Conditions for an In Vitro Culture of Murine Mixed Hematopoietic Colonies and Their Putative Cellular Origin

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The supernatant fluid of stimulated spleen cells (PHA-SCM) supported in vitro colony growth of murine marrow. In the absence of exogenous erythropoietin, it stimulated the growth of (1) myeloid colonies and (2) distinct mixed colonies containing erythroid cells, granulocytes, macrophages, and infrequently megakaryocytes in a setting structurally resembling biopсид marrow. The cells that form mixed colonies reside in a density range of 1.058-1.068 g/ml in a discontinuous albumin gradient. Active supernatant was produced by T cells in combination with a macrophage factor. DNA synthesis correlated with activity. PHA-SCM differed from erythropoietin (EPO) when chromatographed on lectin columns and did not contain EPO activity as demonstrated by the fetal mouse liver cell (FMLC) assay. The activity for mixed colony growth could be eluted from an anion exchange column with 0.07 M NaCl and eluted in a gel filtration column at a distance corresponding to an apparent molecular weight of 39,000. Mixed colony-forming cells responsive to PHA-SCM were found to be H-2<sup>i</sup> but showed significant sensitivity to anti-la antisera reflecting variable antigenic density. The mixed colony-forming cell appeared less differentiated than myeloid or erythroid progenitor cells examined, and its antigenic determinants are consistent with those observed for the pluripotent stem cell assayed in vivo (CFU-S).

CONDITIONED MEDIUM prepared from stimulated lymphocytes contains a factor(s) that stimulates the growth of hematopoietic progenitor cells in vitro. Pokeweed-mitogen-stimulated spleen cell supernatant (PWM-SCM) stimulates the growth of mixed colonies containing myeloid, erythroid, and megakaryocytic cells in agar cultures of fetal liver cells. Also present were neutrophil-macrophage and erythroid colonies. SCM prepared from mouse spleen cells incubated with allogeneic irradiated spleen cells has been found to stimulate the growth of CFU-Es in the absence of EPO. Conditioned medium from PHA-stimulated lymphocytes and subsequent addition of EPO promotes the growth of mixed colonies (CFU-GEMM) in human marrow. Evidence exists that mixed colonies are clones derived from single multipotent hematopoietic stem cells.

The objectives of this article are to describe (1) conditions required for the production of this growth factor(s), (2) characterization of the factor(s), (3) the nature of mixed colonies, and (4) the antigenic determinants of the mixed colony-forming progenitor cell. This report confirms the observation that lymphoid tissue can produce growth factor(s) for hematopoietic cells. It further provides evidence that the in vitro mixed colonies structurally resembling marrow hematopoietic cords occurring in nature originate from progenitor cells that are H-2<sup>i</sup> and la<sup>-</sup>, an antigenic expression compatible with the pluripotent stem cell (CFU-S) assayed in vivo.

MATERIALS AND METHODS

Mice

Inbred mice strains used in production of spleen cell supernatant were 2-4-mo-old C57BL/6J and those used for bone marrow culture were 2-4-mo-old CBA/J (haplotype k). All were purchased from Jackson Laboratories, Bar Harbor, Me.
Mφ) from 2-4-month-old C57BL/6J mice were incubated for 3 days at 37°C in 5% CO₂ in air at a cell concentration of 2 x 10⁶ cells/ml in RPMI with 25 mM HEPES buffer (GIBCO, Grand Island, N.Y.), containing 2.5 µg/ml purified PHA (Burroughs Wellcome, Greensville, N.C.), 3% heat-inactivated pooled human serum, 10⁻⁴ M 2-mercaptoethanol, 2 mM glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin. Cells were incubated in 2-3 ml volumes in loosely capped tissue culture tubes. After incubation, the media was centrifuged at 130 g for 10 min, the supernatant fluid filtered through a 0.45-µ filter, and stored at -70°C until used.

**L-Cell Conditioned Media for Growth of CFU-C-Derived Colonies**

Mouse L-cells were a gift from Dr. Bryce Weinberg of our institution. Supernatant was prepared by incubating 2 x 10⁶ cells in 15 ml of alpha media (GIBCO) containing 10% fetal calf serum (FCS) (GIBCO), 100 U/ml penicillin, and 100 µg/ml streptomycin in 75 cm² tissue culture flasks for 7 days at 37°C in 5% CO₂ in air. After incubation, the media were centrifuged at 130 g for 10 min, filtered through a 0.45-µ filter, and stored at -20°C until used.

