derived from peritoneal exudate (PE),

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Characteristics of the *in Vitro* Monocyte-Macrophage Colony-Forming Cells Detected within Mouse Thymus and Lymph Nodes,

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pleural effusion, and alveolar cells as well as those CFU-c derived from bone marrow (BM), spleen (SPL), and peripheral blood leukocytes (PBL). Properties were studied such as cluster-to-colony ratio, sensitivity to the absence of PMUE in culture, radiation and drug sensitivity, and the fraction in cell cycle.

MATERIALS AND METHODS

TISSUE SOURCE

Thymuses, cervical and mesenteric lymph nodes, spleens, peripheral blood leukocytes, peritoneal exudate cells, and femoral bone marrow cells were obtained from 8- to 12-week-old male and female mice (Cumberland View Farms, Clinton, Tenn.) of strains AKR/Cum-BR and BALB/C Cum-BR, B6D2F1/Cum-BR, C57BL/6 Cum-BR, and random-bred Ha/ICR-BR. The animals were maintained on a 6:00 AM to 6:00 PM (light-dark) cycle. Wayne Lab-Blox and acidified (pH 2.5) water were available *ad libitum*. All mice were acclimated to laboratory conditions for 2 weeks. During this time they were certified free of lesions of murine pneumonia complex, and of oropharyngeal *Pseudomonas* spp. Approximate numbers of thymus, lymph node, spleen, and bone marrow cells were prepared and suspended in McCoy's 5A medium with 25 mM Hepes buffer and 15% fetal calf serum (McCoy's 5A/15% FCS). Peripheral blood leukocytes were obtained by dextran sedimentation of heparinized blood obtained by cardiac puncture of mice under ether anesthesia. Exudate cells from the peritoneal cavity were stimulated to migrate there by an injection of 2.5% thioglycollate medium (TM) 3 days prior to lavage with 5 ml of Spinner modified Hanks' balanced salt solution (HBSS) containing 5 μ g heparin/ml (15). Harvested exudate cells were pooled, centrifuged, and washed two times and resuspended in McCoy's 5A/15% FCS prior to counting and dilution for culture.

IN VITRO CULTURE

The culture technique used was similar to that described by Bradley et al. (2) using the extract of pooled mouse placentae, membranes, and gravid uteri (PMUE) as the source of CSA. PMUE was prepared in the same manner, using water extraction, ammonium sulfate fractionation, dialysis, and heating. Each pooled extract was made up to a final volume so that 1 ml of extract was obtained per gram of wet weight tissue used.

Also used were L-cell conditioned medium (LCM) as prepared by Austin et al. (1), and mouse sera collected after stimulation with bacterial endotoxin (*Salmonella typhosa*, Difco, Detroit, Mich.) as described by Quesenberry et al. (24). The serum was diluted 1:6 with McCoy's 5A prior to use.

The ability of the blood leukocytes, marrow, spleen, peritoneal exudate, lymph node, and thymus cells to form colonies in culture was determined by using each of the various stimulating factors mixed (v/v) with culture medium (CMRL 1066/10% FCS + 10% trypticase soy broth + 5% horse serum and supplemented with 30 μ g/ml of L-asparagine) plus 0.5% agar. The cell types were each suspended in various concentrations in an upper layer of culture medium and 0.3% agar. Cultures were incubated at 37°C in a humidified CO₂ in air atmosphere. Colonies of more than 50 cells counted after 7 to 10 days of incubation were considered derived from CFU-c, while those colonies counted after 22 to 25 days of culture were considered to be derived from CFC. In order to determine the survival of CFU-c and CFC in the absence of PMUE, a group of marrow and thymus cell cultures were prepared without PMUE. At selected days after incubation, PMUE was added to the culture dish in a 0.5 ml aliquot of 0.33% agar:media. Control cultures were initiated immediately after they had jelled. Respective marrow-derived and thymus cell-derived cultures were then incubated for an additional 10 to 24 days after addition of PMUE.

COLONY MORPHOLOGY

Morphology was determined by removing individual colonies from the agar by means of a pasteur pipette and placing in a small volume of McCoy's 5A/15% FCS. Glass slides were prepared through use of a cytospin centrifuge (Shandon Southern Instruments Limited, Sewickley, Penn.), air-dried, and stained with a Wright-Giemsa solution.

