Human Erythroid Burst-forming Units

GROWTH IN VITRO IS DEPENDENT ON MONOCYTES, BUT NOT T LYMPHOCYTES

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ABSTRACT The roles of monocytes and T lymphocytes in the regulation of human peripheral blood erythroid burst-forming units (BFU-E) were studied in erythropoietin-containing plasma clot cultures of subpopulations of human blood mononuclear cells. BFU-E growth was decreased significantly after depletion of monocytes alone (mean 11% of expected, range 0 to 42% of expected) or depletion of both monocytes and T cells (mean 6.5% of expected, range 0.5 to 12% of expected) from mononuclear cells. T cell depletion did not impair BFU-E growth in vitro. Using 10^5 monocyte- and T lymphocyte-depleted mononuclear cells as target cells (<1% monocytes, <5% T cells), BFU-E growth was restored to 40% of expected by addition of 10^4 monocytes, and to 96% of expected by 10^5 monocytes alone. Addition of as many as 2×10^5 T cells but no monocytes resulted in stimulation to only 34% of expected BFU-E growth. Addition of 2×10^4 T cells, which alone did not affect BFU-E growth, could augment significantly the stimulatory effect of $5–20 \times 10^3$ monocytes on BFU-E growth. Thus, monocytes alone appear to be capable of stimulating BFU-E growth in vitro in the presence of erythropoietin. T cells also may make small quantities of BFU-E stimulators. However, it seems more likely that the most important role of T lymphocytes in BFU-E regulation in vitro is a result of interactions with monocytes and augmentation of monocyte production of stimulators of BFU-E growth.

INTRODUCTION

Considerable evidence exists that the early erythroid progenitor cells, the burst-forming units (BFU-E)[1], are not dependent on erythropoietin for their maintenance or proliferation in vivo (1–3) or in vitro (4, 5). Several investigators have provided evidence for the existence of another regulatory protein for BFU-E in vitro, which has been termed burst-promoting activity (4, 6, 7), burst-feeder activity (8), erythroid-potentiating activity (9), or lymphocyte mitogenic factor (10). Regulators of murine BFU-E have been reported to be derived from unfractionated, mitogen-stimulated spleen cells (4), plastic-adherent radioresistant bone marrow mononuclear cells (8), or adherent mouse peritoneal cells (macrophages) (11). There has been a great deal of controversy regarding the cellular source of regulators of human BFU-E. Although some studies have suggested that T lymphocytes are required for BFU-E growth in vitro (10, 12), other investigators have found that BFU-E growth in culture is not dependent on the presence of T cells (7, 13–15). Monocytes and macrophages (adherent mononuclear cells) have been reported by several investigators to be critical for optimal growth of BFU-E in vitro (7, 16–18). Also, there have been reports that unstimulated peripheral blood mononuclear cells (7, 16), mitogen-stimulated mononuclear cells (5, 6, 16, 17), unstimulated peripheral blood monocytes (7), unstimulated T lymphocytes (7), antigen-stimulated T lymphocytes or mononuclear cells (10), unfractionated bone marrow cells (19), and a human T lymphoblast-derived cell line (9) may produce conditioned media containing BPA-like stimulator(s) of human BFU-E growth in vitro.

The purpose of the present study is to delineate more clearly the roles of monocytes and T lymphocytes in the regulation of human BFU-E proliferation and differentiation in vitro.

METHODS

Human subjects. All studies were approved by the institutional committee for the protection of human subjects at the University of Michigan. After informed consent, periph-

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[1] *Abbreviations used in this paper*: BFU-E, erythroid burst-forming unit; BPA, burst-promoting activity; CFU-GM, granulocyte-monocyte-macrophage colony-forming units; IMDM, Iscove's modified Dulbecco's medium.

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eral blood was obtained by venipuncture from normal human volunteers ranging in age from 21 to 54 yr. Blood was anticoagulated with preservative-free heparin, 10 U/ml of blood.

