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Macrophage Regulation of Myelopoiesis Is Altered by Exposure to the Benzene Metabolite Hydroquinone[1]

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Macrophage Regulation of Myelopoiesis Is Altered by Exposure to the Benzene Metabolite Hydroquinone. THOMAS, D. J., REASOR, M. J., AND WIERDA, D. (1989). *Toxicol. Appl. Pharmacol.* **97**, 440–453. Hydroquinone, a myelotoxic metabolite of benzene, decreases the ability of murine bone marrow stromal cells to support myelopoiesis *in vitro*. Bone marrow stroma consists of macrophages and fibroblastoid stromal cells that participate coordinately in regulating myelopoiesis. The goal of this study was to determine if macrophage or fibroblastoid cell function is more sensitive to the myelotoxic actions of hydroquinone. To address this question, we developed purified populations of macrophages and fibroblastoid stromal cells and treated each population with hydroquinone. These cells were reconstituted together with nontreated cells of the opposite type and assayed for their ability to support the formation of granulocyte and macrophage colonies in an agar overlay. Reconstituted cultures containing hydroquinone-treated macrophages supported fewer colonies than did corresponding cultures containing untreated macrophages. Reconstituted cultures containing hydroquinone-treated fibroblastoid stromal cells were not affected. Moreover, hydroquinone reduced detectable interleukin-1 activity in purified macrophage cultures stimulated with lipopolysaccharide. These results indicate that hydroquinone selectively interferes with macrophage function possibly, in part, via alteration of macrophage interleukin-1 secretion.

Benzene is a chemical intermediate in the synthesis of industrial compounds and a component of unleaded gasoline. Human exposure has been reported to cause a variety of hematological disorders including leukopenia, lymphocytopenia, agranulocytosis, thrombocytopenia, aplastic anemia, and myelogenous leukemia (Cronkite, 1987; Laskin and Goldstein, 1977; Snyder *et al.*, 1977). Toxicity is generally accepted to be the result of metabolism to highly reactive intermediates including hydroquinone, benzoquinone, phenol, and catechol. Other potentially reactive metabolites are 1,2,4-benzenetriol, *trans–trans*-muconaldehyde, 2,2-biphenol, and 4,4-biphenol (Bolcsak and Nerland, 1983; Eastmond *et al.*, 1986; Goldstein *et al.*, 1982; Lee *et al.*, 1974; Sawahata and Neal, 1983; Snyder *et al.*, 1982). Benzene metabolism occurs in both liver and bone marrow. In the liver, benzene is metabolized via mixed-function oxidase to phenol, hydroquinone, and catechol (Andrews *et al.*, 1977; Tunek and Oesch, 1982). These compounds are carried to the bone marrow via the blood where hydroquinone and catechol have been shown to concentrate within the bone marrow

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(Rickert *et al.*, 1979). It is postulated that these and other metabolites can undergo further metabolism in the bone marrow, presumably via cellular peroxidase enzymes (Eastmond *et al.*, 1986; Irons *et al.*, 1980; Sadler *et al.*, 1988).

Within the bone marrow microenvironment, the stroma acts as a supportive matrix providing sites for hemopoiesis to occur (Allen and Dexter, 1984; Dexter, 1982; Lichtman, 1981). The stroma produces soluble mediators which stimulate hemopoiesis (Cronkite *et al.*, 1982a; Dexter *et al.*, 1984; Zipori *et al.*, 1982) and simultaneously provides a supporting framework for immature stem cells. It has been well established that marrow-derived adherent stromal cell monolayers support lymphopoiesis and myelopoiesis *in vitro* (Dexter *et al.*, 1977; Gaido and Wierda, 1984, 1985; Zipori *et al.*, 1982). Without the presence of adherent stromal cells *in vitro* hemopoietic cells rapidly die (Dexter *et al.*, 1977; Reimann and Burger, 1979). This point is further illustrated in mice carrying the Steel (Sl) mutation which results in defective hemopoiesis. The anemia seen in these mice cannot be cured by the infusion of normal marrow stem cells, but can be alleviated by the transplantation of normal stromal tissue (Bernstein, 1970; McCulloch *et al.*, 1965; Dexter and Moore, 1977). It follows then that because stromal cells are essential for hemopoiesis, damage to them may be a key factor in benzene-induced marrow hematotoxicity.

Cultures of adherent bone marrow stroma generally consist of two cell types which have been classified as fibroblastoid stromal cells and macrophages. The fibroblastoid stromal cell appears to be responsible primarily for hemopoictic growth factor production. Cultured marrow stromal fibroblastoid cells when transplanted to other areas of the body are able to form organ-specific hemopoietic microenvironments (Friedenstein *et al.*, 1974, 1982). These cultured cells can support the growth and development of granulocytes, monocytes/macrophages, and granulocyte/macrophage (G/M) progenitors from stem cells via the production of colony-stimulating factors (Brockbank *et al.*, 1986; Brockbank and van Peer, 1983; Metcalf, 1984). Although controversy remains as to whether macrophages are true stromal cells, they produce interleukin-1 (IL-1), which in turn, stimulates fibroblastoid cells to increase production of colony-stimulating growth factors (Bagby *et al.*, 1983; Lee *et al.*, 1987; Lovhaug *et al.*, 1986; Rennick *et al.*, 1987; Zucali *et al.*, 1986). *In vitro*, adding increasing numbers of macrophages to fibroblastoid stromal cells results in an increase in the quantity of colony-stimulating activity (CSA) produced. However, purified macrophage cultures alone do not support the maturation of G/M progenitors (Zucali *et al.*, 1986). Thus, it has become apparent that macrophages can regulate fibroblastoid stromal cell CSA production through the secretion of IL-1.

Our goal in the present studies was to determine if decreased support of myelopoiesis by bone marrow stroma after *in vitro* hydroquinone exposure was due to selective toxicity to either bone marrow macrophages or fibroblastoid stromal cells. Independent populations of fibroblastoid stromal cells and macrophages were obtained from mouse bone marrow and exposed in culture to hydroquinone. These cells were then reconstituted together in culture and assayed for their ability to support granulocyte/macrophage colony formation. Identical experiments were performed using a bone marrow-derived fibroblastoid stromal cell line identified as LTF and primary fibroblastoid stromal cells derived from bone marrow mixed cultures over a 14-day period. Our results indicate that bone marrow macrophages, rather than bone marrow fibroblastoid stromal cells, are targets for hydroquinone myelotoxicity.

METHODS

Mice. Male B6C3F1 mice, 6 to 10 weeks old when used in our studies, were purchased from Jackson Laboratories (Bar Harbor, ME). The mice were housed four to

a cage in microisolator cages and had free access to feed (Wayne Certified Lab Blox, Allied Mills, Inc., Chicago, IL) and acidified water (pH 2.5–3.0). A 12-hr light cycle was maintained.

Chemicals. Hydroquinone (Sigma Chemical Co., St. Louis, MO) was diluted to the indicated concentrations in pyrogen-free saline (Kendall–McGraw Laboratories, Inc., Irvine, CA). Solutions of hydroquinone were prepared immediately prior to addition to cell cultures.

Bone marrow cell suspension. Mice were killed by cervical dislocation and their femurs and tibias removed. Using a sterile syringe with a 23-gauge needle, marrow was flushed from each bone shaft and collected in 5 ml of medium in a 60-mm Petri dish. The media consisted of RPMI 1640 (Whittaker Bioproducts, Walkersville, MD) supplemented with 7.5% horse serum and 7.5% fetal bovine serum (Hyclone Labs, Logan, UT) plus 0.29 mg/ml glutamine (Sigma), 0.02 mg/ml of gentamicin (Whittaker), and 0.1 mg/ml of sodium pyruvate (Sigma). Marrow plugs were gently aspirated through a syringe with a 23-gauge needle to prepare a suspension of single cells. Cell number was determined by counting nucleated cells with a hemacytometer.