CELL CYCLE STATUS OF COLONY-FORMING CELLS

In vitro cell killing with tritiated thymidine (³H-TdR). BM-, T-, and LN-derived cell suspensions were incubated at a concentration of 4–5 × 10⁶ cells/ml in HBSS at 37°C for 10 min. Two hundred microcuries of ³H-TdR (specific activity 30 Ci/mM, New England Nu-

clear, Boston, Mass.) were added per ml of culture volume and incubated for an additional 20 min. Duplicate cell suspensions were incubated in the presence of 20 μ g/ml of unlabeled thymidine. After incubation all culture tubes were diluted with 10 ml of ice-cold HBSS containing 20 μ g/ml of thymidine and centrifuged at 1000 *g* for 10 min. Cell pellets were washed three times with ice-cold HBSS, counted, and cultured in agar at several concentrations.

In vivo killing with hydroxyurea (HU). Mice were injected i.p. with hydroxyurea (Aldrich Chemical Co., Milwaukee, Wis.) (900 mg/kg) 2 hr prior to sacrifice and removal of the T, LN, SPL, and BM for preparation of cell suspensions for *in vitro* culture. Peritoneal cells were collected by lavage with Spinner modified HBSS (5 μ g heparin/ml) 2 hr after injection with HU. All cell suspensions were cultured at several concentrations.

IRRADIATION AND DRUG SENSITIVITY

Whole body radiation (WBI) of mice was performed by midline bilateral exposure from the Armed Forces Radiobiology Research Institute ⁶⁰Co source at a dose rate of 42rad/min. Cyclophosphamide (CY) (Cytosan, Mead Johnson, Evansville, Ind.) and Vinblastine (VLB) ("Velbe", Lilly and Company, Indianapolis, Ind.) were administered i.v. as a single injection. Animals were

euthanatized 24 hr after radiation or exposure to cytotoxic drugs; tissues were removed and prepared for culture.

RESULTS

RELATIVE INCIDENCE OF CFC, CFU-c AND CLUSTER-TO-COLONY RATIOS

T-CFC and LN-CFC had significantly lower concentrations relative to both TM-stimulated PE-CFC as well as BM-derived and SPL-derived CFU-c. In addition, T-derived and LN-derived cultures had markedly lower cluster-to-colony ratios (0.8 to 1.4) than those observed for PE-CFC cultures (5.8) and those CFU-c cultures derived from BM (6.4), SPL (3.6), and PBL (10.8) (Table 1).

Significant differences were observed in the number of T-CFC in several strains of mice (Table 2). The AKR strain had a greater concentration ($p < 0.001$) while BALB/C and C57BL/6 were lower ($p < 0.001$) than Ha/ICR and B6D2F₁ strains. On an organ basis, only the T-CFC in BALB/C and C₅₇BL/6 remained less than the other strains tested.

SURVIVAL OF T-CFC AND BM CFU-c IN THE ABSENCE OF PMUE

T-CFC were markedly resistant to the absence of PMUE in culture (Fig. 1). The more sensitive BM CFU-c were reduced to 60% of control

TABLE 1 The Frequency and Cluster-to-Colony Ratios of Colony-Forming Cells (CFC, CFU-c) from Various Hematolymphopoietic Sites in the Mouse^a

	COLONIES PER 10 ⁶ CELLS	COLONIES PER ORGAN	CLUSTER-TO- COLONY RATIO
CFC			
Peritoneal exudate, TM	27.3 $\pm$ 4.7	2,317 $\pm$ 369 ^c	5.8 (4) ^b
Peritoneal exudate, normal	8 $\pm$ 3	18 $\pm$ 4	(4)
Thymus	31 $\pm$ 4	2,506 $\pm$ 295	0.8 (20)
Cervical lymph node	22 $\pm$ 4	915 $\pm$ 267	1.3 (18)
Mesenteric lymph node	20 $\pm$ 6	329 $\pm$ 93	1.4 (12)
CFU-c			
Bone marrow (femur)	1,802 $\pm$ 122	39,284 $\pm$ 3,902	6.4 (15)
Spleen	62 $\pm$ 9	6,363 $\pm$ 1,168	3.6 (16)
Peripheral blood	10 $\pm$ 5	74 $\pm$ 17 ^d	10.8 (8)

^aPMUE used at 3.0% (final v/v) of culture medium-agar mix. Mean values $\pm$ SEM. Peritoneal exudate values $\times 10^3$. B6D2F₁ male and female mice.

^bNumber of experiments.

^cExpressed as total CFC harvested from peritoneal cavity.

^dExpressed as CFU-c per milliliter of blood.