Isolation of cell populations. Blood was diluted 1:1 with Iscove's modified Dulbecco's medium (IMDM) (Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.). Mononuclear cells were isolated by density centrifugation over Ficoll-Hypaque (d = 1.077 g/cm^3; Pharmacia Fine Chemicals, Piscataway, N. J.) (20). The mononuclear cells that were recovered from the interface above the Ficoll-Hypaque contained 15–28% monocytes and 72–85% lymphoid cells, with 58–70% T lymphocytes. Monocytes were identified by Wright-Giemsa and nonspecific (alpha-naphthyl butyrate) esterase staining (21) and sometimes by phagocytosis of zymosan or latex particles (22). Disparity between Wright-Giemsa and esterase staining techniques for counting monocytes was ≤3%. Lymphoid cells were identified by absence of homogenous esterase staining and by morphology on Wright-Giemsa staining. T lymphocytes were identified using the sheep erythrocyte rosetting technique (23).

Adherent cells, which were >96% monocytes and <4% lymphoid cells, were obtained by a slight modification of the technique described by Shaw et al. (24). Mononuclear cells were incubated in IMDM with 10% autologous serum in polystyrene tissue culture dishes for 90 min at 37°C. Nonadherent cells were removed initially by gentle pipetting and then by repeated vigorous washing of the plates with cold IMDM. The adherent cells were then incubated for 3 min in cold Seligman's balanced salt solution (Gibco Laboratories) containing 0.1% bovine serum albumin and 0.2% sodium EDTA. The adherent cells were then removed from the dishes by gentle scraping with a rubber policeman. The cell suspension was subsequently maintained at 4°C to prevent cell clumping. The adherent cells were washed twice in IMDM with 10% AB serum. These monocyte-enriched cell populations are designated operationally in the remainder of this paper as "monocytes."

Nonadherent cells were obtained after two consecutive 90-min adherence procedures in polystyrene tissue culture dishes. Only the cells that could be obtained by gentle aspiration of the supernatant cell suspension into a pipet were used. Nonadherent cells were >96% lymphoid and <4% monocytes.

T lymphocytes were enriched from suspensions of nonadherent mononuclear cells by rosetting with neuraminidase-treated sheep erythrocytes, followed by Ficoll-Hypaque density centrifugation, recovery of rosetted cells from the pellet below the Ficoll-Hypaque, physical disruption of the rosettes by vortex mixing and vigorous pipetting, and osmotic lysis of the erythrocytes (23). The T cell-enriched suspensions always contained >95% erythrocyte rosette positive cells and <3% monocytes. These T lymphocyte-enriched cell populations are designated operationally as "T cells" in the remainder of this paper.

Mononuclear cell populations depleted of both monocytes and T lymphocytes were obtained by collection of the cells at the interface above the Ficoll-Hypaque after centrifugation of sheep erythrocyte-T lymphocyte rosettes through the Ficoll-Hypaque. These cell populations were contaminated with <1% monocytes and <5% T lymphocytes.

All cell populations used in experiments were >97% viable as determined by trypan blue dye exclusion.

Human peripheral blood BFU-E assay. The plasma clot culture technique of Tepperman et al. (25) was modified in several ways. IMDM was used in place of minimum essential medium with Hank's balanced salt solution and NCTC-109. Human AB serum (obtained from normal volunteers) was used in a 10% concentration in place of 30% fetal calf serum. Human urinary erythropoietin (generously provided by the National Heart, Lung, and Blood Institute) or step III sheep plasma erythropoietin (Connaught Laboratories, Willowdale, Ontario) was used in concentrations of 1–2 U/ml of culture. Cells were plated at low concentrations to minimize even further the slight contamination of cultures with cells which were to have been removed from the populations being evaluated, as described in the cell isolation procedures above. Thus, the cultures contained only 5–10 × 10^4 "target" cells being evaluated for their content of BFU-E. The "target" cells analyzed for BFU-E content were unfractionated mononuclear cells or mononuclear cells depleted of monocytes, T cells, or both monocytes and T cells. To evaluate the effects of monocytes and/or T lymphocytes on BFU-E growth, 10^3 to 2 × 10^5 "monocytes" and/or "T cells" were added to target cell populations of 5 × 10^4 or 10^5 mononuclear cells depleted of both monocytes and T cells. Control groups containing 5 × 10^4 or 10^5 "monocytes," "T cells," or unfractionated mononuclear cells were assayed for their content of BFU-E. Cultures of "monocytes" and "T cells" alone or in combination usually contained no BFU-E and never contained >4 BFU-E/10^5 "monocytes" or "T cells." In all experimental groups, three to six 0.5-ml plasma clot cultures were analyzed.