Establishment of bone marrow-derived adherent layer. Adherent stromal cell layers were established using a modification of the method of Zipori and Bol (1979) as reported by Gaido and Wierda (1984, 1985). Bone marrow cell suspensions were diluted to 2×10^6 cells/ml and plated into 35-mm tissue culture dishes (2 ml/well). The cultures were incubated at 37°C in the presence of 5% CO_2 in air to establish foci of adherent cells. Cultures were refed by complete RPMI medium replacement on Day 3, a 50% medium replacement on Day 7, and a 100% medium replacement on Days 9 and 14.

Isolation of primary fibroblastoid stromal cells. Bone marrow cell suspensions were plated at a concentration of 2×10^6 cells/ml, 2 ml, in 35-mm tissue culture dishes. The cells were incubated in the presence of 10% L-929-conditioned medium (L-929-CM), a source of colony-stimulating factor, and 300 μg of protein-coated silica. Protein-coated silica is prepared by adding 1.5 mg of autoclaved silica (particle size 0.014 μm; Sigma) to medium containing 15% serum. The suspension is sonicated for 2 min, stored at 4°C, and resonicated prior to use (O'Rourke *et al.*, 1978). L-929-CM stimulates maturation of macrophages and phagocytosis of silica particles (Metcalf, 1984) which results in the selective killing of macrophages (O'Rourke *et al.*, 1978). Fibroblastoid stromal cells cannot phagocytize silica and, therefore, are unaffected by silica addition (Schmidt *et al.*, 1984). After 3 days of incubation, medium containing nonadherent cells was removed and replaced with 1 ml fresh medium containing 150 μg silica. Two days later, medium was again removed and the cultures were refed with 1 ml medium containing 0.1 ml nystatin (Lederle) (100 U/ml). Nystatin is toxic to macrophages when phagocytized but does not alter fibroblastoid stromal cell viability (Boniver *et al.*, 1981). Concentrated (10×) fibroblastoid stromal cell conditioned medium (0.05 ml) was also added to each culture. Fibroblastoid conditioned medium was prepared by ultrafiltration (Amicon Corp.) of medium removed from Day 5 bone marrow fibroblastoid cultures. The conditioned medium was a source of fibroblast stimulatory activity which was used to stimulate fibroblast growth (Schmidt *et al.*, 1984). After 2 days of culture in the presence of nystatin and conditioned medium, old medium was removed and replaced with 1 ml fresh medium and 0.1 ml protein-coated silica. On Day 9, the culture media and silica were removed and replaced with 1 ml of fresh medium and the cultures were incubated. On Day 12, 0.1 ml nystatin was added to the cultures and the cultures were incubated for an additional 2 days. Primary fibroblastoid stromal cell cultures were approximately 90% fibroblastoid stromal cells with approximately 10% macrophage contamination based on morphology (Werts *et al.*, 1980). These cultures were subsequently assayed for ability to support myelopoiesis.

Fibroblastoid stromal cell line (LTF). Single cell clones of fibroblastoid stromal cells were obtained from 6-month long-term bone marrow cultures. Adherent cells were diluted to 100 cells per milliliter and plated in 100-mm tissue culture dishes. Single cell clones were isolated using glass rings (Bellco, MA). A single cell clone, designated LTF, was used in the studies described here. These cells are maintained in RPMI 1640 with glutamine containing 5×10^{-5} M 2-mercaptoethanol (2-ME; Sigma), 0.02 mg/ml gentamicin, and 10% FBS. LTF cells were characterized histochemically for alkaline phosphatase, acid phosphatase, α-naphthyl acetate esterase, and myeloperoxidase enzymes, and for reaction to Periodic acid–Schiff using diagnostic kits (Sigma). For the outlined experiments, LTF cells were plated at 1×10^4 cells/ml, 1 ml/dish, and used 3 days later.

Isolation of macrophages. Macrophages were isolated from bone marrow cell suspension using a modification of the procedure of Tushinski *et al.* (Hume *et al.*, 1987; Tushinski *et al.*, 1982). The bone marrow cells were cultured in the presence of 10% L-929-CM as a source of macrophage colony-stimulating factor to stimulate macrophage growth and maturation. On Days 1 and 3 of culture, the medium plus nonadherent cells was removed by trituration and replated into a new culture dish. L-929-CM, at a concentration of 10%, was added to the cultures. On Day 5, the nonadherent cells were removed and replated with medium plus 10% L929-CM. The replated cultures were incubated for 2 more days and the adherent layer that developed was refed with RPMI complete medium containing 10% L-929-CM and glucose (2.7 mg/ml). Medium was replenished on Days 9 and 14. These adherent macrophages were used in the granulocyte/macrophage colony-forming (G/M-CFU-C) coculture assay. It was later determined that Day 5 macrophages were sufficiently purified and, therefore, were used in subsequent reconstitution and IL-1 assays.

Hydroquinone exposure and reconstitution. Cultures of macrophages or fibroblastoid cells were individually exposed to hydroquinone for 48 hr in serum-reduced medium, reconstituted together, and assayed for the ability of the reconstituted culture to support granulocyte/monocyte colony-forming cells in coculture. A 48-hr treatment period was chosen for these studies based upon the survivability of macrophages grown in culture without a source of colony-stimulating factor. Longer time periods are detrimental unless L929-conditioned medium or stromal cells are added to cultures. Day 5 macrophage cultures, Day 3 LTF cultures, or Day 14 primary fibroblast cultures were exposed to different doses of hydroquinone for 48 hr. Cell viability was determined via trypan blue exclusion. Based upon viability data, 10^{-6} M hydroquinone was chosen for initial studies involving reconstitution cultures. This concentration was the highest concentration that did not reduce cell viability. After hydroquinone treatment, macrophage cultures were rinsed thoroughly to remove residual hydroquinone. The cells were removed from adherence by scraping with a rubber policeman and aspirated gently to disperse cell clumps. Hydroquinone-treated macrophages were added at 5×10^3 cells per culture to nontreated LTF cells or to nontreated primary fibroblastoid cells. Alternatively, nontreated macrophages were added at the same cell density to hydroquinone-treated LTF cells. After a 24-hr incubation to allow for macrophage adherence, the reconstituted stromal layer was assayed for ability to support myelopoiesis in soft agar coculture. Macrophage numbers from 10 to 10^5 were examined.

Coculture of adherent layer. Cocultures consisting of tissue culture dish-adherent cells and bone marrow cells in agar were established as a modification of the procedure described by Gaido and Wierda (1984, 1985). The adherent layer was overlaid with RPMI:0.5% Bacto-agar (Difco) to ensure sufficient nutrient supply. A second layer consisting of 0.5 ml of freshly isolated bone marrow cells at a concentration of 2×10^5 cells/ml in medium plus 0.5 ml of a 50/50 mixture of 2× RPMI and 1.5% Bacto-agar was layered over the first layer. The 2× RPMI medium was supplemented with 15% horse serum and 15% fetal bovine serum, 2 μg/ml DEAE-dextran, 1.12×10^{-2} M glucose (Sigma), 1×10^{-4} M 2-mercaptoethanol, 11.2 mM glutamine, 200 μg/ml sodium pyruvate, and 0.04 mg/ml gentamicin. Cocultures were incubated for 7 days at 37°C in 5% CO_2 in air after which the granulocyte/macrophage colonies (G/M-CFU-C) that developed in agar were scored by examination of the cultures with a stereomicroscope (Olympus).