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TABLE 2 The Incidence of Colony-Forming Cells (CFC) and Cell Clusters in Thymus Cell Suspensions from Various Strains of Mice^a

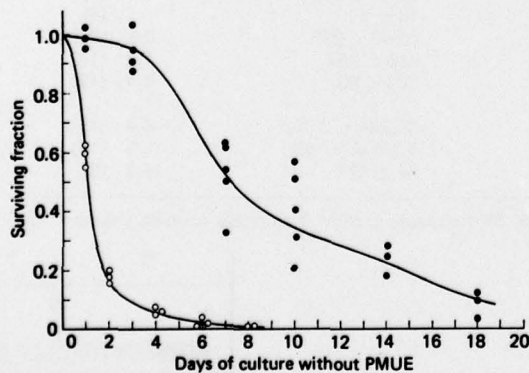
MOUSE STRAIN	CFC PER 10 ⁶ CELLS $\pm$ SEM	CFC PER ORGAN $\pm$ SEM	CLUSTER-TO- COLONY RATIO
AKR/Cum	41.4 $\pm$ 2.2	3,198 $\pm$ 237	0.9 (7) ^b
Ha/ICR	31.6 $\pm$ 4.2	2,647 $\pm$ 346	1.1 (14)
B6D2F1/Cum	31.9 $\pm$ 3.6	2,506 $\pm$ 295	0.8 (20)
B6CBF1/Cum	26.7 $\pm$ 5.1	2,136 $\pm$ 320	0.8 (10)
BALB/c Cum	12.2 $\pm$ 3.3	1,013 $\pm$ 137	1.4 (12)
C57BL/6 Cum	19.8 $\pm$ 6.0	1,531 $\pm$ 125	1.1 (12)

^aPMUE used at 3.0% (final v/v) of culture medium-agar mixture.^bNumber of experiments.

within 24 hr of culture without PMUE, whereas it took 6 days without PMUE for an equivalent effect with T-CFC.

APPEARANCE OF COLONIES IN CULTURE

The marked delay (6–10 days) in appearance of colonies derived from T-CFC and LN-CFC was reported earlier (16) and is similar to that previously observed for PE-CFC (15) and confirmed here (Fig. 2). Once colony formation is initiated, the rate of appearance of colonies derived from T-CFC and LN-CFC is much slower than that observed for colonies derived from PE-CFC. The rate of appearance of colonies from PE-CFC is not significantly different from that observed for BM, SPL, and PBL colonies derived from CFU-c. The number of T-CFC-derived and LN-CFC-derived colonies doubled every 58 and 42 hr, respectively. The number of PE-CFC and colonies derived from BM, SPL, and PBL CSU-c doubled within a range of every 17 to 23 hr.

FIGURE 1. The surviving fraction of bone marrow- (○) and thymus- (●) derived CFU-c versus time in culture in the absence of PMUE. Values are individual means of four culture dishes from each experiment.

FRACTION OF COLONY-FORMING CELLS IN S-PHASE OF THE CELL CYCLE

The fraction of T-CFC in S-phase of the cell cycle did not differ significantly from that fraction measured for BM or SPL CFU-c when measured either by the ³H-TdR or the HU techniques (Table 3). Mean values of the percentage reduction using ³H-TdR ranged from 37.0 to 43.8%, while mean values using HU ranged from 37.0 to 40.1%. The percentage reduction in colony formation of PE-CFC was slightly higher, at 48.9% of control values.

COMPARATIVE RADIOSENSITIVITY OF CFU-c DERIVED FROM BM AND SPL AND CFC DERIVED FROM PE, T, AND LN CELLS

The radiosensitivity of T-CFC, LN-CFC, and PE-CFC did not differ significantly from that observed from BM CFU-c (Fig. 3). Thymus-CFC and LN-CFC, however, were found to be significantly more radiosensitive than PE-CFC and PL-CFC (5). The T-CFC and LN-CFC had respective D₀ values of 85 $\pm$ 5 rad (SEM) and 80 $\pm$ 10 rad, while PE-CFC and PL-CFC had reported D₀ values of 117 rad and 116 rad, respectively. The D₀ value for PE-CFC observed in our laboratory was 100 $\pm$ 7 rad. Spleen CFU-c were consistently more radiosensitive than BM-derived CFU-c.