Calculations used to determine expected number of BFU-E per 10^5 cells. Since monocyte-enriched and T cell-enriched cell populations contained only rare BFU-E, removal of these cells from the mononuclear cells should result in an increased concentration of BFU-E per 10^5 cells in the populations remaining after monocyte and or T cell depletion. Therefore, the observed number of BFU-E per 10^5 target cells was compared with the expected number of BFU-E, which was based on the number of BFU-E per 10^5 unfractionated mononuclear cells and the percentage of monocytes and/or T cells in the mononuclear cell population. The following general formula was used to compute the expected number of BFU-E per 10^5 cells from which monocytes and/or T cells have been removed:

$$n = \text{BFU-E}/10^5 \text{ mononuclear cells} \times \frac{100}{100 - \text{percent monocytes and/or T cells}}.$$

Statistics. Whenever statistical analysis was warranted, the Student's t test or the nonparametric Wilcoxan rank order test was used (26).

RESULTS

BFU-E growth after depletion of both monocytes and T lymphocytes. In eight experiments, monocytes and T lymphocytes were removed from an aliquot of peripheral blood mononuclear cells, and BFU-E were assayed from unfractionated mononuclear cells and the monocyte- and T lymphocyte-depleted mononuclear cells, Results are shown in Table I. The expected number of BFU-E per 10^5 non-T lymphocyte, non-monocyte mononuclear cells was calculated as discussed above. BFU-E growth from the mononuclear cells that had been depleted of both monocytes and T lymphocytes consistently ranged from only 0.5 to 12% of expected ($P < 0.005$).

BFU-E growth from monocyte-depleted mononuclear cells. Monocytes were removed from peripheral

TABLE I

Effects of Monocyte and T Lymphocyte Depletion on Human Peripheral Blood BFU-E Growth In Vitro

Experiment	Peripheral blood MNC		M- and T-depleted MNC			
	M	T	MNC	Observed	Expected*	Observed/expected
	%		*BFU-E/10⁵ target cells cultured*			
1	24	63	28.0±4.2	23.5±4.9	215.4	0.11
2	18	70	3.4±1.1	3.4±4.2	28.3	0.12
3	20	67	24.0±1.4	21.8±2.5	184.6	0.12
4	21	64	22.5±1.6	9.5±1.5	150.0	0.06
5	20	66	12.4±2.2	2.2±1.1	88.6	0.02
6	19	66	98.6±5.8	3.0±0.6	657.3	<0.01
7	16	58	45.0±3.8	13.8±1.8	173.1	0.08
8	21	60	10.0±1.4	0.3±0.3	52.6	<0.01

All values are mean±SE (three to six culture plates per experiment). M, monocyte; T, T lymphocyte; MNC, mononuclear cells.

* Expected number of BFU-E per 10^5 T cell- and monocyte-depleted MNC takes into account the concentration effect of BFU-E after monocytes and T cells are depleted but the same total number of target cells is cultured.

$$\text{Expected BFU-E} = \text{BFU-E}/10^5\ \text{MNC} \times \frac{100}{100 - \%\ \text{T cells and monocytes in MNC}}.$$

