

## Production of Erythroid Potentiating Factor(S) by a Human Monocytic Cell Line

By Joao L. Ascensao, Neil E. Kay, Theresa Earenfight-Engler, Hillel S. Koren, and Esmail D. Zanjani

**U-937 is a human monocytic cell line that has been shown to elaborate factors that affect normal human hematopoiesis in vitro. Studies on the effects of these factors demonstrated an erythroid potentiating factor (EPF) and a potent inhibitor of granulocyte-macrophage (CFU-GM) colony growth. The EPF was present in both serum-containing and serum-free U-937 conditioned media, had a dose-dependent effect on erythroid colony formation and was remarkably heat stable. The CFU-GM inhibitory activity was also detected in serum-free conditioned medium, was**

**dose-dependent, heat labile and its effect was reversed by Indomethacin. Indomethacin (Sigma, St. Louis, Mo.) did not alter the erythroid effects of the U-937 conditioned medium. No colony stimulating factor (CSF) or erythropoietin (Ep) could be detected in this medium. The existence of a human cell line capable of producing EPF without simultaneous CSF production will permit further studies on the biochemical and biologic nature of these factors.**

**D**EVELOPMENT of in vitro semi-solid culture techniques that facilitate proliferation and differentiation of erythroid precursors has refined our concepts of the regulation of erythropoiesis by permitting the operational identification of at least two major classes of erythroid progenitors, erythroid burst-forming (BFU-E), and erythroid colony-forming units (CFU-E),<sup>1,2</sup> the latter being a progeny of BFU-E.<sup>2</sup>

At least two different substances which influence in vitro erythropoiesis have been recognized. Erythropoietin (Ep) regulates the growth of CFU-E and controls the differentiating process leading to the hemoglobinization of the erythrocyte precursors.<sup>1,3</sup> "Burst promoting factor" (BPF) is a substance(s) different from Ep that acts on more primitive erythroid precursors (BFU-E) and may be responsible for the precursor's acquisition of a response to Ep.<sup>1,2,4-6</sup> BPF has been detected in media conditioned by a variety of cells and tissues including leucocytes,<sup>4</sup> macrophages,<sup>6</sup> placenta,<sup>7</sup> T cells,<sup>8</sup> and is present in serum.<sup>1</sup>

Recently, a human T-lymphoblastoid cell line was found to elaborate CSF as well as factors capable of enhancing the proliferation and/or differentiation of both BFU-E and CFU-E in vitro.<sup>8</sup> We report here that

a human monocytic cell line (U-937) produces a factor(s) that enhances the growth of human BFU-E and CFU-E in vitro. Because the product of this cell line affected both CFU-E and BFU-E growth in vitro, the term erythroid potentiating factor (EPF) was used to designate this substance, presumably to distinguish it from agents that only enhance BFU-E growth in vitro.<sup>1,2,4,5</sup> This erythroid potentiating activity occurs in the absence of any detectable Ep or CSF activities. The U-937 provides a continuous source of potent cell-mediated factors that can be used for further characterization of EPF.

### MATERIALS AND METHODS

#### *U-937 Cell Line*

The U-937 cell line was derived from a pleural effusion of a patient with histiocytic lymphoma and shown to possess macrophage-like characteristics.<sup>9</sup> They were adapted to rapid growth<sup>10</sup> and upon activation exhibit marked augmentation of ADCC against erythroid and tumor target cells.<sup>11</sup> They are, under usual conditions, nonadherent and do not express Ia antigens. Cells are routinely maintained in RPMI 1640 supplemented with 5% fetal calf serum (FCS). Cultures are fed thrice weekly.

#### *Preparation of Conditioned Media (U-937)*

Conditioned media used in these studies were obtained as follows: either by collecting direct supernatants from the U-937 cells grown in 5% FCS, or U-937 cells after washing in serum-free media were incubated at a concentration of  $1 \times 10^6$  cells per plate in McCoy's 5A media with or without FCS and 0.5% agar. The petri dishes were overlaid with McCoy's, with or without FCS. Conditioned medium was collected at 3 days for serum-free, and 7 days for serum-containing medium. The supernatants were kept at 4°C until used.