**Measurement of DNA Synthesis**

The proliferation of spleen cells in response to PHA was assessed by labeling the cultures with [³H]-thymidine ([³H]-Tdr) at 2 mCi/ml, specific activity 20 Ci/m mole (New England Nuclear, Boston, Mass.) for the last 4 hr of culture. The acid-precipitable fractions of cells were then harvested for radioactivity counting as previously described.²¹

**Lectin Chromatography**

Twelve milliliters of PHA-SCM dialyzed overnight against phosphate-buffered saline (PBS) were applied to a column (0.9 x 24 cm) of concanavalain-A-Sepharose 4B (Pharmacia, Piscataway, N.J.), which was previously equilibrated with 20 ml PBS containing 1 mM CaCl₂, 1 mM MnCl₂, and 1 mM MgCl₂. The column was then eluted with the identical buffer. The bound protein was eluted with 0.1 M 1-0-methyl-α-D-glucopyranoside (Sigma) dissolved in buffer. Flow rate of the column was 10-15 ml/hr. Fractions (1.5 ml), beginning with application of the supernatant, were collected, dialyzed overnight against PBS, filtered, and frozen at -20°C until cultured.

Fifteen milliliters of PHA-SCM dialyzed overnight against PBS were applied to a column (0.9 x 7.0 cm) of wheat germ lectin-Sepharose 6MB (Pharmacia) that was previously equilibrated with 20 ml PBS. The column was then eluted with 29 ml of PBS. Bound protein was eluted with 0.1 M N-acetyl-β-D-glucosamine (Tridom Chemical Inc., Hauppauge, N.Y.) dissolved in PBS. Flow rate of the column was 10-15 ml/hr. Fractions (1.5 ml) beginning with application of the supernatant were collected, dialyzed overnight against PBS, filtered, and frozen at -20°C.

The protein profile of these and other columns was monitored by absorbance at 280 nm. Colony-stimulating activity was assayed in methylcellulose culture.

**Gel Filtration Chromatography**

One milliliter of PHA-SCM was applied to a column (1.6 x 24 cm) of Bio-Gel P200 (Bio-Rad, Rockville Center, N.Y.) equilibrated with PBS. The column was developed with PBS at a flow rate of 7 ml/hr. Colony-stimulating activity of selected fractions (0.69 ml/fraction) was assayed as described above. For molecular weight determination, the column was calibrated with standard markers, including Blue Dextran for determination of void volume (V₀), transferrin (mol wt 80,000), BSA (mol wt 67,000), ovalbumin (mol wt 45,000), and chymotrypsinogen (mol wt 26,000). The distribution coefficient (Kₐ) was calibrated for standard molecular weights and for the PHA-SCM fraction showing maximum mixed colony-stimulating activity and compared on a semilog plot to determine the molecular weight of the active PHA-SCM fraction.

**Iron-Exchange Chromatography**

Eighty milliliters of PHA-SCM were concentrated to 20 ml by dialyzing against 20% polyethylene glycol 20,000 (Fisher Scientific, Norcross, Ga.) in PBS and applied to a column (6 x 12 cm) of P200 equilibrated with PBS. The column was developed with PBS at a flow rate of 12 ml/hr. Colony-stimulating activity was determined in methylcellulose culture.

Active fractions were pooled, dialyzed against 0.05 M TRIS (Trizma base, Sigma) pH 7.0, and applied to a column (0.9 x 9.0 cm) of DEAE-Sephacel (Pharmacia) equilibrated with 0.05 M TRIS, pH 7.0. Flow rate was 12 ml/hr. Fractions (0.8 ml) beginning with the gradient were collected, dialyzed against PBS, filtered, and frozen at -70°C. Sodium concentration of active fractions was assayed by flame photometry.

**Fetal Mouse Liver Cell Assay**

Since erythroid colonies grew actively in the PHA-SCM-stimulated cultures, it was essential that PHA-SCM be tested for contamination with EPO. A modification¹¹ of the basic in vitro FMLC¹³ was used for the detection of EPO.

**Buoyant Density Separation of Bone Marrow Cells**

Bone marrow density was used to determine the potential density difference of each progenitor cell and to enrich each population. Bone marrow cells were separated using a density gradient of BSA (fraction V powder, Sigma) prepared by the method of Worton et al.¹⁹ Cells from CBA/J mice were washed once in suspension media and layered in 10% BSA over a discontinuous BSA gradient of 5 densities: 21% (1.0545 g/ml), 23% (1.0583 g/ml), 25% (1.0643 g/ml), 28% (1.0710 g/ml), and 37% (1.0941 g/ml). The gradient was centrifuged at 2000 g at 10°C for 45 min. Cells of each density were separated, washed once in Hank's balanced salt solution (HBSS) (Sigma), and counted after resuspension in media. Total cells of each density, total cells recovered from the gradient, and percentage of total cells of each density were calculated.