TABLE II

Effects of Monocyte Depletion on Human Peripheral Blood BFU-E Growth In Vitro

Experiment	Monocytes in peripheral blood MNC	Monocyte-depleted MNC			
		MNC	Observed	Expected*	Observed/expected
	%	*BFU-E/10⁵ target cells cultured*			
1	19	31.3±5.1	5.0±1.6	38.6	0.13
2	22	39.5±0.7	0.7±0.8	50.6	0.01
3	21	14.8±1.9	5.3±1.2	18.7	0.28
4	30	40.8±3.8	8.8±2.3	58.2	0.15
5	25	5.8±0.7	0.7±0.7	7.7	0.09
6	26	11.7±1.1	0.3±0.4	15.8	0.02
7	20	28.0±1.9	14.8±1.7	35.0	0.42
8	25	33.8±1.1	4.2±1.1	45.1	0.09
9	16	18.8±1.8	1.5±0.8	22.4	0.07
10	15	24.2±1.7	3.7±1.0	28.5	0.13
11	15	31.7±1.5	0.5±0.6	37.3	0.01
12	20	18.7±2.3	0.8±0.6	23.1	0.04
13	24	17.3±1.5	0	22.8	0

Values are mean±SE (three to six culture plates per experiment). MNC, mononuclear cells.

* Expected number of BFU-E per 10^5 monocyte-depleted MNC takes into account the concentration effect of BFU-E after monocytes are depleted but the same total number of target cells is cultured.

$$\text{Expected BFU-E} = \text{BFU-E}/10^5\ \text{MNC} \times \frac{100}{100 - \%\ \text{monocytes in MNC}}.$$

blood mononuclear cells in 13 separate experiments. BFU-E growth from whole mononuclear cells and growth from monocyte-depleted mononuclear cells are compared in Table II. In all 13 experiments, monocyte removal resulted in >50% inhibition of BFU-E, and in 11 of 13 experiments there was >85% inhibition of BFU-E in monocyte-depleted cultures ($P < 0.001$).

BFU-E growth from T lymphocyte-depleted mononuclear cells. T lymphocytes were removed from peripheral blood mononuclear cells in five experiments. The comparisons between BFU-E growth from T cell-depleted mononuclear cells and from whole mononuclear cells are listed in Table III. The mean effect of T cell depletion was no change (101% of expected) in BFU-E growth. In no experiment was >40% inhibition of BFU-E growth noted from T cell-depleted mononuclear cells.

BFU-E growth after addition of monocytes or T lymphocytes to mononuclear cells depleted of both monocytes and T cells. BFU-E growth was impaired significantly and reproducibly only when monocytes or both monocytes and T cells were removed from mononuclear cells. Therefore, the next set of experiments was designed to determine whether the decreased BFU-E growth after depletion of both monocytes and T cells could be corrected by readdition of "monocytes" or "T cells" alone. In these experiments, unfractionated mononuclear cells were cultured to determine the expected number of BFU-E in the population of mononuclear cells that had been depleted of monocytes and T cells. In the experimental groups, $5–10 \times 10^4$ monocyte- and T cell-depleted mononuclear cells were cultured alone and in the presence of $1–200 \times 10^3$ "monocytes" or "T lymphocytes." In addition, to rule out a BFU-E-enhancing or -inhibiting effect of sheep erythrocytes from the T cell separation procedure, sheep erythrocyte lysates were added to control cultures and were found to have no effect on BFU-E growth. As was shown in Table I, the number of observed BFU-E per 10^5 mononuclear cells or monocyte- and T cell-depleted mononuclear cells varied greatly among experiments. Therefore, the results of "monocyte" or "T cell" addition to the non-monocyte, non-T cell mononuclear cells in three to six experiments are expressed in Fig. 1 as percentage of expected numbers of BFU-E. Addition of as few as 10^4 "monocytes" to 5×10^4 or 10^5 monocyte- and T cell-depleted mononuclear target cells resulted in significant stimu-

TABLE III

Effects of T Lymphocyte Depletion on Human Peripheral Blood BFU-E Growth In Vitro

Experiment	T cells in peripheral blood MNC	MNC	T cell-depleted MNC		
			Observed	Expected*	Observed/expected
	%	*BFU-E/10⁵ target cells cultured*			
1	62	21.0±3.2	111.3±0.8	55.3	2.01
2	63	28.0±4.2	64.0±4.2	75.7	0.85
3	65	4.7±0.8	10.3±2.3	13.4	0.77
4	60	4.0±1.1	6.0±1.4	10.0	0.60
5	60	18.7±2.3	39.4±12.6	46.8	0.84
6	57	15.3±2.1	36.0±1.9	35.6	1.01

Values are mean±SE (three to six culture plates per experiment). MNC, mononuclear cells.