#### *Preparation of Mononuclear Cells From Bone Marrow (BM)*

BM aspirates were obtained from paid, normal, human volunteers after informed consent was given. Approval was obtained from the Subcommittee on Human Studies at the Minneapolis VA Medical Center. They were prepared as described before.<sup>12</sup> We deplete BM of monocytes by adherence to plastic at 37°C for 60 min in the presence of 20% fetal calf serum (FCS).

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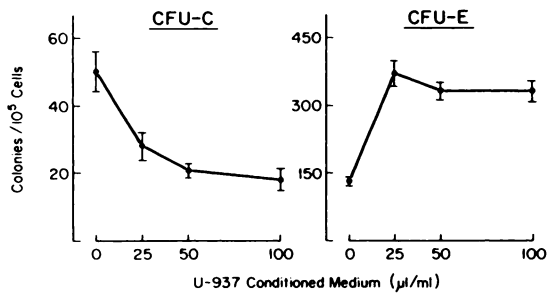


Fig. 1. Effect of U-937 CM on human BM granulocyte-macrophage (CFU-C) and erythroid (CFU-E) colony formation in vitro. Each point represents mean  $\pm$  1 SD of quadruplicate determinations.

### Hematopoietic Clonal Assays

**CFU-GM.** This assay was performed as described before.<sup>13</sup> For CSF, either placental conditioned media (PCM) (50–100  $\mu$ l/ml) or feeder layers ( $10^6$  normal human leucocytes) were used.  $1 \times 10^5$  bone marrow cells were cultured in 0.3% agar and scored at 7 days.

**BFU-E and CFU-E Assays.**<sup>11,12,14</sup> Erythroid progenitors were assayed in plasma cultures as described by Tepperman et al.<sup>14</sup> Human bone marrow cells ( $2-6 \times 10^5$  cells/1.2 ml) were cultured in the presence or absence of 1.5 IU Ep. The Ep (25 IU/mg) used in these studies was prepared from human urine and did not contain significant EPF or CSF activities. The cultures were examined at days 7 and 15 for CFU-E and BFU-E derived colony formation, respectively.

### RESULTS

The U-937 cell line elaborates factor(s) that inhibit human CFU-GM in vitro (Fig. 1). The degree of suppression was dependent upon the concentration of CM used, but plateaued at a concentration of 100  $\mu$ l of CM/ml. This inhibition could be demonstrated using either feeder layers or PCM as a source of CSF. Also, depletion of monocytes from bone marrow cells did not alter the inhibitory profile. Repeated attempts failed

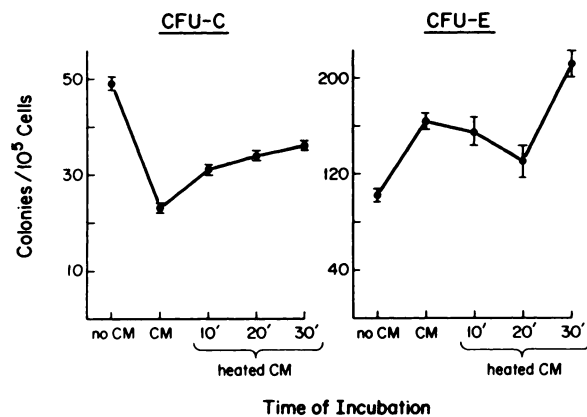


Fig. 2. Effect of boiled U-937 CM on human bone marrow CFU-C and CFU-E colony formation in vitro. Each point represents mean  $\pm$  1 SEM of quadruplicate determinations.

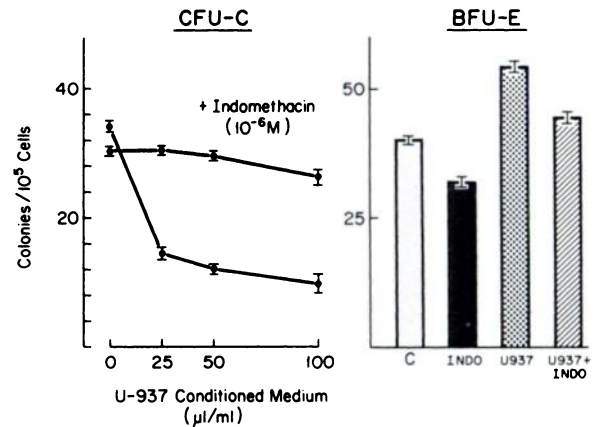


Fig. 3. Effect of Indomethacin on the hematopoietic activity of U-937 CM. Indomethacin ( $10^{-6}$  M) was added at the start of each culture. Each point represents mean  $\pm$  1 SEM of quadruplicate determinations.