IL-1 bioassay. Macrophage LPS-stimulated conditioned medium (1-ml samples) was dialyzed at 4°C against 50 vol of phosphate-buffered saline, pH 7.2, containing 10% heat-inactivated FBS in a 28-well microdialysis system [Bethesda Research Laboratories (BRL), Gaithersburg, MD] to remove low-molecular-weight contaminants. Dialysis membranes (BRL; molecular weight cutoff 6000–8000) were extensively washed to remove the ethanol and azide packing solution. Samples were dialyzed for 48 hr, collected, and sterile filtered using Millex-GV 0.22-μm low protein binding filter units (Millipore Corp., Mississauga, Ontario). IL-1 activity was determined by the ability of LPS-stimulated macrophage-conditioned medium to stimulate proliferation of 4-week-old C3H/HeJ thymocytes in the presence of submitogenic concentrations of phytohemagglutinin (PHA; Burroughs Wellcome) (Mizel *et al.*, 1978; Lyle and Bick, 1986). C3H/HeJ mouse thymocytes were cultured in U-shaped 96-well plates at a concentration of 5×10^5 cells/well for 72 hr in RPMI culture medium containing 10% FBS, 2-ME, and 2.0 μg/ml PHA, plus LPS-stimulated macrophage-conditioned medium. Triplicate cultures were pulsed for the final 18 hr of culture with 1 μCi/well [^{3}H]thymidine. Cells were harvested using a cell harvester (Skatron, Sterling, VA) and the filters were washed to remove unincorporated [^{3}H]thymidine. Uptake of [^{3}H]-thymidine in the presence of LPS-stimulated macrophage-conditioned medium was compared with human recombinant IL-1 standard (Genzyme, Boston, Mass.). One unit of IL-1 activity was defined as the volume of conditioned medium required to cause 50% of maximum thymidine uptake.

Statistical analysis. A paired *t* test was used as a test of the null hypothesis at a level of significance of $p \leq 0.05$.

RESULTS

Isolation and Characterization of Cell Populations

To determine if hydroquinone selectively alters the function of specific cell types we used purified populations of cells obtained from adherent bone marrow cell cultures. Two major cell types were identified in adherent bone marrow cultures (Fig. 1a). The first cell type is a large, bipolar cell (30–50 μm) with an oval, light-staining nucleus (Wright–Giemsa). The second cell type is smaller (10–12 μm) with a round, dark-staining nucleus. Under culture conditions similar to those we employed, other investigators have observed cells with similar characteristics and have classified the first type as fibroblastoid stromal cells and the second cell type as macrophages (Werts *et al.*, 1980).

Purified macrophages (Fig. 1b) were readily obtained by continuous, short-term culture of bone marrow cells in the presence

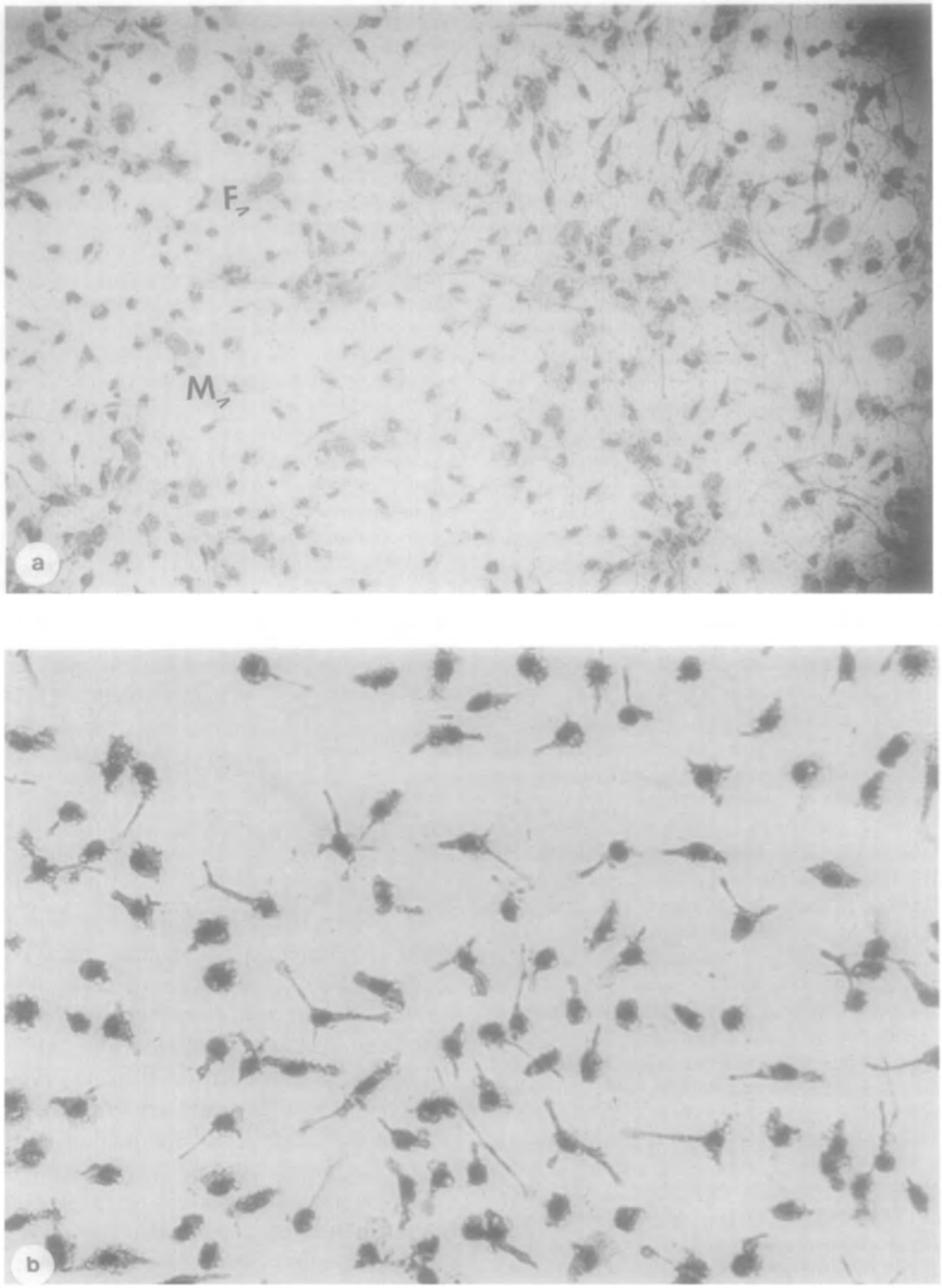

FIG. 1. Photomicrographs of bone marrow adherent stromal cells from (a) mixed populations consisting of macrophages (M) and fibroblastoid stromal cells (F) (19×); (b) purified populations of macrophages (38×); and (c) LTF fibroblastoid stromal cell line derived from 6-month long-term bone marrow cultures (19×). Wright–Giemsa.