SENSITIVITY OF T-CFC, BM CFU-c, AND SPL CFU-c TO CYCLOPHOSPHAMIDE (CY) AND VINBLASTINE (VLB)

CY is an agent classified as proliferation-dependent in its action on target cells (4). The dose-survival curves are exponential in form through 300 mg/kg of body weight of CY (Fig. 4). No significant difference in sensitivity was ob-

In Vitro Monocyte-Macrophage Colony-Forming Cells in Mouse Thymus and Lymph Nodes

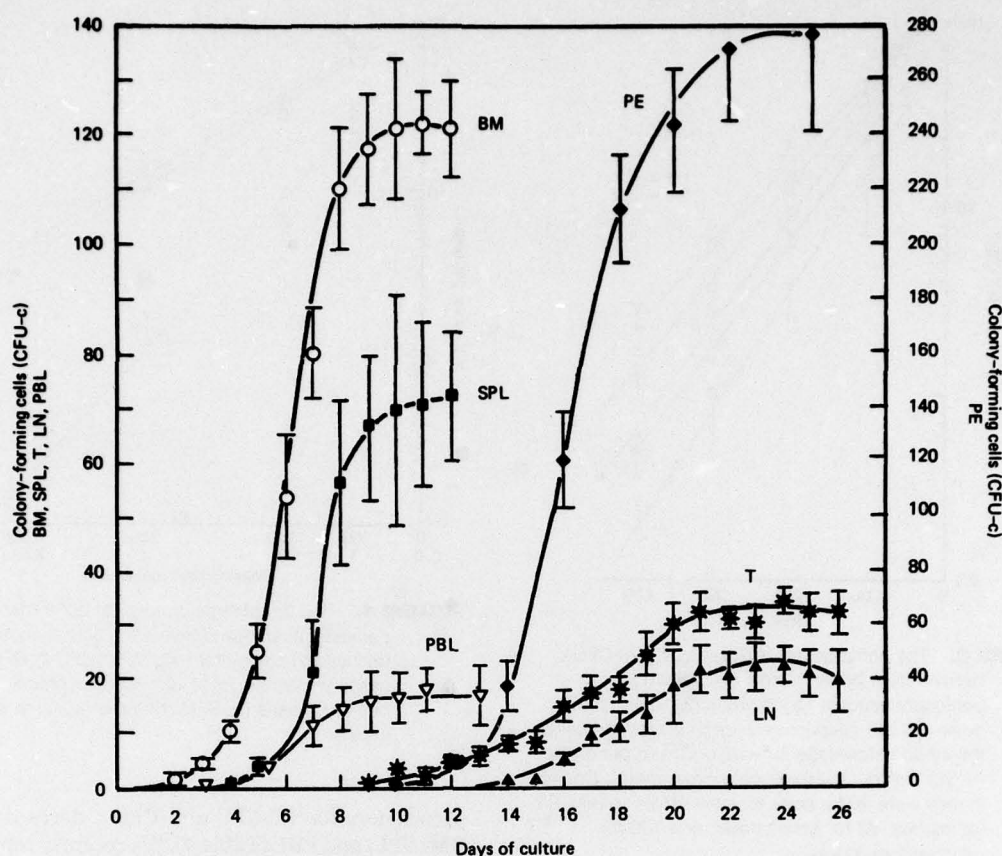


FIGURE 2. The appearance of CFU-c derived from 5×10^4 bone marrow ($\circ$), 1×10^6 spleen ($\blacksquare$), 1×10^6 peripheral blood leukocytes (∇), 1×10^4 peritoneal exudate stimulated with thioglycollate ($\bullet$), 1×10^6

thymus ($*$), and 1×10^6 cervical lymph node ($\blacktriangle$) cell suspensions. Values are means ($\pm$ SEM) from four to six replicate experiments.

TABLE 3 Tritiated Thymidine (*in Vivo*) and Hydroxyurea (HU) (*in Vitro*) Killing of Colony-Forming Cells Derived from Bone Marrow, Spleen, Thymus, and Peritoneal Exudate Cultures^a

BONE MARROW		SPLEEN		THYMUS		PERITONEAL EXUDATE ^b
HU	³ H-TdR	HU	³ H-TdR	HU	³ H-TdR	HU
Percent Depression in Colony Formation						
37.3	25.0	38.6	45.8	39.1	48.1	55.5
38.6	38.7	35.8	40.2	39.3	43.1	44.2
54.5	48.2	41.7	30.6	40.6	33.3	46.9
42.0	47.3	39.1	31.3	39.0	34.3	—
46.4	46.2	—	—	—	—	—
Mean Values ($\pm$ SEM)						
43.8 $\pm$ 4.2	40.1 $\pm$ 4.6	38.8 $\pm$ 1.4	37.0 $\pm$ 3.7	37.0 $\pm$ 2.8	39.5 $\pm$ 3.6	48.9 $\pm$ 3.9

^aHU values ($\pm$ SEM) are percent depression per organ (femur, spleen, and thymus) with the exception of peritoneal exudate (percent depression per 10^4 CFC). ³H-TdR values are percent depression per concentration of CFU-c, CFU in culture (BM, 10^5 cells; SPL, 10^6 cells; T, 10^6 cells).