to demonstrate CSF activity in U-937 CM. Similarly, the use of U-937 cells as feeder layers ( $2.5 \times 10^5-2 \times 10^6$ /plate) did not result in stimulation of CFU-GM growth, in vitro. Moreover, in agreement with prior reports,<sup>9</sup> we were not able to demonstrate formation of colonies/clusters by U-937 cells in the presence or absence of CSF. The inhibitory activity was also present in serum-free CM, and was at least partially inactivated by boiling for 30 min. (Fig. 2). Figure 3 shows that addition of Indomethacin ( $10^{-6}$  M/ml) prevented the inhibition of CFU-GM by U-937 CM. Figure 1 also shows that the addition of U-937 CM at low concentrations (25  $\mu$ l/ml) to normal human bone marrow in presence of Ep significantly enhanced CFU-E growth in vitro; potentiation at higher doses was frequently not seen presumably due to interacting inhibitors. The U-937 CM also significantly enhanced

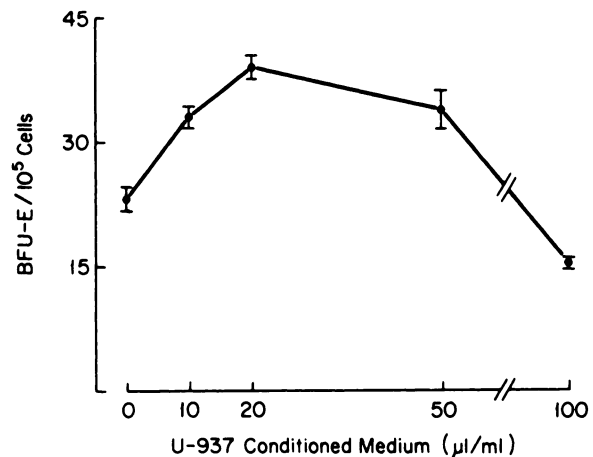


Fig. 4. Effect of U-937 CM on human BM erythroid (BFU-E) colony growth in vitro. Each point represents the mean  $\pm$  1 SEM of quadruplicate determinations.

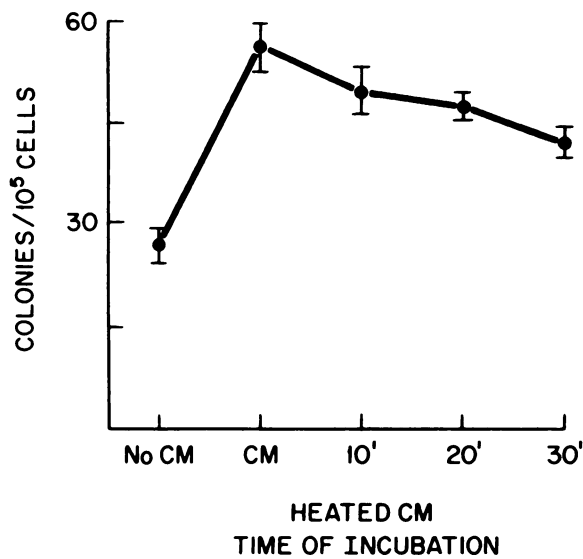


Fig. 5. Effect of boiled U-937 CM on human bone marrow BFU-E growth in vitro. Each point represents the mean  $\pm$  1 SEM of quadruplicate determinations.

BFU-E growth in vitro (Fig. 4). At higher concentrations, however, a reduction in BFU-E derived colony formation was observed. Figures 2 and 5 show that this EPF was heat stable and not affected by boiling the U-937 CM up to 30 min. The erythroid potentiating activity was also demonstrated in serum-free preparations of U-937 CM (Fig. 6); and was not affected by addition of Indomethacin at  $10^{-6}$  M per culture (Fig. 3). Similar results were obtained with peripheral blood BFU-E (data not shown). That this erythroid potentiating activity could not be attributed to the presence of Ep, was demonstrated by the fact that (1) injections of U-937 CM into ex-hypoxic polycythemic mice<sup>15</sup> failed to stimulate erythropoiesis, and (2) no erythroid colonies were observed in any of the cultures in the absence of exogenous Ep (Table 1).