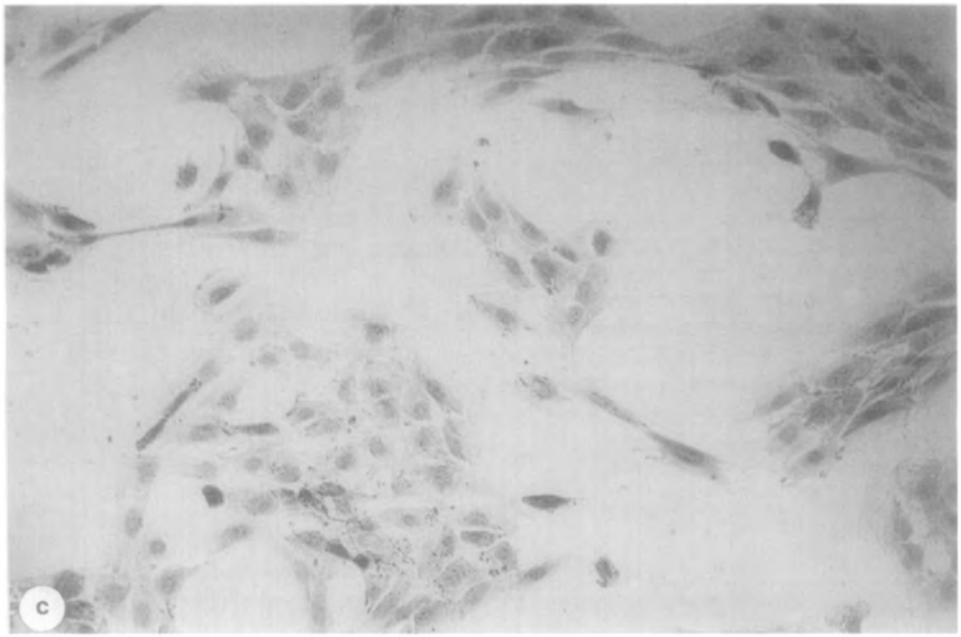

FIG. 1—*Continued*

of L-929-CM (Hume *et al.*, 1987; Tushinski *et al.*, 1982). By replating only nonadherent cells over a 5-day period, a population composed of greater than 95% macrophages was obtained, with the remainder of cells identified as granulocytes. Primary fibroblastoid stromal cell cultures from bone marrow were obtained by selectively killing macrophages with silica and nystatin as described under Methods. This procedure has been used previously to obtain purified populations of fibroblasts (Boniver *et al.*, 1981; O'Rourke *et al.*, 1978; Schmidt *et al.*, 1984).

Previous studies have demonstrated that bone marrow adherent cell cultures (mixed) support granulocyte/macrophage colony (G/M-CFU-C) growth in coculture (Gaido and Wierda, 1984; Lichtman, 1981; Zipori and Bol, 1979). This activity was also demonstrated for mixed adherent cultures in the present study (Fig. 2). In contrast, purified cultures of macrophages were unable to support significant G/M-CFU-C formation, regardless of the number of macrophages present (data not shown) (Zucali *et al.*, 1986). Purified primary fibroblastoid stromal cell cultures, however, supported an average of 55 colonies. This is in comparison to an average of 100 colonies supported by adherent mixed stroma containing both macrophages and fibroblasts. These results demonstrate

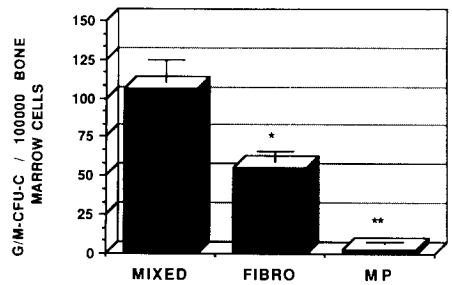

FIG. 2. Ability of primary cultures to support myelopoiesis. Day 14 mixed stromal cell cultures, fibroblastoid stromal cell cultures, and bone marrow-derived macrophage cultures were assayed for their ability to support granulocyte/macrophage colony (G/M-CFU-C) formation in a coculture assay. Values are presented as means ± SEM from three experiments. *Significantly different from mixed ($p < 0.05$). **Significantly different from mixed ($p < 0.001$) and from fibroblast ($p < 0.05$).

TABLE 1

EFFECT OF LTF CELL NUMBER ON GM-CFU-C FORMATION[a]

Number of cells plated	GM-CFU-C[b]
0.25×10^4	92.7 ± 12.35
1.00×10^4	170.7 ± 1.33
2.00×10^4	189.3 ± 7.42
8.00×10^4	106.6 ± 5.93

[a] LTF cells were initially plated as indicated in 1 ml of medium and cocultured 3 days later with an agar overlay. After an additional 7 days incubation, granulocyte/macrophage colony formation (G/M-CFU-C) was determined.

[b] Values represent means ± SEM for three experiments.

the ability of macrophages to enhance fibroblastoid stromal cell-dependent G/M-CFU-C growth. This enhancement could be observed with the addition of as few as 100 macrophages to the fibroblastoid stromal cell cultures.

The total number of G/M-CFU-C supported by primary fibroblastoid cells was likely related to the number of cells producing CSA. This is illustrated with the LTF cell line (described below) which supported a maximum of approximately 200 G/M-CFU-C in coculture. Average G/M-CFU-C declined coincident with decreasing numbers of LTF cells plated at initiation of culture (Table 1). The decrease in G/M-CFU-C observed in cultures with initially the highest number of LTF cells plated is attributed to LTF stromal cell overgrowth and subsequent deterioration of culture conditions.

The LTF cell line (Fig. 1c) was derived from 6-month bone marrow cultures as described under Methods. The LTF cells appear histologically as large cells with cytoplasmic processes. They have large nuclei with prominent nucleoli and most of their chromatin in the euchromatin form. These cells have survived greater than 50 passages and have been histochemically characterized as acid phosphatase positive, myeloperoxidase negative, α-naphthyl acetate esterase negative, periodic acid–Schiff positive, and mac-1 negative. This pattern of characterization indicates that the LTF cell line is not of macrophage origin as macrophages are positive for both myeloperoxidase and α-naphthyl acetate esterase (Brockbank *et al.*, 1986; Crocker and Gordon, 1985; Cronkite *et al.*, 1982a; Rennick *et al.*, 1987). The LTF cell line constitutively produces CSA (Table 1).

Macrophage Regulation of LTF Fibroblastoid Stromal Cell Colony-Stimulating Activity Production

Macrophages can regulate or influence the production of growth factors from fibroblasts or endothelial cells (Lovhaug *et al.*, 1986; Rennick *et al.*, 1987; Zucali *et al.*, 1986). In our system, reconstitution of fibroblastoid stromal cells with macrophages enhanced the ability of the fibroblastoid stromal cells to support myelopoiesis (data not shown). These results were similar to those obtained from coculture of mixed adherent layers in comparison to fibroblasts (Fig. 2).

Forty-eight-hour treatment of macrophages with hydroquinone (10^{-6} M) did cause some cell death as determined by trypan blue exclusion (Fig. 3A). Macrophage viability was significantly decreased at concentrations of 10^{-5} M and higher. To determine if hydroquinone interfered with macrophage regulation of fibroblastoid production of colony-stimulating activity, purified macrophages were exposed for 48 hr to hydroquinone followed by LTF cell reconstitution with 1×10^4 viable treated macrophages. As shown in Fig. 4, a reduction in macrophage activity was detected at hydroquinone concentrations of 10^{-7} M with statistically significant reductions in activity noted at 10^{-6} M and above. Viability of LTF cells was essentially unaltered by hydroquinone except at 10^{-4} M (Fig. 3B). In contrast to macrophage cultures, LTF cultures previously exposed to hydroquinone and then reconstituted with normal macro-

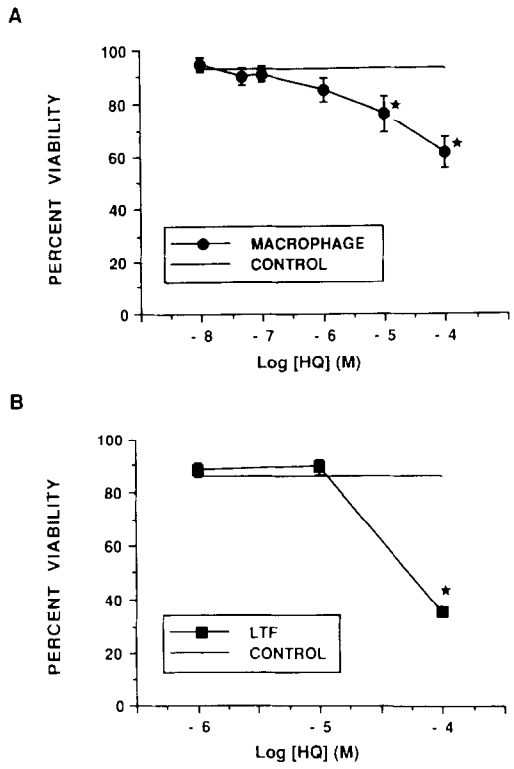

FIG. 3. Effect of hydroquinone on (A) macrophage and (B) LTF viability. Macrophages or LTF cells were incubated with hydroquinone for 48 hr. Cell viability was determined by trypan blue exclusion. Values are presented as means ± SEM from four experiments. *Significantly different from control ($p < 0.05$).

phages supported numbers of G/M-CFU-C approximately equal to untreated LTF cultures (Fig. 5). A decrease in constitutive production of CSA by LTF cells was seen only when the cells were treated with a cytotoxic concentration of 10^{-4} M hydroquinone (Fig. 5).