^bPeritoneal CFC stimulated to peritoneal site by injection of thioglycollate medium 3 days prior to harvest.

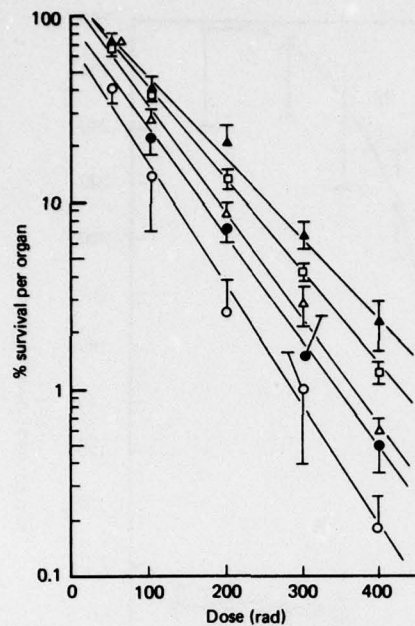


FIGURE 3. The comparative radiosensitivity of CFU-c derived from bone marrow ($\square$), spleen ($\circ$), peritoneal exudate ($\blacktriangle$), thymus ($\triangle$), and lymph node ($\bullet$) cell suspensions. Each point represents the mean percentage survival of CFU-c per organ ($\pm$ SEM) of four to 10 replicate experiments. Doses in rads were 90 for bone marrow, 70 for spleen, 85 for thymus, 80 for lymph node, and 100 for peritoneal exudate.

served between BM-derived CFU-c and T-derived CFC, while the SPL CFU-c showed a significantly greater sensitivity to the drug. An LD₁₀ dose of 200 mg/kg resulted in 10–12% survival for BM CFU-c and T-CFC, while SPL CFU-c survival was approximately 1%.

VLB is phase specific in its mode of action (4). The survival curves decreased exponentially with low doses of VLB, then approached constant values of survival with dose at a saturation concentration of VLB (Fig. 5). The percentage survival of colony-forming cells per organ at maximum drug concentration was 12% for BM CFU-c, 2% for T-CFC, and 0.3% for SPL CFU-c.

DISCUSSION

In these experiments, we compared the cluster-to-colony ratios, survival in the absence of CSA, radiosensitivity, sensitivity to the proliferation and cell cycle, phase-specific drugs, CY and VLB, respectively, and the fraction in cell cycle of T-derived and LN-derived CFC with the same

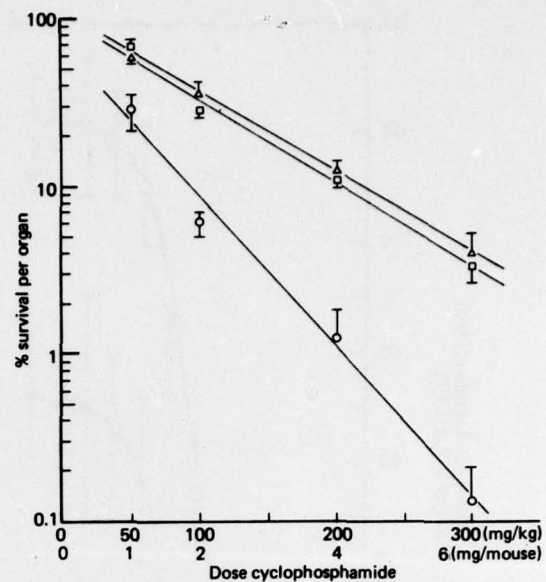


FIGURE 4. The percentage survival of bone marrow-derived ($\square$), spleen-derived ($\circ$), and thymus-derived ($\triangle$) colony-forming cells (CFU-c, CFC) per organ versus the dose of cyclophosphamide. Values are means ($\pm$ SEM) of three replicate experiments.

parameters for PE-CFC and CFU-c derived from BM, SPL, and PBL (Table 4). We recently reported (16) their low incidence, singular direction toward monocyte-macrophage differentiation, apparent sole specificity of PMUE to initiate colony formation, the long delay prior to colony formation, and the more gradual appearance of colonies in culture compared to BM-derived CFU-c. In addition the present data indicate that the T-CFC and LN-CFC were significantly different from CFU-c derived from BM, SPL, and PBL with respect to their survival in the absence of CSA and their cluster-to-colony ratios. Similarities with the CFU-c were observed in radiosensitivity, response to cyclophosphamide, and the fraction in cell cycle. In comparison with the CFC derived from peritoneal exudate, two additional similarities were observed: namely, the marked survival in the absence of CSA and the same fraction in cell cycle. Thymus and LN-CFC were, however, significantly lower in cluster-to-colony ratio and more sensitive to ^{60}Co γ radiation than PE-CFC.

