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LA-UR--84-2663

DE84 016464

TITLE: Clonal Analysis of Bone Marrow and Macrophage Cultures

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SUBMITTED TO: Proceedings of the Fourth Leiden Conference on Mononuclear Phagocytes,
Noordwijk, The Netherlands, May 9-16, 1984.

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CLONAL ANALYSIS OF BONE MARROW AND MACROPHAGE CULTURES

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Key Words: bone marrow
macrophages
macrophage cell lines
growth factors
monoclonal antibodies
proliferation
differentiation

INTRODUCTION

The identification and organization of the major cell types that make up the mononuclear phagocyte system is based on conventional histological staining patterns. This system is discussed in the chapters by Van Furth (1), Van der Meer (2), Ginsel (3), and Hoefsmit (4). About two decades ago a similar situation existed for the identification of cells of the lymphoid system when only large, medium and small lymphocytes were delineated by histological and morphological characteristics. Today, the lymphoid system has been subdivided into a multitude of functional subpopulations based on surface membrane differences resolved by antibodies.

The mononuclear phagocyte system has evolved from the most primitive cells into a complex heterogenous system of cells that exhibit four major functions: 1) the removal of effete cells and the products of tissue breakdown; 2) synthesis and secretion of a multitude of regulatory molecules; 3) microbicidal and tumoricidal activity; and 4) initiation of specific immune responses through antigen presentation.

The diversity of function raises the question as to whether mononuclear phagocytes comprise a single lineage of cells or whether there are several different lineages, each exhibiting unique functions. This latter possibility is consistent with what we know about lymphocytes as represented by B-cells and T-cells and their distinctive subpopulations. For example, the expression of certain markers, such as Ia and complement receptors, might be the result of clonal expansion of discrete lineages (5). Such subpopulations, when separated, should produce stable phenotypes in culture. Alternatively, heterogeneity may be the result of maturational stages through which all mononuclear phagocytes pass in a linear developmental pathway. Properties such as adherence, phagocytosis and expression of Fc receptors may be accommodated within this single lineage differentiation scheme, with mononuclear phagocytes acquiring these properties as they mature (6,7). To further confuse the picture, there is also evidence that certain lymphokines may enhance or induce functional expression, e.g., tumoricidal (8,9) and microbicidal activities (10), and the expression of Ia (11). Other inducible properties of mononuclear phagocytes

include plasminogen activator (12), collagenase (13), elastase (14) and neutral protease secretion (15). These inducible, often transiently expressed activities, may not be acquired via true differentiation events and, as suggested by Ia induction experiments (11), may be found to be a property of all mononuclear phagocytes. Alternatively, the susceptibility to induction by various inducing agents may be acquired by certain mononuclear phagocytes populations as a consequence of their differentiation. Such populations may provide a means to order these cells into a maturational sequence.

Since mononuclear phagocytes proliferate in vitro from single progenitor cells when they are cultured with CSF-1 (16), it might be possible to establish lineages that can be used to study this functional heterogeneity. In this report, we review work on the proliferation and differentiation of bone marrow derived mononuclear phagocytes and the lineages that have been derived from them.

MATERIALS AND METHODS

C3H bone marrow was used for all the studies dealing with primary cultures. Bone marrow was flushed from the femur using 3 ml of culture medium. Culture medium consisted of α -MEM supplemented with 10% fetal bovine serum. Growth medium consisted of culture medium supplemented with 10% L-cell conditioned medium. The complete details for obtaining and culturing murine bone marrow have been previously described (17). For mass cultures, cells were adjusted to a concentration of 10^5 cells per ml in either culture medium or growth medium, and 5 ml were plated in 60 mm culture dishes. To determine colony formation, cells were adjusted to 1,000 or 100 cells per ml in growth medium, and 3 ml was plated into 35 mm dishes. After 7 days of incubation in a 10% CO₂ in an air atmosphere, the plates were rinsed and stained with methylene blue, and the number of clusters containing 50 or more cells were counted. The establishment of cell lineages has been previously described in detail (18).