Macrophage Regulation of Primary Fibroblastoid Stromal Cell Cultures

Experiments similar to those described above with the LTF cell line were performed using purified, primary fibroblastoid cells isolated from fresh bone marrow adherent cells (Fig. 6). These cultures contain greater than 95% fibroblastoid cells but are not entirely devoid of contaminating macrophages. Reconstitution with 10^{-6} M hydroquinone-treated macrophages (5×10^3 cells) induced inhibition of the production of colony-stimulating activity. A similar response was noted when other macrophage cell numbers were used. These results reproduced the effect observed when hydroquinone-treated macrophages were added to LTF fibroblastoid cultures (Fig. 4). Moreover, the decrease in G/M-CFU-C observed in reconstituted primary fibroblastoid cultures was more marked in comparison to reconstituted LTF cultures. This may be attributed to a lower constitutive level of CSA production by primary fibroblastoid stromal cells in contrast to LTF cells. Primary fibroblasts were treated with hydroquinone and, following reconstitution, were assayed for their ability to support G/M-CFU-C formation. Exposure of these unmanipulated primary fibroblastoid cell cultures to 10^{-6} M hydroquinone for 48 hr did not affect innate colony-stimulating activity (Fig. 7). The results demonstrate resistance of primary fibroblastoid cells to the toxic effects of hydroquinone similar to that seen with LTF stromal cells.

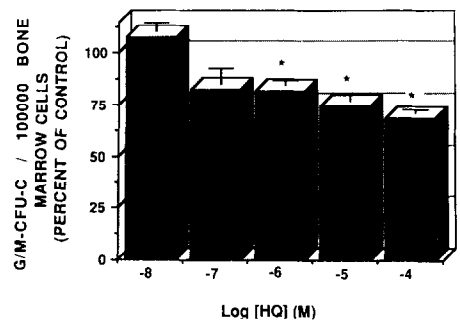

FIG. 4. Dose response of hydroquinone on ability of macrophages to regulate fibroblastoid production of colony-stimulating factor activity. Macrophages were treated for 18 hr with indicated concentrations of hydroquinone. LTF cells were reconstituted with 10^4 treated macrophages. The reconstituted cultures were cocultured 24 hr later and the number of G/M-CFU-C that formed after 7 days was determined. Values are presented as means ± SEM from three experiments. *Significantly different from control ($p < 0.05$).

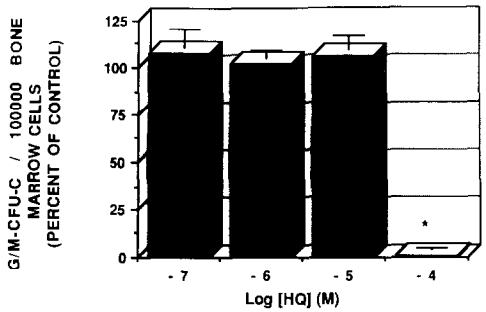

FIG. 5. Dose response of hydroquinone on constitutive CSA production by LTF cells. After treatment with hydroquinone for 48 hr and reconstitution with macrophages, the LTF cells were cocultured. The number of granulocyte/macrophage colonies (G/M-CFU-C) that formed after an additional 7-day incubation was determined. Values are presented as means ± SEM from two experiments of triplicate cultures. *Significantly different from control ($p < 0.05$).

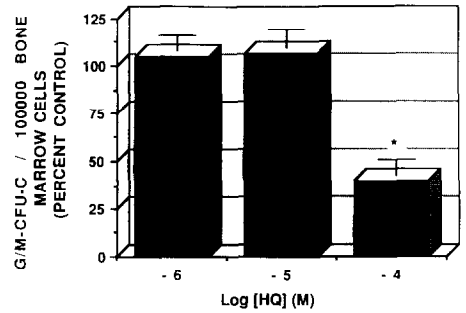

FIG. 7. Ability of cultures containing hydroquinone-treated primary fibroblasts and nontreated macrophages to support G/M-CFU-C formation. Primary fibroblast cultures were developed over a 14-day period as described and exposed to hydroquinone for 48 hr. These cultures were then reconstituted with macrophages, cocultured, and incubated for 7 days, and the number of G/M-CFU-C was determined. Values are presented as means ± SEM from three experiments of triplicate cultures. *Significantly different from control ($p < 0.05$).

Decreased IL-1 Activity in Hydroquinone-Treated Macrophages

IL-1 produced by macrophages has been shown to increase CSA production by fibroblast cultures (Lovhaug *et al.*, 1986; Rennick *et al.*, 1987; Zucali *et al.*, 1986). To determine if hydroquinone could inhibit IL-1 production, dialyzed conditioned media from lipopolysaccharide (LPS)-activated macrophages, previously exposed to hydroquinone, were bioassayed for IL-1 activity (Table 2). LPS-inducible IL-1 activity was significantly reduced in macrophage cultures treated with 10^{-6} M hydroquinone in both experiments when compared with control macrophages. A similar decrease in production of IL-1 activity by hydroquinone-treated macrophages has been seen in our laboratory after only 1-hr exposures (King *et al.*, 1988). The increase seen with 10^{-5} M hydroquinone in experiment 2 is postulated to be due to excessive death of cells and release of internal IL-1. An increase in IL-1 in conditioned medium is seen when cells are treated with 10^{-4} M hydroquinone, a concentration that causes significant cell death. This observed decrease in IL-1 after treatment with 10^{-6} M hydroquinone activity may account, in part, for the decreased ability of hydroquinone-exposed macrophages to stimulate CSA activity in bone marrow fibroblastoid stromal cell cultures (Figs. 4A and 6). Very little, if any, hydroquinone was expected to be carried over into the conditioned medium and affect the IL-1 bioassay. To alleviate any carryover fol-

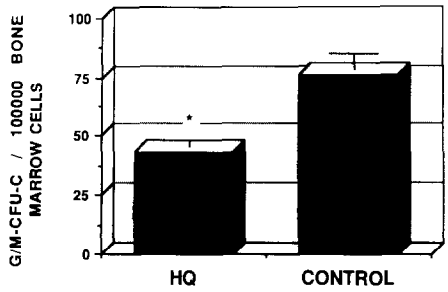

FIG. 6. Ability of reconstituted cultures containing hydroquinone-treated macrophages and nontreated primary fibroblastoid stromal cells to support G/M-CFU-C formation. Macrophages were exposed to hydroquinone (10^{-6} M) for 48 hr and then added to Day 14 purified primary fibroblastoid stromal cells. The cultures were cocultured 24 hr later and the number of granulocyte/macrophage colonies (G/M-CFU-C) that formed after 7 days was determined. Values are presented as means ± SEM from two experiments of triplicate cultures. *Significantly different from control ($p < 0.05$).

lowing hydroquinone exposure, macrophage cultures were rinsed to remove residual hydroquinone and fresh medium was added. After an additional 24 hr in culture, conditioned medium was collected and dialyzed against PBS containing 10% FBS. The action of hydroquinone, therefore, would appear to occur at the cellular level. It should be noted that the decrease in thymocyte proliferation could be due to inhibition of interleukin-6 (IL-6) production and/or release as well as IL-1. IL-6 in the presence of PHA has been shown to stimulate thymocyte proliferation (Uyttenhove *et al.*, 1988). IL-6 was not examined in these studies.