The similarities between T-CFC, LN-CFC and PE-CFC, PL-CFC and alveolar CFC with respect to (a) the long lag time prior to colony formation, (b) singular production of monocyte-macrophage colonies, (c) survival in the absence

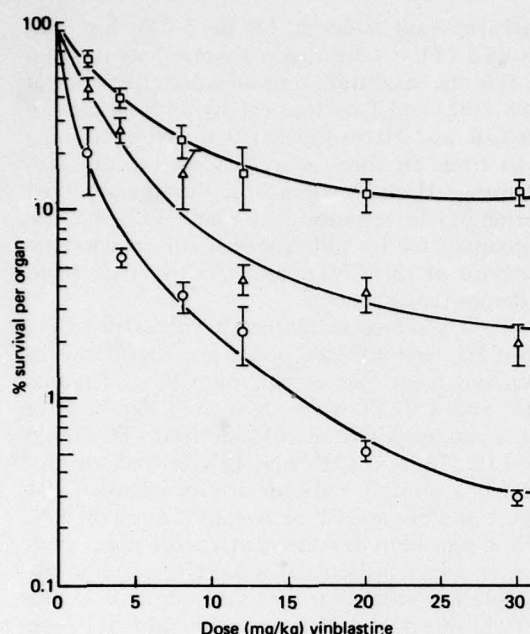


FIGURE 5. The percentage survival of bone marrow-derived ($\square$), spleen-derived ($\circ$), and thymus-derived (Δ) colony-forming cells (CFU-c, CFC) per organ, versus the dose of vinblastine. Values are means ($\pm$ SEM) of three replicate experiments.

of CSA, and (d) the fraction in cell cycle support the contention that these CFC are probably of the same class or population of progenitor cells (5, 16). A number of investigators have shown that monocyte-macrophage cells detected within inflammatory exudate, peritoneal exudate, and

lung alveoli have their origin in a bone marrow precursor (7, 8, 30, 31). It is most likely that the CFC have a similar origin in the heterogeneous bone marrow CFU-s and/or CFU-c population(s). The granulocyte-to-macrophage transition is a characteristic feature of the CFU-c culture system (20). If we assume the existence of a common progenitor cell for both granulocyte and macrophage cell lines, the persistence of monocyte-macrophage morphology in CFC cultures may be a product of the inductive influence of lymphoid organ specific microenvironment and/or locally produced factors to commit the heterogeneous marrow-derived CFU-c to a single line of differentiation. The same influence might be expected within inflammatory tissue, the serous cavities, and the alveolar tissue.

The relatively low incidence of T-CFC and LN-CFC as well as the low cluster-to-colony ratio of approximately 1.0, would be indicative of a resident population of progenitor cells with a limited monocytopoietic capacity within the lymphoid microenvironment. This is based on the assumption that cell clusters are the near progeny of the CFC, a relationship similar to that ascribed to clusters forming in CFU-c cultures (17). Cultures derived from CFU-c within BM, SPL, and PBL as well as those derived from PE-CFC were characterized by larger cluster-to-colony ratios ranging from 3.6 to 10.8 (Table 1). The higher ratios were characteristic of cell populations with extensive granulo- and/or monocytopoiesis. A similar situation may be found within certain fetal tissues. CFU-c are found within the fetal yolk sac and liver. Those organs, however, are characteristi-

TABLE 4 Characteristic Parameters of Colony-Forming Cells^a

	CFC			CFU-c		
	T	LN _c	PE	PBL	SPL	BM
Incidence per 10 ⁶	31	22	27,300	10	63	1,800
Morphology ^b	M	M	M	G-M	G-M	G-M
CSA ^c	A	A	A,B,C,+	A,B,C,+	A,B,C,+	A,B,C,+
Cluster-to-colony ratio	1.0	1.0	5.8	10.8	3.6	6.4
Lag time (days)	9-12	13-15	10-12	3	3	2
Rate of colony appearance ^d	58	43	22	28	17.5	17.5
Survival minus CSA ^e	60%	—	40%	—	—	3%
Radiosensitivity, D ₀ (rad)	85	80	100	—	70	90
Cell cycle status	Rapid	Rapid	Rapid	—	Rapid	Rapid

^aB6D2F1, adult, male and female mice. Source: T = thymus, LN_c = cervical lymph node, PE = thioglycollate-stimulated peritoneal exudate, PBL = peripheral blood leukocytes, SPL = spleen, BM = bone marrow.