For staining with monoclonal antibodies, nonadherent cells were first removed from primary cultures of bone marrow. The adherent cells were then removed with sodium pyrophosphate as previously

described (19). For the cell lines, cells were cultured on plastic petri dishes, and the cells were removed by a jet stream (directed at the cells) from a 22-gauge needle on a plastic syringe.

Cells were adjusted to 20×10^6 cells/ml, and 50 μ l were added to 10 X 75 mm glass tubes containing 50 μ l of culture supernatant derived from the hybridoma cells producing the monoclonal antibody. All procedures were carried out at 4°C. After 45 min, cells were washed in 4 ml of a buffer consisting of 1% sodium azide and 0.5% bovine serum albumin in phosphate buffered saline. After centrifugation, the supernatant was discarded, the cells were resuspended in 50 μ l of a 1/10 dilution of fluoresceinated goat anti-rat Ig. After a 15 min incubation, the cells were again washed in 2 ml of buffer and analyzed in the presence of 2 μ g/ml of propidium iodide using a flow cytometer. The propidium iodide is taken up by dead cells that can then be excluded from the analysis.

RESULTS AND DISCUSSION

Growth kinetics of murine bone marrow mononuclear phagocytes

Mononuclear phagocyte heterogeneity may be studied by taking advantage of the proliferative ability of their bone marrow progenitors. The proliferation of C3H bone marrow cells in culture medium and growth medium is shown in Figure 1. For these studies, the non-adherent cells were rinsed from the cultures and counted separately from the adherent cells. As shown in Figure 1A, when no LCM was present the nonadherent cells decreased continuously with a half time of 1.8 days. When LCM was present, the number of nonadherent cells decreased during the first 3 days of culture, and then their total number increased. As shown in Figure 1B, on day 0 only 4% of the cells were adherent after two hours of incubation. In the absence of LCM, there was a slow decrease in adherent cell survival over the 7 day culture period. In the presence of LCM these adherent cells increased so that by day 7 there were 30 times as many adherent cells. Thus, in the absence of LCM, no proliferation occurs, but when LCM is present, some nonadherent and adherent macrophages proliferate in culture.

Using flow cytometry, the cell volume and DNA distribution of bone

marrow cells cultured for various times in growth medium was determined, and the results are shown in Figure 2. On day 0, there were three distinct cell volume regions that could be identified. These populations have been identified by sorting the cells in each peak (19). Region one, representing 47% of the cells, consisted primarily of lymphoid cells. Region two (35% of cells) was predominantly myeloid, and region three (18% of cells) was comprised of large blastlike cells (19). As cells were cultured in growth medium, there was a continuous decrease in the fraction of cells in regions 1 and 2 with a concomitant increase in the fraction of cells in region 3. By day 6, a small peak appeared between region 1 and 2, accounting for 6% of the cells. This small population contained the nonadherent cells (19). The remaining cells (94%) were in region 3 and consisted of adherent macrophages. On day 0, 5% of the cells were in DNA synthesis as shown by the DN⁺ distribution of mithramycin stained cells. The fraction of cells in DNA synthesis increased to 23% by day 4 and then decreased to only 3% by day 6. This decrease in cycling cells was due to a depletion of growth factor to nonstimulating levels.

The above results suggest that nonadherent cells might contain precursors for the adherent macrophages. To test this possibility, the nonadherent cells were removed each day from different replicate cultures that had been initiated on day 0. These cells were appropriately diluted and replated in fresh growth medium. After 7 days incubation the proportion of cells that gave rise to macrophages was determined. From this proportion the total number of macrophage colony forming cells was calculated (proportion times the number of nonadherent cells shown in Figure 1A for that day). As shown in Figure 3, on day one, 0.8% of the cells in the nonadherent fraction could give rise to macrophage colonies. This number increased significantly with time in culture so that by day 6, 100,000 (or 30%) of the recovered nonadherent cells were now capable of forming macrophage colonies. This represents a 40-fold increase in the number of colony forming cells.