DISCUSSION

Treatment of bone marrow stromal cells with the benzene metabolite hydroquinone reduces the ability of mixed stromal cell cultures to support myelopoiesis (Gaido and Wierda, 1984, 1985). In the present studies, we used purified cultures on macrophages and fibroblastoid stromal cells to determine possible cell-specific toxicity to hydroquinone. Macrophages and fibroblastoid stromal cells coordinately regulate myelopoiesis (Bagby *et al.*, 1983; Lee *et al.*, 1987; Lovhaug *et al.*, 1986; Rennick *et al.*, 1987; Zucali *et al.*, 1986) and, therefore, reconstitution cultures were used to examine cell sensitivity. Our results support the hypothesis that macrophages are specific targets of hydroquinone toxicity. Reconstituted cocultures containing 10^{-6} M hydroquinone-treated macrophages had a reduced capacity to support G/M-CFU-C formation. In contrast, the same concentration of hydroquinone had no effect on LTF fibroblastoid stromal cell production of CSA when assayed in a reconstitution system or on primary fibroblastoid CSA production assayed without reconstitution. A decrease in both LTF fibroblastoid stromal cell function and viability was noted only with treatment with 10^{-4} M hydroquinone.

The reduced ability of LTF cultures reconstituted with hydroquinone-treated macrophages to support G/M-CFU-C was slight but statistically significant in the present studies. Moreover, this reduction in colony-stimulating activity was consistently observed in other experiments which examined the effects of adding different numbers of hydroquinone-treated macrophages to LTF cultures (data not shown). This effect could conceivably become biologically important when stromal cells must be recruited to produce colony-stimulating factors as shown in Fig. 2 or during increased hemopoietic demand *in vivo*. Addition of hydroquinone-treated macrophages to primary fibroblastoid stromal cells had a more dramatic effect as illustrated by a 45% reduction in colony-

TABLE 2

EFFECT OF HYDROQUINONE ON THE ABILITY OF MACROPHAGES TO PRODUCE IL-1 WHEN STIMULATED WITH LPS[a]

Concentration (M)	Experiment 1	Experiment 2
Control	70.92[b] (100%)[c]	30.03 (100%)
10^{-7}	70.42 (99%)	28.57 (94%)
10^{-6}	32.40 (45%)	<10.0 (<33%)[d]
10^{-5}	10.49 (15%)	30.3 (100%)

[a] Bone marrow-derived macrophages obtained after a 5-day culture period were exposed to hydroquinone for 12 hr. The macrophages were then rinsed thoroughly to remove residual hydroquinone and stimulated with LPS for 24 hr. The conditioned medium from the 24-hr stimulation period was collected and analyzed for IL-1 via thymocyte proliferation assay.

[b] Values were expressed as units/ml, with 1 unit equaling the volume of conditioned medium required for half-maximal stimulation for each experiment. Units/ml for each concentration of hydroquinone was determined from graphs representing volume of conditioned medium (μl) versus counts per minute (cpm). On these graphs, values for each concentration point represent mean cpm for triplicate cultures. Variability for each concentration point did not exceed 10% of mean values. Experiments 1 and 2 were performed on different days.

[c] Numbers in parentheses represent percentages of control.

[d] The volume required for half-maximal stimulation was greater than 100 μl, the largest volume examined. 100 μl of conditioned medium corresponds to 10 U/ml.

stimulating activity (Fig. 6). If this translated to a 45% reduction in granulocyte/macrophage precursors in the bone marrow after benzene exposure, it would be expected that an equal reduction in circulating nucleated blood cells would be observable within one transit time for precursors through the marrow (Cronkite *et al.*, 1982b).

Another parameter that was taken into account was the potential effect of hydroquinone on IL-1 production by the macrophage. IL-1 is generally believed to induce stromal cells, including fibroblastoid cells and endothelial cells, to produce CSA (Lovhaug *et al.*, 1986; Rennick *et al.*, 1987; Zucali *et al.*, 1986). IL-1 secretion by macrophages is believed to be responsible for enhanced G/M-CFU-C formation by fibroblastoid stromal cells in reconstituted stromal cell cultures (Bagby *et al.*, 1983; Lee *et al.*, 1987). In a separate series of experiments, we have observed that purified recombinant IL-1 can enhance interleukin-4 production by stromal fibroblastoid cells (King *et al.*, 1988). To address the effect of hydroquinone on macrophage IL-1 production, we measured IL-1 activity in conditioned medium removed from hydroquinone-treated macrophage cultures following subsequent stimulation with lipopolysaccharide (LPS) (Mizel *et al.*, 1978). LPS is a powerful stimulus for IL-1 production by macrophages. Pretreatment of macrophages with hydroquinone reduced the amount of detectable IL-1 activity secreted into conditioned medium. This effect correlates with the inability of hydroquinone-treated macrophages to induce colony-stimulating activity in reconstituted cultures as efficiently as untreated macrophages.

We cannot rule out the possibility that enhanced prostaglandin production by macrophages also contributed to reduced CSA in reconstituted cultures. We have previously detected concentrations of $0.4–1.1 \times 10^{-9}$ M PGE_2 in mixed stromal cultures containing 10^{-5} to 10^{-7} M hydroquinone (Gaido and Wierda, 1987). Macrophages are an excellent source of prostaglandin, and such concentrations of PGE_2 are sufficient to inhibit G/M-CFU-C formation in agar (Pelus *et al.*, 1981). PGE_2 concentrations were not monitored in these studies. Further experiments are necessary to address this aspect of toxicity.

Benzene exposure reduces pluripotent stem cells in bone marrow as well as the committed precursors that arise from these stem cells (Uyeki *et al.*, 1977, Cronkite *et al.*, 1982b). The present studies provide further evidence that stem cells are not the exclusive target for benzene toxicity and that damage to bone marrow stromal cells can contribute to bone marrow hypoplasia induced by benzene. However, benzene exposure can also lead to the formation of leukemias in humans as well as in animals. Could an alteration in stromal cell growth factor production be involved in the etiology of this disease? Studies by Zipori and co-workers (Zipori, 1981; Zipori and Bol, 1979) suggest an affirmitive answer to this question. Their studies indicate that bone marrow-derived stromal cell lines can either promote or inhibit the growth of selected hemopoietic tumor cells *in vitro*. If stromal cell produced inhibitory factors are reduced upon benzene exposure, the growth of tumor cells may increase. In light of this information, it would be interesting to speculate that the interaction of benzene or its metabolites with the hemopoietic microenvironment could select for stromal cells which support the development of leukemic stem cells. Such stem cells may arise "spontaneously" or be initiated by exposure to benzene or other environmental contaminants. Formal proof of this hypothesis must await development of an appropriate *in vitro* model which allows for the growth and detection of stem cells in the presence of bone marrow stromal cells.

These studies support and extend observations made by Kalf and co-workers (Kalf *et al.*, 1982, 1985; Post *et al.*, 1986) that benzene and its metabolites can inhibit RNA synthesis in mouse peritoneal macrophages by demonstrating that bone marrow stromal macrophages are specific targets for hydroquinone toxicity. Our results also suggest that

toxicity may be manifested by a reduction in IL-1 activity. Benzene and metabolites have been shown to interfere with RNA synthesis and the formation of interleukin-2 in peritoneal macrophages (Post *et al.*, 1986) and hydroquinone may be acting in a similar fashion to inhibit IL-1 production.

Toxicity to macrophages may be a direct effect of hydroquinone or may result from compounds formed by cellular metabolism of hydroquinone. Macrophages contain peroxidase enzymes which have been shown to convert polyhydroxybenzene metabolites into reactive molecules (Eastmond *et al.*, 1986; Irons *et al.*, 1980; Sadler *et al.*, 1988). Macrophage lysates, which contain large amounts of prostaglandin H synthetase can convert hydroquinone into a compound capable of covalently binding to protein (Schlosser and Kalf, 1988). Hydroquinone may be converted into benzoquinone which is capable of altering a broad range of macrophage functions including H_2O_2 release and F_c-mediated phagocytosis (Lewis *et al.*, 1988). Conversion of hydroquinone to reactive species which can interact with RNA synthesis or covalently bind to cellular proteins is a possible occurrence.