^bMorphology: M = monocyte-macrophage, G = granulocyte.

^cCSA, A = PMUE, B = L-cell conditioned media, C = endotoxin-stimulated mouse sera, + = other CSA's active.

^dRate of colony appearance in culture as doubling time (hours) of colonies per culture.

^ePercentage survival of CFC and CFU-c after 6 days in absence of CSA PE = CFC value (ref. 13).

cally nongranulopoietic and subsequently have low cluster-to-colony ratios of approximately 2.0 (20, 23).

Thymus CFC survival in the absence of PMUE was remarkably similar to that observed for the peritoneal exudate CFC in the absence of L-cell conditioned medium (15). Both showed relatively little sensitivity to the absence of stimulating factor during the first 3 days of incubation, during which time marrow CFU-c had decreased to less than 20% of control values. The marked sensitivity of marrow CFU-c is in agreement with that previously shown by Metcalf (18). It took approximately five times as long (15 days) in the absence of stimulating factor to reduce colony formation in thymus and peritoneal exudate cultures to values 20% of their control.

The inordinate lag period prior to colony formation, marked survival in the absence of PMUE, and slower rate of appearance in culture indicated that perhaps the CFC population detected within the T and LN had proliferative characteristics different from those observed for BM- and SPL-derived CFU-c. Bruce and coworkers (4) have classified cyclophosphamide and vinblastine as agents which are proliferation-dependent and phase-specific (mitosis) in their respective cytotoxic effects on target cells. We used these agents in addition to the S-phase specific, *high specific activity* tritiated thymidine and hydroxyurea to determine the proliferative state of the colony-forming cell population detected within the thymus relative to PE-CFC and CFU-c within Femoral BM and SPL.

The marked depression of colony-forming cells by *in vitro* ^3H -TdR and *in vivo* HU incorporation suggested that the T-CFC and PE-CFC are in a state of rapid cycle similar to that observed for BM and SPL CFU-c. Spleen-derived CFU-c, however, have been reported to be less sensitive to ^3H -TdR incorporation (19) and therefore have a lesser percentage of the CFU-c in active cycle. This is in contrast to our results using the B6D2F₁ strain of mouse which showed no significant difference between the reduction of CFU-c derived from BM and SPL.

The response of T-CFC to cyclophosphamide did not differ from that of the BM-derived CFU-c, while SPL CFU-c showed a marked sensitivity to the action of the drug. Marrow CFU-c and T-CFC were reduced to 10–12% of control values by a dose of 200 mg/kg. This falls within the response observed for BM CFU-c of 7–30% survival noted by Brown and Carbone (3) and Millard et al. (22), respectively. Although the sensitivity of BM CFU-c to VLB was within previ-

ously reported values (3, 22), the T-CFC and SPL-derived CFU-c were markedly more sensitive to VLB at the saturation concentration. Exposure of BM-, SPL- and T-derived colony-forming cells to ^3H -TdR and HU suggested that colony-forming cells from all three sources were rapidly proliferating. Hence it is probable that the observed variability in response to CY and VLB may be accounted for by differences in the phenotypic makeup of the CFU-c and CFC derived from different tissue sources.

The radiosensitivities (D_0 values) of T-CFC, LN-CFC, and PE-CFC were not significantly different from that of marrow CFU-c. Thymus CFC and LN-CFC were, however, significantly more radiosensitive than PE-derived CFC (Table 4). LIN (12) and CHU and LIN (5) recently reported a similar radiosensitivity between BM CFU-c and PE- and PL-derived CFC. Only the SPL CFU-c proved to be consistently more radiosensitive in terms of both D_0 value (70 rad) and extrapolation number (<0.7). Guzman and Lajtha (9) observed a similar relationship between pluripotent stem cells (CFU-s) derived from marrow and spleen. They reported D_0 values of 69 rad and 82.5 rad with extrapolation numbers of 0.8 and 1.5 for SPL- and BM- derived CFU-s, respectively. The SPL-derived CFU-c may retain the characteristic sensitivity to radiation and drugs through a parent-progeny relationship, and/or the marked sensitivity may be solely a function of the splenic microenvironment which altered the phenotype of its resident stem and progenitor cells. This heterogeneity within a class of progenitor or stem cells was also observed for the T-CFC and LN-CFC. They differed from those CFC derived from peritoneal exudate, pleural effusion, and alveolar space in terms of (a) the sole specificity of PMUE to initiate colony formation (16), (b) their low cluster-to-colony ratio, (c) their greater radiosensitivity, and (d) their more gradual appearance in culture (16).