These results indicate that the nonadherent cell fraction indeed contained precursors for macrophages as they could be passed to new cultures and exclusively produced macrophage colonies. These nonad-

herent precursor cells also increased in number as they were cultured and by day 6 represented about one-third of the nonadherent cells recovered from the culture. This preculturing technique, therefore, has utility in increasing the proportion and number of macrophage precursors.

Extent of proliferation

Since the nonadherent cell fraction became enriched for cells capable of forming macrophage colonies, these cells offered a convenient way to passage mononuclear phagocytes. To determine the extent of the nonadherent cells' proliferative capacity, 10,000 nonadherent cells in 3 ml of growth medium obtained from 6 day primary cultures were seeded into 35 mm dishes. Every three or four days the nonadherent cells were removed and serially passed into new dishes. Three ml of fresh growth medium were added to the dishes from which the nonadherent cells were derived, and the number of macrophages on these dishes was determined 7 days after the initial seeding. Figure 4A shows that each passage resulted in the recovery of about the same number of nonadherent cells as was originally plated. The passage of these nonadherent cells produced a new culture of adherent cells that also proliferated. Thus, the nonadherent population contained cells that could produce a new population of nonadherent cells as well as a new population of adherent cells. The adherent cells increased with a doubling time of about 24 hours.

We were, however, unable to passage indefinitely the nonadherent cells and, as shown in Figure 4B, no proliferating cells could be recovered from the dishes after 24 days of culture as the doubling time increased to an undetermined time. By this time, however, a single precursor would have gone through an average of 27 divisions and produced 134 million progeny. An average of 10^7 cells per femur from C3H mice were found to contain 30,000 cells capable of forming macrophage colonies. Thus, colony forming cells from a single femur can produce 4×10^{12} progeny or 4 kg of macrophages. One would need about 8,000 liters of growth medium to produce this many cells in 24 days from one femur.

A model to describe mononuclear phagocyte proliferation and differentiation

We have developed a conceptual model (Fig. 5) to describe mononuclear phagocyte proliferation and differentiation based on the above results. Similar models have been proposed by others to describe fibroblast senescence (20,21,22). In this model, we assume that as cells divide they also differentiate and pass through recognizable classes of mononuclear phagocytes. We propose the existence of a stem cell (committed to the mononuclear phagocyte lineage) which can divide and produce another stem cell (self renewal) as well as a cell that begins the differentiation process. The cell that differentiates divides and differentiates continuously, thereby producing, with each division, more differentiated progeny as it approaches the formation of an end cell lineage. While doing so, progeny pass through each classical differentiation stage, designated monoblast (Mb), promonocyte (PM), monocyte (M), exudate macrophage (EM) and senescent macrophage (SM) (Fig. 5). In this model we have indicated that all cells from the monoblast stage through the monocyte retain proliferative capacity (even if they don't actually proliferate in situ). Most exudate macrophages can proliferate, but senescent macrophages cannot proliferate. In vitro, all cells capable of proliferation would do so as long as an adequate concentration of CSF-1 was present.

With each division of the stem cell, a whole new end cell lineage is initiated as it produces another stem cell and a cell that differentiates. As depicted in Figure 6, at any sampling in time, there will be multiple end cell lineages with cells in various stages of completion of their differentiation cycle. At this time, the colony that arises from a single stem cell will consist of cells in every stage of differentiation, but the most frequent cells will be the most mature cells.

A summary of the number of cells in each stage of differentiation is shown in Table 1 for different numbers of divisions after adding a single stem cell to a culture. As shown in this conceptual model, there will never be more than one stem cell at any point in time, even though it continually divides and produces, with each division, an exponentially expanding population of differentiating cells. At any time the frequency of cells within the least mature population will

decrease with the population doubling time. Thus, the frequency of stem cells decreases with each round of division of the differentiating cells even though the number of stem cells remains relatively constant. In the experiment shown in Figure 3, there would be a decreasing proportion of nonadherent cells that are stem cells due to this proliferative dilution until finally no stem cells would be passed. This would eventually result in an inability to establish a new culture of macrophages, and was the result that was found in this study.