Nucleated cells from each density layer were cultured as described below. BFU-E per 4.5 x 10⁵ cells, CFU-C, and mixed colonies per 1.5 x 10⁵ cultured cells were scored. Colony-forming cells of each density and of the total gradient as well as percentage of colony-forming cells of each density were calculated for each factor.

The density of each BSA fraction was calculated as a ratio of weight and volume corrected by the density of water at 25°C.

**Bone Marrow Culture**

Bone marrow cells were obtained by flushing the femurs of CBA/J mice with suspension media consisting of 15% FCS, 0.17% bovine serum albumin (BSA) (Sigma), 100 U/ml penicillin, and 100 µg/ml streptomycin in alpha media.

The methylcellulose culture technique described by Iscove¹⁵ was used with modifications. Routinely, 1.5-2.0 x 10⁵ nucleated marrow cells of density between 1.058 and 1.068 were cultured in 35-mm Lux plastic tissue culture dishes (MA Bioproducts, Walkersville, Md.) in 1 ml of media containing 0.8% methylcellulose, 24% FCS selected for mixed colony growth, 0.8% BSA, 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM glutamine, and 5 x 10⁻⁵ M 2-mercaptoethanol. Cultures contained the appropriate growth factor (20% PHA-SCM for mixed colony growth or 20% mouse L-cell supernatant for CFU-C). A quantity of 4.5 x 10⁵ marrow cells was
cultured with 2 U/ml erythropoietin (step III, Connaught Laboratories, Ontario, Canada) for BFU-E. Petri dishes were incubated at 37°C in 7.5% CO₂ in air in a humidified atmosphere for 10–12 days. Cultures were scored at 10–12 days using an Olympus inverted microscope to identify aggregates of greater than 50 cells as colonies.

**Antisera**

Anti-la 1,2,3,7 antiserum (A.TH anti-A.TL) (Catalog no. Y 1-7-02-02-02) (anti-la⁴) and anti-H-2Kk serum [(A.TL x 129)F, anti-A.AL] (Catalog no. Y 1-9-03-15-03) were obtained from the NIH Serum Bank, National Institute of Allergy and Infectious Diseases, Bethesda, Md.

The anti-la 1,2,3,7 antiserum was produced under NIAID Contract 1-A1-62502 to Washington University and characterized by standard microcytotoxicity and absorption methods. Fresh rabbit serum was used as the complement source. This antiserum was found to be specific for the I region, A and E subregions (k haplotype).

The anti-H-2Kk antiserum was produced and characterized in the Dept. of Immunology, Mayo Medical School, Rochester, Minn. under contract to Dr. Chella S. David. Using the microcytotoxicity test, it was shown to be specific for the K region (k haplotype).

**Complement**

Lowtox rabbit complement (Accurate Chemical Co., Hicksville, N.Y.) was used at a final dilution of 1:5. This dilution of complement was optimum in the microcytotoxicity test with the above antisera.

**RESULTS**

**Mixed Hematopoietic Colonies From CBA Marrow Cells**

Supernatant fluid from 3-day PHA cultures of whole spleen cells stimulated growth of hematopoietic colonies containing mixtures of erythrocytes, normoblasts, segmented granulocytes, macrophages, occasional giant megakaryocytes, and some unidentified cells in a cohesive and compact association (Fig. 1). In addition to these very large colonies, separate myeloid colonies were observed.

**Fig. 1.** Cohesive growth of a mixed colony consisting of erythroid cells (normoblasts designated NB and mature erythroid, right upper corner of photograph), myeloid cells (mature myeloids, MM) and macrophages (MP). Megakaryocytes (not shown) are sometimes present in small numbers. The photograph was taken at 1000x magnification of a formalin-fixed colony stained with hematoxylin and eosin.
colonies were also present in the same culture dishes. The number of colonies was enhanced by separating the marrow cells on a BSA gradient and culturing only those cells of density 1.058-1.068. Growth of colonies increased in proportion to the amount of SCM added with mixed colonies, reaching a maximum at 20% concentration (v/v). At this concentration, there was a linear relationship between the number of cells plated and the number of colonies. All surface antigen determination experiments were done at this concentration.