These differences observed for progenitor cells assumed to be within the same population emphasize the fact that the colony detected in the culture plate—just as the colony observed in the *in vivo* spleen assay—is not homogeneous with respect to several characteristic properties. The pluripotent stem cell (6, 9, 11, 25, 26), the granulocyte-monocyte progenitor cell (10, 19–21, 27, 32), and monocyte-macrophage progenitor cells are heterogeneous populations. These differences may in part reflect the influence of tissue specific microenvironment on determining the phenotypic expression of resident stem and/or progenitor cells.

SUMMARY

The enigmatic presence of *in vitro* colony-forming cells (CFC) within the thymus (T) and lymph node (LN) organs prompted us to determine additional characteristics of this cell population in an effort to examine their relationship to other colony-forming cells (CFC, CFU-c) derived from several hematopoietic sites. Their cluster-to-colony ratio, survival in the absence of colony-stimulating activity (CSA), radiosensitivity, drug sensitivity, and fraction in cell cycle were compared to the same parameters for CFU-c derived from bone marrow (BM), spleen (SPL), and peripheral blood leukocytes (PBL) and those CFC derived from thioglycollate-stimulated peritoneal exudate (PE). When compared with the parameters characteristic of CFU-c derived from BM, SPL, and PBL, the T-CFC and LN-CFC differed markedly in cluster-to-colony ratio, sensitivity to absence of pregnant mouse uterus extract (PMUE) in culture, and rate of appearance of colonies in culture. Similarities were observed in sensitivity to radiation (D_0 value) and cytotoxic drugs as well as the fraction in cell cycle. In comparison with the CFC derived from the peritoneal exudate, two similarities were observed; namely, the marked survival in the absence of CSA in culture and the same fraction in cell cycle. However, T-CFC and LN-CFC were significantly lower in cluster-to-colony ratio and more sensitive to ^{60}Co γ radiation than PE-CFC.

Data are provided on the nature of a subpopulation of the ubiquitous monocyte-macrophage CFC located in the thymus and lymph nodes of the mouse. The results indicated that the CFC is a heterogeneous population in several respects and support the implication that the tissue microenvironment may alter the phenotypic expression of the resident CFC population.

ACKNOWLEDGMENTS

The authors wish to acknowledge the excellent technical assistance of Ms. E. McCarthy, Mr. R. Brandenburg, Mr. J. Atkinson, and HMC R. Mitchell.

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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) CHARACTERISTICS OF THE IN VITRO MONOCYTE- MACROPHAGE COLONY-FORMING CELLS DETECTED WITHIN MOUSE THYMUS AND LYMPH NODES		5. TYPE OF REPORT & PERIOD COVERED
7. AUTHOR(s) T. J. MacVittie and T. L. Weatherly		6. PERFORMING ORG. REPORT NUMBER
9. PERFORMING ORGANIZATION NAME AND ADDRESS Armed Forces Radiobiology Research Institute (AFRRI) Defense Nuclear Agency Bethesda, Maryland 20014		8. CONTRACT OR GRANT NUMBER(s)
11. CONTROLLING OFFICE NAME AND ADDRESS Director Defense Nuclear Agency (DNA) Washington, D.C. 20305		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		12. REPORT DATE
		13. NUMBER OF PAGES
		15. SECURITY CLASS. (of this report) UNCLASSIFIED
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release; distribution unlimited		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES Published in Book <u>Experimental Hematology Today</u>		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number)		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The enigmatic presence of <u>in vitro</u> colony-forming cells (CFC) within the thymus (T) and lymph node (LN) organs prompted us to determine additional characteristics of this cell population in an effort to examine their relationship to other colony-forming cells (CFC, CFU-c) derived from several hematopoietic sites. Their cluster-to-colony ratio, survival in the absence of colony-stimulating activity (CSA), radiosensitivity, drug sensitivity, and fraction		

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20. ABSTRACT (continued)

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Data are provided on the nature of a subpopulation of the ubiquitous monocyte-macrophage CFC located in the thymus and lymph nodes of the mouse. The results indicated that the CFC is a heterogenous population in several respects and support the implication that the tissue microenvironment may alter the phenotypic expression of the resident CFC population.

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