While this is a conceptual model, and the actual kinetics of stem cell renewal and subsequent differentiation of daughters is not known, it lends itself to experimental verification. The model indicates that an established lineage of cells could only occur if stem cells were always passaged.

Established cell lineages

Permanent cell lines of mononuclear phagocytes have been established from several strains of mice by Johnson et al. (18). They were successful in this endeavor by growing bone marrow cells in agar containing an enriched growth medium. After 3-5 weeks the largest colonies (containing 31,000 to 250,000 cells) were selected for subsequent passage. Some of these colonies emerged into cell lines that still require the presence of CSF-1 for growth. Seventy separate cell lines were established using this technique. A list of some of the mouse strains from which these lines have been derived is shown in Table 2.

Characterization of mononuclear phagocytes

We have used several monoclonal antibodies (MABs) to characterize mononuclear phagocytes derived from bone marrow cultured for six days in growth medium. Table 3 summarizes the monoclonal antibodies that were found to stain cells in these cultures as well as their previously known specificity. For these studies the nonadherent cell fraction was separately characterized from the adherent cells. A more complete description of these studies has been previously described (19).

MAC-1 was found to stain virtually all the cultured cells. The staining pattern for MAC-1 is shown in Figure 6. MAC-1 binds to a

structural protein of the CR3 receptor (22); nearly all derived cells have this receptor. 30-H11 and G-2 were for the nonadherent cells. The staining pattern for 30-H11 is in Figure 6. Since nearly all nonadherent cells were stained 30-H11, we believe this marker is an excellent discriminator for the earliest precursor cells, some of which may be the MAC-3 was found on only a small proportion of nonadherent cells. Due to the high degree of autofluorescence of cultures and relatively weak staining by MAC-3, it is likely that there was a greater proportion of positive cells than could be detected. E-2 (Fig. 6) was found on the majority of adherent cells and the nonadherent cells.

The γ 2a myeloma protein MAR18.5 binds to the γ 2a Fc receptor on macrophages, and it was found to label about half of the adherent cells and a small proportion of nonadherent cells. The staining of adherent cells by MAR18.5 was not very bright, and autofluorescence may have interfered with our ability to resolve a greater number of positive cells by flow cytometry. These data suggest that CR3 receptors develop later than does the CR3 receptor (MAC-1).

Ten cell lines derived from normal bone marrow have been characterized using these monoclonal antibodies. Unfortunately, these ten cell lines are known to be contaminated with mycoplasma. While their usefulness may be compromised, we believe our data on them is sufficiently important to report. These cell lines provide a basis to believe such lines can be used to aid in the understanding of macrophage heterogeneity and differentiation. In writing new cell lines free of mycoplasma have been developed. We are attempting to free some of the existing lines of mycoplasma by passage through nude mice.

Among all the lines tested, there was a variation in the proportion of cells positive for any particular monoclonal antibody (Table 4). These variations could be interpreted as an indication of variation in the proportion of positive cells among lines, or they could be related to our ability to resolve positive cells due to autofluorescence. Thus, for the same epitope density, as autofluorescence increases, there is a concomitant decrease in the relative

of stained cells, and this decreased our ability to explicitly resolve them as positive. In an attempt to determine if autofluorescence was the problem, we reasoned that the relative proportion of positive cells would bear the same relationship for each monoclonal antibody within each cell line, but the actual proportion of positive cells among cell lines would be different depending on the relative epitope density to autofluorescence. Accordingly, we selected three markers which stained a relatively high proportion of cells in all the lines: MAC-1, MAC-2 and G-12. The results, shown in Figure 7, are consistent with our hypothesis that there were probably about the same proportion of positive cells in all lines, but our ability to detect them varied with each line due to autofluorescence.