Mixed colonies were identified as those with small, medium, and large cells surrounded by fibroblasts and mesh-like matrix resembling marrow histology, while myeloid colonies had a more homogeneous population of medium-sized cells. Extensive plucking and staining of colonies and staining of cells with benzidine-Giemsa for erythroid cells, periodic acid Schiff for megakaryocytes, or Wright's stain provided positive identification of cell types. Correlation of stained cells with colony appearance under an inverted microscope showed the mixed colonies to be composed of erythroid cells, granulocytes, macrophages, megakaryocytes, and fibroblasts.

Some cultures were grown on top of agar-coated (0.5%) Petri dishes in methylcellulose media as described above. On the day of harvest, 1 ml of 0.5% agar was gently poured on top of the methylcellulose culture. Petri dishes with tops removed were placed in stainless steel cassettes with numerous perforations and fixed in formalin overnight. The fixed colonies in double layers of agar were sectioned in 6 μ thickness and stained with hematoxylin-eosin for microscopy (Fig. 1).

**PHA-SCM Production**

PHA at 2.5 μg/ml and 3% heat-inactivated pooled human serum were required for production of active PHA-SCM capable of stimulating mixed colony growth in CBA marrow. FCS could not substitute for the pooled human serum used in these experiments. The most active supernatant resulted from spleen cell concentrations of 2-3 x 10^6 cells/ml cultured for 3 days. DNA synthesis paralleled the colony-stimulating activity (Fig. 2). When PHA-SCM was prepared from spleen cells treated with mitomycin-C, a marked reduction of mixed colony growth was observed (1-2 mixed colonies versus 6 mixed colonies), and DNA synthesis as assessed by 3H-TdR uptake was inhibited (119 ± 7 versus 11,480 ± 1260).

Table 1 shows the results of experiments performed using different combinations of enriched lymphocyte subpopulations and peritoneal Mφs. In T + Mφ and B + Mφ cultures, Mφs comprised 3% of the total cell population and the lymphocytes 97%. T + B cultures were comprised of 60% T cells and 40% B cells to approximate the ratio found in the spleen. Cultures containing media and PHA without cells served as controls. Studies shown in Table I indicate that T^+^ M is the minimum cellular requirement for supernatant with mixed colony-stimulating activity. Some activity seen with B^+^ M is either we were unable to remove all of the T cells responsible for the production of PHA-SCM or there is a contributing factor(s) being produced by Mφs.

**Table 1. Growth Promotion by Supernatants From Different Cell Combinations**

<table>
<thead>
<tr>
<th>Source of Cells</th>
<th>Growth of Mixed Colonies*</th>
<th>Average Growth ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>WSC</td>
<td>8,4,6,7</td>
<td>6 ± 0.4</td>
</tr>
<tr>
<td>T + B + Mφ†</td>
<td>6,6,5</td>
<td>6 ± 0.2</td>
</tr>
<tr>
<td>T + Mφ</td>
<td>7,7,4,4,8</td>
<td>6 ± 0.4</td>
</tr>
<tr>
<td>B + Mφ</td>
<td>1,2,2</td>
<td>2 ± 0.2</td>
</tr>
<tr>
<td>T + B</td>
<td>2,1,2</td>
<td>2 ± 0.2</td>
</tr>
<tr>
<td>T</td>
<td>1,1,1</td>
<td>1 ± 0</td>
</tr>
<tr>
<td>B</td>
<td>1.1</td>
<td>1</td>
</tr>
<tr>
<td>Mφ</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>T + LAF</td>
<td>5.3</td>
<td>4</td>
</tr>
<tr>
<td>LAF</td>
<td>0.0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Colonies per 2 x 10^5 cells plated with 10% v/v PHA-SCM
†T + B + Mφ reconstituted.
Spleen cells from congenitally athymic nu/nu mice were unable to produce active supernatant. When T cells from normal littermates (purified on nylon wool) were added in varying concentrations, the colony-stimulating activity increased with the rise in percentage of T cells supplemented. Activity at the 50% T-cell level was comparable to the growth obtained from normal spleen cells (Fig. 3).

To determine if macrophages were required to produce the factor(s), experiments were carried out with a soluble Mφ factor (LAF)\textsuperscript{2} substituting for Mφs in T + Mφ suspensions. PHA-SCM thus prepared stimulated growth of mixed colonies similar to the growth seen with supernatant from T + Mφ cell suspension but to a lesser extent (Table I).