* Expected number of BFU-E per 10^5 T cell-depleted MNC takes into account the concentration effect of BFU-E after T cells are depleted but the same total number of target cells is cultured.

$$\text{Expected BFU-E} = \text{BFU-E}/10^5\ \text{MNC} \times \frac{100}{100 - \%\ \text{T cells in MNC}}.$$

lation of BFU-E ($P < 0.05$). Addition of 5–100 × 10^3 "monocytes" resulted in a progressive sharp increase in BFU-E, and 2 × 10^5 added "monocytes" resulted in no increment in BFU-E stimulation over that induced by 10^5 added "monocytes." Maximum BFU-E stimulation by "monocytes" added to monocyte- and T cell-depleted mononuclear cells reached 96% of expected values. In contrast, 1–200 × 10^3 "T lymphocytes" alone stimulated a more modest increase in BFU-E, reaching a maximum of 34% of expected values when 2 × 10^5 "T cells" were added to a culture. Thus, 10^5 "monocytes" alone could correct completely the BFU-E growth deficiency induced by depletion of both monocytes and T lymphocytes, and as few as 10^4 "monocytes" resulted in a greater increase in BFU-E growth than did as many as 2 × 10^5 "T cells."

BFU-E growth after addition of both monocytes and T lymphocytes to mononuclear cells previously depleted of monocytes and T cells. Because low numbers of "monocytes" resulted in detectable stimulation of BFU-E, but high numbers of "T lymphocytes" were required to achieve similar degrees of BFU-E recovery, one possible explanation for the modest stimulation of BFU-E growth with higher T cell concentrations could be that 1–2% contamination of the "T cells" by monocytes provided a sufficient number of monocytes to produce BPA. Another possibility is that T lymphocytes could enhance the production of BPA by the small number of monocytes contaminating the "T cells" or that the 0.5–2% contaminating monocytes could enhance BPA

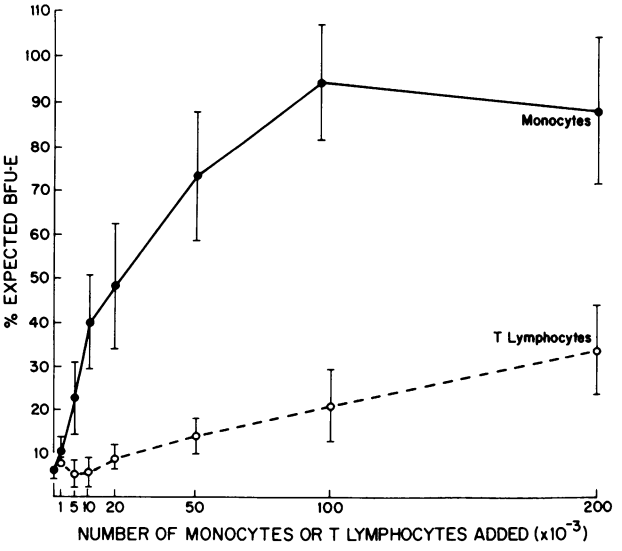

FIGURE 1 Effects of monocytes and T lymphocytes on BFU-E growth in vitro. Variable numbers of highly purified monocytes (●) or T lymphocytes (○) were added directly to 0.5-ml plasma clot cultures containing 10^5 monocyte- and T cell-depleted peripheral blood mononuclear cells. Results are expressed as percentage of expected number of BFU-E per 10^5 monocyte- and T lymphocyte-depleted mononuclear cells (±SEM for three to six experiments). This value was calculated as described in Methods and in Table I.