Assuming that autofluorescence has limited our ability to detect positive cells, each cell line, shown in Table 4, has been listed from least to most autofluorescent. The results suggest that all the lines have a generally similar staining profile, except for 30-H11. Since this monoclonal antibody stained cells brightly and 30-H11 positive cells were absent in examples of both high and low autofluorescent cell populations, their absence could not be attributed to the autofluorescence problem. Thus, lines 10, 63, 18 and 35 did not have detectable 30-H11 positive cells, while all other lines contained cells positive for this marker. These data are consistent with the hypothesis that this marker is actually detecting, within the line, the presence or absence of cells that express the 30-H11 epitope. From the studies described in Table 3 (using normal bone marrow), we found that 30-H11 positive cells were only found in the nonadherent fraction, which contained the precursors for mononuclear phagocytes. Thus, some lines may be more or less differentiated, and the frequency of 30-H11 positive cells may be indicating this differentiation.

The phenotypes of the various lines do not appear to vary among mouse strains. No distinctive epitope or spectrum of epitopes could be found unique to lines derived from a particular strain of mice or their F_1 hybrids. Furthermore, their staining profile was similar to the cells derived from normal C3H bone marrow after 6 days of culture (Table 3) after correcting for autofluorescence (data not shown). We interpret these results to mean that, except for a great variation in

autofluorescence, these cells have maintained a stable phenotype on long term passage and they may be useful in providing a convenient supply of mononuclear phagocytes for functional studies.

Some lines were analyzed for their ability to respond to WEHI3 conditioned medium (WCM). This medium is a good source of IL-3, a factor that causes immature hematopoietic progenitor cells to proliferate (28). The results are summarized in Table 5. For these studies, 10^4 cells were plated in 3 ml culture medium containing either LCM or WCM in 35 mm culture dishes, and cell counts were performed every 2 or 3 days. All lines except line 30 responded to both growth factors. Line 9, however, did not immediately respond but exhibited a lag period of 8 days prior to entering an exponential growth phase. Line 16, while it responded, exhibited a considerably longer doubling time than when it was grown in LCM. Line 35 had a 5 day lag period (prior to exponential growth) after replating in either of the two conditioned media. These data show that these lines, with the exception of line 30, are capable of responding to both CSF-1 and IL-3. Further characterization of these responses will be necessary before any further conclusions can be made.

SUMMARY

Mononuclear phagocytes from normal murine bone marrow proliferate in response to CSF-1. After 6 days of culture, a population of nonadherent cells that have epitopes on their plasma membranes for the monoclonal antibodies 30-H11, G2 and MAC-1, can be isolated, and when cultured in CSF-1, will produce macrophage colonies. These nonadherent cells will produce adherent macrophages for a limited number of passages. When very large macrophage colonies are passaged, however, permanent cell lines can be established that depend on the continuous presence of CSF-1 for growth. These cell lines appear to maintain the same phenotype (as the original primary cells from which they were derived) as determined by a battery of monoclonal antibodies. These lines provide a convenient source of mononuclear phagocytes for functional studies.

ACKNOWLEDGEMENTS

This work was performed under the auspices the Department of Energy and was supported by U.S. Public Health Service Grants AI 19490, AI 15355 and AI 16228; by DHHS; by Grant #IM371 from the American Cancer Society; and by the Los Alamos National Flow Cytometry and Sorting Research Resource funded by the Division of Research Resources of NIH (Grant P41-RR01315-02).

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TABLE 1

DISTRIBUTION OF PROLIFERATING CELLS IN MACROPHAGE CULTURES

<u>Cell Type</u>	Number and Proportion of Cells After:					
	<u>First Division</u>		<u>Fifth Division</u>		<u>Tenth Division</u>	
	Number*	%	Number	%	Number	%
Stem Cell	1	50	1	3.1	1	0.9
Monoblast	1	50	1	3.1	1	0.9
Promonocyte	0	0	2	6.2	2	1.8
Monocyte	0	0	4	12.5	4	3.5
Exudate Macrophage	0	0	8	25.0	8	7.0
Senescent Macrophage	0	0	16	50.0	96	85.0
Total Cells	2	-	32	----	112	----

The data represent the number of cells in a colony produced by a single stem cell. The number of cells was determined by counting cells shown in Figure 6 in each differentiation stage after each round of division. The Table illustrates the dilution of the stem cells that occurs when one daughter remains a stem cells and the other daughter continues to divide as it differentiates. Thus, after the 10th round of divisions, 85% of the cells are senescent macrophages and there is only 1 stem cell, representing 0.9% of all cells in the colony.