**Lectin Chromatography**

When PHA-SCM was chromatographed on concanavalin-A-Sepharose, most of the protein passed through the column, while all detectable colony-stimulating activity bound to the column (Fig. 4A). Recovery following elution with 0.1 \( M \) 1-0-methyl-\( \alpha \)-D-glucopyranoside was 59% for mixed and 98% for myeloid colony-stimulating activity.

When PHA-SCM was chromatographed on wheat germ lectin-Sepharose, most protein again failed to bind (Fig. 4B). Bound protein was eluted with 0.1 \( M \) N-acetyl-D-glucosamine. Most of the colony-stimulating activity, 89% myeloid and 97% mixed, was detected in the unbound fractions. Only 11% of myeloid and 3% of mixed activity were eluted with the bound protein. Total recovery was 90% of mixed and 100% of myeloid colony-stimulating activity.

**Gel Filtration and Molecular Weight Determination**

Gel filtration of PHA-SCM on P200 beads separated detectable colony-stimulating activity from the majority of protein (Fig. 5A). The peaks of myeloid and mixed colony-stimulating activity cochromatographed and the estimated molecular weight of this fraction was 39,000.

**Ion-Exchange Chromatography**

DEAE-Sephacel chromatography was performed to determine binding characteristics of the active factors of PHA-SCM. The diluteness of the active factor and loss of activity during concentration made quantitative assessment of recovery from the P200 and DEAE columns difficult; recovery was poor from both. Less than half of the protein loaded on the DEAE column bound (not shown), whereas most of the detectable active factor was eluted from the DEAE column with the continuous NaCl gradient (Fig. 5B). Peak mixed
Density Gradient Distribution of BFU-E, Myeloid, and Mixed Colonies

Cells responsible for mixed colony growth resided in a density range of 1.058–1.068 g/ml. BFU-E sedimented in a density range between 1.058 and 1.064 g/ml, and the peak density for CFU-C was 1.064 g/ml. For experiments with H-2 and Ia antisera, cells of density between 1.058 and 1.068 g/ml were used. At this density, BFU-Es grown in the presence of erythropoietin were present at 10.0 ± 0.7/4.5 × 10⁶ cells. CFU-Cs grown in the presence of L-cell-conditioned supernatant were present at 56.9 ± 1.9/1.5 × 10⁶ cells. PHA-SCM stimulated the growth of two morphologically distinct colonies: myeloid at a frequency of 17.3 ± 0.9 and mixed at a frequency of 7.7 ± 0.5/1.5 × 10⁶ cells. The lower number of BFU-E colonies from fractionated cells may be due to the absence of auxiliary cells, which may be critical for their growth.²¹,²²

Treatment With Anti-Ia Antisera

CBA bone marrow cells of density 1.058–1.068 g/ml were treated with complement as described in Materials and Methods. Slight inhibition of colony formation was noted when growth with complement-treated cells was compared to that with untreated cells, but since the decreased growth of BFU-E, CFU-C, and PHA-SCM responsive colony-forming cells was equivalent, loss of colony-forming cells with complement alone was attributed to nonspecific killing.

Anti-Ia antisera dilutions of 1/4, 1/10, 1/20, 1/40, and 1/80 were used to treat marrow as described above, and results of different experiments were standardized by expressing colony growth following antisera treatment as percent of complement control. BFU-E, CFU-C, and PHA-SCM myeloid colony-forming cells were moderately sensitive to anti-Ia antisera at dilutions of 1/4 and 1/10 (Fig. 6). No significant loss of PHA-SCM mixed colony-forming cells was noted at any anti-Ia antisera dilution used. At an intermediate anti-Ia antisera dilution of 1/10, in comparison to complement control, colony growth ± SEM was 57.8% ± 14.0% for BFU-E, 60.1% ± 7.1% for CFU-C, and 58.0% ± 7.4% for PHA-SCM myeloid colony-forming cells, but was 97.0% ± 2.0% for PHA-SCM mixed colony-forming cells. Sensitivity of BFU-E, CFU-C, and PHA-SCM myeloid colony-forming cells to anti-Ia antisera was equal, and these progenitor cells were considered inseparable by the Ia determinant. In contrast, the PHA-SCM mixed colony-forming cell was insensitive to anti-Ia antisera.