#### DISCUSSION

The U-937 is a well characterized human tumor cell line<sup>9,10</sup> of monocyte origin, that can be shown to differentiate into macrophages using appropriate stimuli.<sup>11</sup> We have demonstrated that media conditioned by U-937 contain a heat stable factor(s) capable of potentiating the growth of human BFU-E and CFU-E in vitro. Production of EPF by a human T cell line has recently been reported.<sup>8</sup> However, unlike the T cell line, the U-937 does not produce CSF,<sup>7</sup> a fact that may prove useful in distinguishing between the effects of CSF and EPF in the regulation of erythroid progenitor activity. In this regard, the addition of U-937 medium to cultures of human bone marrow significantly suppressed CFU-GM development. This inhibitory

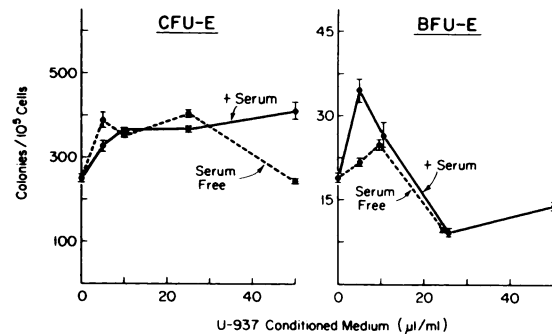


Fig. 6. Effect of U-937 CM on human BM CFU-E and BFU-E colony formation in vitro. Media containing 15% FCS or prepared in a serum-free system was added to cultures containing serum in the plasma clot assay. Each point represents the mean  $\pm$  1 SEM of quadruplicate determinations.

effect on CFU-GM appears to be mediated by prostaglandins of the E series, since addition of Indomethacin prevented the U-937 mediated inhibition of CFU-GM in vitro. By contrast, Indomethacin did not change the stimulatory effect of U-937 medium on erythroid progenitors suggesting that it is not mediated through PGE production. Recent observations by Korn et al.,<sup>16</sup> suggest that human monocytes secrete factors that stimulate endogenous production of prostaglandins by fibroblasts; a similar mechanistic effect might be operative in the hemopoietic clonal assays. The EPF produced by the U-937 cells is present in serum-free medium and is heat stable, similar to what has been reported using medium conditioned by the T-cell line.<sup>8</sup>

These studies suggest that different mechanisms operate at the levels of CFU-GM and the erythroid progenitor series, lending additional support to the long-held view that separate regulatory factors control leukopoiesis and erythropoiesis.<sup>6</sup>

Table 1. Lack of Effect of U-937 CM on Erythropoiesis in Ex-Hypoxic Polycythemic Mice\*

Material Assayed	% RBC- <sup>59</sup> Fe Uptake†
Saline	0.3 $\pm$ 0.04
0.05 IU Ep‡	1.9 $\pm$ 0.21
0.20 IU Ep	5.8 $\pm$ 0.42
McCoy's + 5-15% FCS§	0.5 $\pm$ 0.05
U-937 CM (1 ml/mouse)	0.4 $\pm$ 0.10
U-937 CM (2.5 ml/mouse)	0.4 $\pm$ 0.06
U-937 CM (4 ml/mouse)	0.6 $\pm$ 0.15

\*Mice were rendered polycythemic by exposure to 0.4 atmosphere of air (19 hr/day for 219 hr). Samples were injected on days 5 and 6 posthypoxia, radiolabel was given on day 7, and % RBC-<sup>59</sup>Fe uptake determined on day 10. Five mice/group were employed.

†Each value represents mean  $\pm$  1 SEM of results from two separate assays.

‡International units of erythropoietin (step III, sheep plasma Ep).

§Fetal calf serum.

The U-937 cell line provides a continuing source of potent cell mediated factor(s) that can be employed

for further biochemical characterization and dissection of their biologic role.

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