In summary, these studies provide further evidence for the central role that macrophages exert in regulating stromal growth factor production in the bone marrow. An imbalance in this regulation caused by benzene or its metabolites could account for the wide variety of blood dyscrasias reported after exposure to these compounds. Additionally, these studies suggest that replacement of certain growth factors may be feasible in therapy of patients who present with myelotoxic symptoms caused by exposure to benzene or other chemical agents.

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REFERENCES

ALLEN, T. D., AND DEXTER, T. M. (1984). The essential cells of the hemopoietic microenvironment. *Exp. Hematol.* **12,** 517–521.

ANDREWS, L. S., LEE, E. W., WITMER, C. M., KOCSIS, J. J., AND SNYDER, R. S. (1977). Effects of toluene on the metabolism, disposition and hemopoietic toxicity of [^{3}H]-benzene. *Biochem. Pharmacol.* **26,** 293–300.

BAGBY, J. C., MCCALL, E., AND LAYMAN, D. L. (1983). Regulation of colony stimulating activity production: Interactions of fibroblasts, mononuclear phagocytes, and lactoferrin. *J. Clin. Invest.* **71,** 340–344.

BERNSTEIN, S. E. (1970). Tissue transplantation as an analytic and therapeutic tool in hereditary anemias. *Amer. J. Surg.* **119,** 448–451.

BOLCSAK, L. E., AND NERLAND, D. E. (1983). Inhibition of erythropoiesis by benzene and benzene metabolites. *Toxicol. Appl. Pharmacol.* **69,** 363–368.

BONIVER, J., DECLEVE, A., DAILEY, M. O., HONSIK, C., LIEBERMAN, M., AND KAPLAN, H. S. (1981). Macrophage and lymphocyte depleted thymus reticuloendothelial cell cultures: Establishment and functional influence on T-lymphocyte maturation. C-type virus expression and lymphomatous transformation *in vitro*. *Thymus* **2,** 193–213.

BROCKBANK, K. G. M., DE JONG, J. P., PIERSMA, A. H., AND VOERMAN, J. S. A. (1986). Hemopoiesis on purified bone-marrow-derived reticular fibroblasts *in vitro*. *Exp. Hematol.* **14,** 386–394.

BROCKBANK, K. G. M., AND VAN PEER, C. M. J. (1983). Colony-stimulating activity production by hemopoietic organ fibroblastoid cells *in vitro*. *Acta Haematol.* **69,** 369–375.

CROCKER, P. R., AND GORDON, S. (1985). Isolation and characterization of resident stromal macrophages and hematopoietic cell clusters from mouse bone marrow. *J. Exp. Med.* **162,** 993–1014.

CRONKITE, E. P. (1987). Chemical leukemogenesis: Benzene as a model. *Semin. Hematol.* **24,** 2–11.

CRONKITE, E. P., HARIGAYA, K., GARNETT, H., MILLER, M. E., HONIKEL, L., AND SHADDUCK, R. K. (1982a). Production of colony-stimulating factor by a murine bone marrow cell line derived from the Dexter adherent layer and other properties of this cell. In *Experimental Hematology Today 1982*. (S. J. Baum *et al.*, Eds.), pp. 11–18. S. Karger, New York.

CRONKITE, E. P., INOU, J., CARSTEN, A. L., MILLER, M. E., BULLIS, J. E., AND DREW, R. T. (1982b). Effects of benzene inhalation on murine pluripotent stem cells. *J. Toxicol. Environ. Health* **9,** 411–421.

DEXTER, T. M. (1982). Stromal cell associated hemopoiesis. *J. Cell. Physiol. (Suppl.)* **1,** 87–94.

DEXTER, T. M., ALLEN, T. D., AND LAJTHA, L. G. (1977). Conditions controlling the proliferation of haemopoietic stem cells in vitro. *J. Cell. Physiol.* **91,** 335–344.

DEXTER, T. M., AND MOORE, M. A. S. (1977). *In vitro* duplication and "cure" of haemopoietic defects in genetically anemic mice. *Nature (London)* **269,** 412–414.

DEXTER, T. M., SIMMONS, P., PURNELL, R. A., SPOONCER, E., AND SCHOFIELD, R. (1984). The regulation of hemopoietic cell development by the stromal cell microenvironment and diffusable regulatory molecules. In *Aplastic Anemia Stem Cell Biology and Advances in Treatment,* (N. S. Young *et al.*, Eds.), pp. 13–33. Alan R. Liss, New York.

EASTMOND, D. A., SMITH, M. T., RUZO, L. U., AND ROSS, D. (1986). Metabolic activation of phenol by human myeloperoxidase and horseradish peroxidase. *Mol. Pharmacol.* **30,** 674–679.

FRIEDENSTEIN, A. J., CHAILAKHYAN, R. K., LATSINIK, N. V., PANASYUK, A. F., AND KEILISS-BOROK, I. V. (1974). Stromal cells are responsible for transferring the microenvironment of the hemopoietic tissues. Cloning *in vitro* and retransplantation *in vivo*. *Transplantation* **17,** 331–340.

FRIEDENSTEIN, A. J., LATZINIK, N. W., GROSHEVA, A. G., AND GORSKAYA, U. F. (1982). Marrow microenvironment transfer by heterotropic transplantation of freshly isolated and cultured cells in porous sponges. *Exp. Hematol.* **10,** 217–227.

GAIDO, K., AND WIERDA, D. (1984). *In vitro* effects of benzene metabolites in mouse bone marrow stromal cells. *Toxicol. Appl. Pharmacol.* **76,** 45–55.

GAIDO, K., AND WIERDA, D. (1985). Modulation of stromal cell function in DBA/2J and B6C3F1 mice exposed to benzene or phenol. *Toxicol. Appl. Pharmacol.* **81,** 469–475.

GAIDO, K., AND WIERDA, D. (1987). Suppression of bone marrow stromal cell function by benzene and hydroquinone is ameliorated by indomethacin. *Toxicol. Appl. Pharmacol.* **89,** 378–390.

GOLDSTEIN, B. D., WITZ, G., JAVID, J., AMORUSO, M. A., ROSSMAN, T., AND WOLDER, B. (1982). Mucondialdehyde, a potential toxic intermediate of benzene metabolism. In *Biological Reactive Intermediates II* (R. Snyder *et al.*, Eds.), pp. 331–339. Plenum, New York.

HUME, D. A., ALLAN, W., FABRUS, B., WEIDEMANN, M. J., HAPEL, A. J., AND BARTELMEZ, S. (1987). Regulation of proliferation of bone marrow-derived macrophages. *Lymphokine Res.* **6,** 127–139.

IRONS, R. D., DENT, J. G., BAKER, T. S., AND RICKERT, D. E. (1980). Benzene is metabolized and covalently bound in bone marrow *in situ*. *Chem.-Biol. Interact.* **30,** 241–245.

KALF, G. F., RUSHMORE, T., AND SNYDER, R. (1982). Benzene inhibits RNA synthesis in mitochondria from liver and bone marrow. *Chem.-Biol. Interact.* **42,** 353–370.

KALF, G. F., SNYDER, R., AND RUSHMORE, T. H. (1985). Inhibition of RNA synthesis by benzene metabolites and their covalent binding to DNA in rabbit bone marrow mitochondria *in vitro*. *Amer. J. Ind. Med.* **7,** 485–492.

KING, A. G., LANDRETH, K. S., AND WIERDA, D. (1988). Bone marrow stromal cell regulation of B-lymphopoiesis. I. The roles of macrophages, interleukin-1 and interleukin-4 in pre-B cell maturation. *J. Immunol.* **141,** 2016–2026.