Treatment With Anti-H-2 Antisera

CBA marrow cells of density 1.058–1.068 g/ml were treated with specific anti-H-2 antisera at dilu-
DISCUSSION

Colony-stimulating factors secreted by murine lymphoid cells incubated with a T-cell mitogen were used to study the regulatory role of lymphocytes in hematopoiesis. While lymphocytes are known to play a part in marrow differentiation, details of this function are unclear. Experimentation with lymphocytes and their factors will contribute to the understanding not only of normal hematopoietic differentiation but also of bone marrow failure states.

PHA-SCM prepared from spleen cells is able to stimulate mixed colonies containing erythroid cells, granulocytes, macrophages, and infrequently megakaryocytes without the addition of EPO. T lymphocytes and a Mφ factor are necessary for production of the growth factor(s), and there is a positive correlation...
with DNA synthesis. This latter finding could offer an explanation for the increased number of colonies observed in adult marrow with PHA-SCM as compared to PWM-SCM, since PHA gives a greater proliferative response of spleen lymphocytes than does PWM.

The growth-promoting component of PHA-SCM was characterized as different from EPO by lectin chromatography and the MLC using $^{59}$Fe. By this assay, EPO-like activity in PHA-SCM was measured to be $0.22 \pm 0.9$ U/ml or 0.05 U of EPO in a milliliter of culture (20% growth factor added), probably enough to affect hemoglobinization but far too little to stimulate erythroid "burst" formation.

Our inability to separate mixed and myeloid colony-stimulating activity by gel filtration of ion-exchange chromatography is in total agreement with the chromatographic properties of PWM-SCM. Inasmuch as fetal liver cells were needed for consistent mixed colony formation with PWM-SCM, it would appear that our culture system using PHA-SCM and density separated marrow cells provides a simpler method for in vitro culture of the progenitor cell of mixed colonies.

In an effort to characterize this progenitor cell, we chose to study Ia and H-2 determinants on the cell surface and compare them with other hematopoietic progenitor cells by their sensitivity to lysis with heteroantisera and complement followed by an assay for the residual colony proliferation. Problems related to this method are (1) cells with low antigen density might not be lysed and (2) results may reflect effects on auxiliary cells essential for their growth rather than on the progenitor cell itself.

Previous work with mice has shown the Ia system to be much less broadly expressed in mice than in humans. Murine CFU-C as well as CFU-S have been reported to be insensitive to anti-Ia antisera, although a recent study suggests the possible presence of the I-E subregion antigens on murine CFU-S. Our mixed colony-forming cell was insensitive to anti-Ia 1,2,3,7, which includes the I-E subregion. Recently, anti-Ia monoclonal antibodies to the A and E subregions and fluorescent cell sorting have been used by one of the authors (BAM) to separate Ia$^+$ and Ia$^-$ populations of marrow cells in BALB/c mice. Preliminary results indicate that the spleen colony-forming cells (CFU-S) are present in the Ia$^+$ population. This finding confirms the observation in this paper using heteroantisera.

BFU-E, CFU-C, and PHA-SCM myeloid colony-forming cells were shown to be sensitive to Ia 1,2,3,7 antisera, but only at high concentrations. The lack of anti-Ia sensitivity of murine CFU-C in previous reports may be due to the use of a lower potency antisera or less effective complement. Also, the heterogeneity of CFU-C and the use of various growth factors by different investigators may influence the apparent expression of Ia determinants in culture.

H-2 determinants on murine CFU-S and CFU-C have been previously examined. In a report by van den Engh, treatment with anti-H-2 antisera at a dilution of 1/30 resulted in an approximate 50% lysis of CFU-S, while lysis of CFU-C was less than 10%. In our experiments, BFU-E, CFU-C, and the PHA-SCM mixed and myeloid colony-forming cells were all extremely sensitive to anti-H-2-K$^k$ antisera to a dilution beyond 1/480. The high potency of the NIH antisera may explain the differences between our observations and those of van den Engh.

In this report, the Ia- H-2$^+$ mixed colony-forming cell expresses the antigenic determinants more compatible with those of CFU-S rather than differentiated progenitors. The assay system described, using PHA-SCM and fractionated bone marrow, should provide a simple and reproducible method for future work in this area.

PHA-SCM prepared in our laboratory stimulates myeloid growth and the growth of mixed colonies containing granulocyes, macrophages, erythroid cells and occasionally megakaryocytes in a cohesive association in methylcellulose cultures of CBA bone marrow. No erythropoietin is required. The method is simple and reproducible and could replace the currently used in vivo spleen colony assay for CFU-S.

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