TABLE 2
MONONUCLEAR PHAGOCYTE LINEAGES DERIVED FROM NORMAL MURINE BONE MARROW

<u>Name</u>	<u>Mouse Strain</u>	<u>Year Derived</u>	<u>Clone or Bulk Culture</u> *	<u>Passage Number</u>
9	B10.A(5R)	1981	C	48
10	B10.A(5R)	1981	B	48
16	DBA/2	1981	C	32
18	DBA/2	1981	B	34
22	(B10XB10.D2)F ₁	1981	C	34
30	(B10XB10.D2)F ₁	1981	C	34
32	(B10XB10.D2)F ₁	1981	C	17
35	DBA/2	1981	C	32
36	DBA/2	1981	C	32
63	(B10XB10.D2)F ₁	1981	B	35
65	DBA/2	1981	C	32

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* Unselected femoral bone marrow cells were dispersed in liquid culture medium containing 10% LCM, and the adherent cell fraction was subcultured continuously as a bulk macrophage cell line. Femoral marrow cells were also dispersed in soft agar medium with 10% LCM. Single cells were permitted to grow to macroscopic colonies and were then isolated by micromanipulation and transferred to liquid culture medium where they have been continuously propagated as clonal macrophage cell lines (18).

TABLE 3
MONOCLONAL ANTIBODIES
THAT STAIN CULTURE DERIVED BONE MARROW MONONUCLEAR PHAGOCYTES^a

<u>Monoclonal Antibody</u>	<u>Percent Positive Cells</u>				
	<u>Nonadherent</u>	<u>Adherent</u>	<u>Known Specificity</u>		
	(%)	(%)	(%)		
MAC-1	60	92	CR3 receptor	(23)	
MAC-2	12	96	M ^b	(24)	
MAC-3	16	35	M	(25)	
30-H11	90	0	B, T	(26)	
G-2	16	8	B, T	(26)	
E-2	<3	93	B, M, E	(26)	
MAR18.5 ^c			Mouse anti-rat κ	(27)	

^a Cells derived from normal C3H bone marrow after 6 days in growth medium.

^b M = macrophages, B = B-cells, T = T-cells, E = erythroid cells.

^c A γ 2a myeloma protein which avidly binds to γ 2a Fc receptors on murine macrophages.

TABLE 4
STAINING PATTERN OF BONE MARROW-DERIVED CELL LINEAGES

Line ²	<u>30-H11</u>	<u>MAC-1</u>	<u>MAC-2</u>	<u>MAC-3</u>	<u>G-2</u>	<u>G-12</u>	<u>E-2</u>	<u>MAR18.5</u>
-----Percent Positive Cells ¹ -----								
10	3	84	40	6	13	82	ND ³	39
9	9	58	45	7	11	64	43	27
22	7	72	51	12	ND	38	12	22
30	11	50	41	10	12	51	15	12
36	26	61	39	7	16	45	7	10
63	3	37	44	8	4	46	5	32
65	9	34	21	4	<3	34	14	9
16	5	31	16	ND	<3	18	13	6
18	<3	9	6	<3	<3	6	4	8
35	<3	21	<3	<3	<3	<3	9	13

1 For these studies, the nonadherent and adherent fractions were not separated.

2 The lines have been listed in decreasing order of percentage of cells stained for MAC-1 or G-12.

3 ND = not done

TABLE 5
RESPONSE OF CELL LINES TO LCM AND WCM

<u>Line</u>	LCM		WCM	
	<u>Lag Period</u> ^a	<u>T_d</u> ^b	<u>Lag Period</u>	<u>T_d</u>
9	None	1.5 days	8 days	1.5 days
16	None	1.8 days	None	2.6 days
30	None	1.4 days	--Does not respond--	
35	5 days	1.0 day	5 days	1 day
36	None	2.3 days	None	2.3 days

---

a Period between plating the cells and their entrance into an exponential growth phase.

b Time required for the cells to double.