LASKIN, S., AND GOLDSTEIN, B. (1977). Benzene toxicity, a critical evaluation. *J. Toxicol. Environ. Health (Suppl.)* **2,** 1–147.

LEE, E. W., KOCSIS, J. J., AND SNYDER, R. (1974). Acute effect of benzene on ^{59}Fe incorporation into circulating erythrocytes. *Toxicol. Appl. Pharmacol.* **27,** 431–436.

LEE, M., SEGAL, G. M., AND BAGBY, G. C. (1987). Interleukin-1 induces human bone marrow-derived fibroblasts to produce multilineage hemopoietic growth factors. *Exp. Hematol.* **15,** 983–988.

LEWIS, J. G., ODOM, B., AND ADAMS, D. O. (1988). Toxic effects of benzene and benzene metabolites on mononuclear phagocytes. *Toxicol. Appl. Pharmacol.* **92,** 246–254.

LICHTMAN, M. A. (1981). The ultrastructure of the hemopoietic environment of the marrow: A review. *Exp. Hematol.* **9,** 391–410.

LOVHAUG, D., PELUS, L. M., NORDLIE, E. M., BOYUM, A., AND MOORE, M. A. S. (1986). Monocyte-conditioned medium and interleukin-1 induce granulocyte-macrophage colony-stimulating factor production in the adherent cell layer of murine bone marrow cultures. *Exp. Hematol.* **14,** 1037–1042.

LYLE, M., AND BICK, P. H. (1986). Modulation of interleukin-1 production by macrophages following benzo(*a*)pyrene exposure. *Int. J. Immunopharmacol.* **8,** 377–381.

MCCULLOCH, E. A., SIMINOVITCH, L., TILL, J. E., RUSSEL, E. S., AND BERNSTEIN, S. E. (1965). The cellular basis of the genetically determined hemopoietic defect in anemic mice of genotype Sl/Sl^d. *Blood* **26,** 399–410.

METCALF, D. (1984). *The Hemopoietic Colony Stimulating Factors.* Elsevier, Amsterdam.

MIZEL, S. B., OPPENHEIM, J. J., AND ROSENSTREICH, D. L. (1978). Characterization of lymphocyte-activating factor (LAF) produced by the macrophage cell line. P388D1. I. Enhancement of LAF production by activated T lymphocytes. *J. Immunol.* **120,** 1497–1503.

O'ROURKE, E. J., HALSTEAD, S. B., ALLISON, A. C., AND PLATTS-MILLS, T. A. E. (1978). Specific lethality of silica for human peripheral blood mononuclear phagocytes, *in vitro*. *J. Immunol. Methods* **19,** 137–151.

PELUS, L. M., BROXMEYER, H. E., AND MOORE, M. A. S. (1981). Regulation of human myelopoiesis by prostaglandin E and lactoferrin. *Cell Tissue Kinet.* **14,** 515–526.

POST, G., SNYDER, R., AND KALF, G. F. (1986). Metabo-

lism of benzene and phenol in macrophages in vitro and the inhibition of RNA synthesis by benzene metabolites. *Cell Biol. Toxicol.* **2,** 231–245.

REIMANN, J., AND BURGER, H. (1979). *In vitro* proliferation of haemopoietic cells in the presence of adherent cell layers. I. Culture conditions and strain dependence. *Exp. Hematol.* **7,** 45–51.

RENNICK, D., YANG, G., GEMMELL, L., AND LEE, F. (1987). Control of hemopoiesis by a bone marrow stromal cell clone: Lipopolysaccharide- and interleukin-1-inducible production of colony stimulating factors. *Blood* **69,** 682–691.

RICKERT, D. E., BAKER, T. S., BUS, J. S., BARROW, C. S., AND IRONS, R. D. (1979). Benzene disposition in the rat after exposure by inhalation. *Toxicol. Appl. Pharmacol.* **49,** 417–423.

SADLER, A., SUBRAHMANYAM, V. V., AND ROSS, D. (1988). Oxidation of catechol by horseradish peroxidase and human leukocyte peroxidase. Reaction of *o*-benzosemiquinone and benzoquinone. *Toxicol. Appl. Pharmacol.* **93,** 62–71.

SAWAHATA, T., AND NEAL, R. A. (1983). Biotransformation of phenol to hydroquinone and catechol by rat liver microsomes. *Mol. Pharmacol.* **23,** 453–460.

SCHLOSSER, M. J., AND KALF, G. F. (1988). Activation of phenol and hydroquinone to covalently binding metabolites by mouse macrophage lysates. *Toxicologist* **8,** 72.

SCHMIDT, J. A., OLIVER, C. N., LEPE-ZUNIGA, J. L., GREEN, I., AND GERY, K. (1984). Silica stimulated monocytes release fibroblast proliferation factors identical to IL-1. *J. Clin. Invest.* **73,** 1462–1472.

SNYDER, R., LEE, E. W., KOCSIS, J. J., AND WITMER, C. M. (1977). Bone marrow depressant and leukemogenic actions of benzene. *Life Sci.* **21,** 1079–1722.

SNYDER, R., LONGACRE, S. L., SAMMETT, D., WITMER, C., AND KOCSIS, J. J. (1982). Relationship between the toxicity and metabolism of benzene. In *Advances in Modern Environmental Toxicology*, (M. A. Mehlman, Ed.), pp. 23–35. Senate Press, Princeton, NJ.

TUNEK, A., AND OESCH, F. (1982). Multi-step metabolic activation of benzene in rat liver microsomes. In *Biological Reactive Intermediates II* (R. Snyder *et al.*, Eds.), pp. 319–329. Plenum, New York.

TUSHINSKI, R. J., OLIVER, I. T., GUILBERT, L. J., TYNAN, P. W., WARNER, J. R., AND STANLEY, E. R. (1982). Survival of mononuclear phagocytes depends on a lineage-specific growth factor that the differentiated cells selectively destroy. *Cell* **28,** 71–81.

UYEKI, E. M., EL ASKHAR, A., SHOEMAN, D. W., AND BISEL, J. U. (1977). Acute toxicity of benzene inhalation to hemopoietic precursor cells. *Toxicol. Appl. Pharmacol.* **40,** 49–57.

UYTTENHOVE, C., COULIE, P. G., AND VAN SNICK, J. (1988). T-cell growth and differentiation induced by interleukin-HP1/IL-6, the murine hybridoma/plasmacytoma growth factor. *J. Exp. Med.* **167,** 1417–1427.

WERTS, E. D., DEGOWIN, R. L., KNAPP, S. K., AND GIBSON, D. P. (1980). Characterization of marrow stromal (fibroblastoid) cells and their association with erythropoiesis. *Exp. Hematol.* **8,** 423–433.

ZIPORI, D. (1981). Conditions required for the inhibition of *in vitro* growth of a mouse myeloma cell line by adherent bone-marrow cells. *Cell Tissue Kinet.* **14,** 479–488.

ZIPORI, D., AND BOL, S. (1979). The role of fibroblastoid cells and macrophages from mouse bone marrow in the *in vitro* growth promotion of haemopoietic tumour cells. *Exp. Hematol.* **7,** 206–212.

ZIPORI, D., SASSON, T., AND FRIEDMAN, S. (1982). Bone marrow resident colony-stimulating factor activity (CSA) produced by stromal cells. In *Experimental Hematology Today 1982,* (S. J. Baum, *et al.*, Eds.), pp. 19–26. S. Karger, New York.

ZUCALI, J. R., DINARELLO, C. A., OBLON, D. J., GROSS, M. A., ANDERSON, L., AND WEINER, R. S. (1986). Interleukin 1 stimulates fibroblasts to produce granulocyte-macrophage colony-stimulating activity and prostaglandin E_2. *J. Clin. Invest.* **77,** 1857–1863.