Figure 1 Growth Kinetics of Bone Marrow Mononuclear Phagocytes

C3H bone marrow cells were cultured in 60 mm culture dishes at a concentration of 10^5 cells per ml in 5 ml of either α MEM supplemented with 10% fetal bovine serum (Δ) or in α MEM containing 10% fetal bovine serum and 10% L-cell conditioned medium (O). The number of nonadherent cells (A) and adherent cells (B) was determined as a function of time in culture.

Figure 2 Volume and DNA Distribution of Cultured Bone Marrow

C3H bone marrow was cultured in growth medium, and harvested on days 2, 4 and 6. The electronic cell count was measured on half of the sample. The other half was fixed in 70% ethanol. After 1 hr, this sample was centrifuged, and the pellet was resuspended in 1 ml of the DNA specific dye mithramycin, 0.15 M NaCl and 0.01 M $MgCl_2$.

Figure 3 Colony Formation by Nonadherent Cells

The nonadherent cells shown in figure 2A were removed on days 1, 3, 5, 6, 7 and 8 from different replicate cultures established on day zero, serially diluted in growth medium, and passaged to new cultures. After 7 more day of incubation, the colonies were stained and counted. The total number of colony forming cells in the original suspension was then determined as the total nonadherent cells recovered times the frequency of colony forming cells.

Figure 4 Passage of Nonadherent Cells

C3H bone marrow was adjusted to 10^4 cells per ml in growth medium, and 3 ml were cultured in 35 mm culture dishes. Every 3 or 4 days the nonadherent cells were removed and passaged to new dishes while 3 ml of fresh growth medium were added to the original dishes. In A, the number of nonadherent cells recovered (X) was determined with each passage. Seven days after the initial plating, the number of adherent cells (O, ●, △, ▲, □, ■, ◇, ◆) was determined on replicate plates at the time nonadherent cells were recovered. In B, the doubling time of adherent cells for each passage is shown.

Figure 5 A Model for Mononuclear Proliferation and Differentiation

A conceptual model of mononuclear phagocyte proliferation and differentiation is depicted. As the stem cell (O) divides it produces one daughter that differentiates and another daughter that remains a stem cell. As the differentiating cells divide, they differentiate through each class of mononuclear phagocytes: monoblast (Mb) promonocyte (PM), monocyte (M), exudate macrophage (EM) and finally into a senescent macrophage (SM). Macrophages with proliferative capability are designated as (●) while senescent macrophages are designated (●). With each division of the stem cell a whole new end cell lineage is created. At any sampling time a colony will consist of the cells shown in each differentiated stage.

Figure 6 Culture-Derived Bone Marrow Mononuclear Phagocytes Stained with Various Monoclonal Antibodies

C3H bone marrow was cultured for 6 days in growth medium. The nonadherent and adherent cells were recovered and stained with the monoclonal antibodies MAC-1, E-2 or 30-H11 and analyzed by flow cytometry. Nonadherent cells above channel 70 and adherent cells above channel 130 were considered positive (arrows). The Coulter volume histogram and the fluorescence histogram of cells preincubated with normal rat serum and the fluoresceinated goat anti-rat serum (second reagent) are also shown.

Figure 7 Staining Pattern of Cell Lines

In this figure we have plotted, in order, 9 of the 10 cell lines shown in Table 4 (line 35 is not plotted). The abscissa has been shifted to the right on the bottom relative to the top to determine if the percentage of positive cells in each line could be accounted for by increasing autofluorescence. The observation that this process causes the endpoint for each of the monoclonal antibodies to nearly line up suggests that increasing autofluorescence compromises our ability to resolve positive cells and that each line probably contains a similar percentage of positive cells.