production by T lymphocytes. Therefore, experiments were designed in which varying numbers of both "monocytes" and "T cells" were added to cultures of mononuclear cells depleted of both monocytes and T cells. The results are shown in Fig. 2. With both concentrations of "T cells" used (2 × 10^4 and 1 × 10^4), the addition of increasing numbers of "monocytes" resulted in a sharp increase in BFU-E growth at low "monocyte" concentrations and a more modest increase with addition of higher concentrations of monocytes. At the lower "monocyte" concentrations examined (5–20 × 10^3), the addition of 2 × 10^4 or 10^5 "T cells" resulted in substantial augmentation of BFU-E growth, whereas the additional stimulatory effect of "T cells" was less obvious when 5 × 10^4 "monocytes" were present in the cultures. Addition of varying quantities of monocytes plus 10^4 "T cells" did not stimulate BFU-E more than did the monocytes alone (data not shown). It should be noted that 2 × 10^4 "T cells" augmented the BPA-like effect of 5–20 × 10^3 added "monocytes," despite the fact that this concentration of "T cells," added alone to monocyte- and T cell-depleted mononuclear cells, had no stimulatory effect on BFU-E growth (Fig. 1).

DISCUSSION

Monocytes and macrophages are known to be major sources of production of colony-stimulating activity,

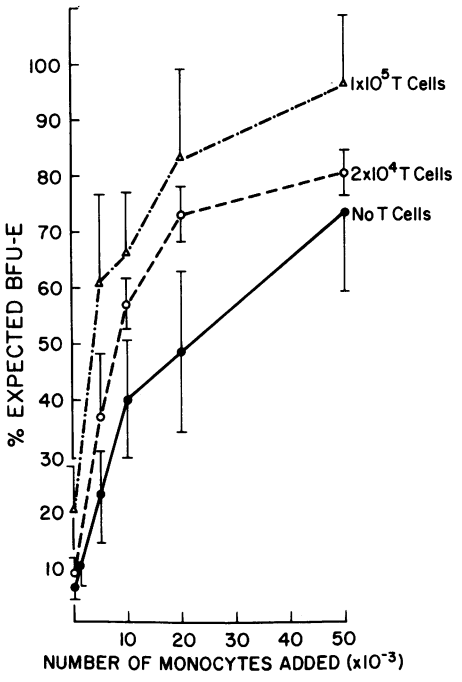

FIGURE 2 Effects of monocyte-T lymphocyte interactions on BFU-E growth in vitro. Variable numbers of monocytes alone (●) or along with 2×10^4 T lymphocytes (○) or 10^5 T lymphocytes (△) were added directly to 0.5-ml plasma clot cultures containing 10^5 monocyte- and T lymphocyte-depleted peripheral blood mononuclear cells. Results are expressed as percentage of expected number of BFU-E per 10^5 monocyte- and T lymphocyte-depleted mononuclear cells (±SEM for three to four experiments). This value was calculated as described in Methods and in Table I.

which is a requirement for proliferation and differentiation of human granulocyte-monocyte-macrophage progenitors (CFU-GM) in vitro (27–30). It has been reported also that T lymphocytes are capable of producing colony-stimulating activity (31–33). It would be of great interest to learn which, if either, of these cell types produce substances that play a role in the regulation of other primitive hematopoietic progenitor cells.

The cellular source(s) of regulators of the primitive erythroid progenitors, BFU-E, is less well defined than those for CFU-GM. Burst-promoting activity (BPA), which is a proposed regulator of the proliferation and initial stages of differentiation of BFU-E, has been detected in media conditioned by unstimulated, mitogen-stimulated, or antigen-stimulated unfractionated mononuclear cells (4–8, 10, 15–18), monocytes or macrophages (7, 8, 11, 34), or T lymphocytes (7, 9, 10, 12, 15).

The present study using peripheral blood BFU-E confirms earlier reports that both peripheral blood and bone marrow BFU-E are dependent on monocytes or macrophages for optimal proliferation and differentiation in vitro (5, 7, 16, 17, 35). In addition to the demonstration of marked impairment of BFU-E growth in the absence of monocytes (Tables I and II), the current series of experiments demonstrates that the impaired BFU-E growth noted in the absence of both monocytes and T lymphocytes can be corrected by the replacement of relatively low numbers of monocytes alone (Fig. 1). In two previous studies (12, 36), removal of monocytes from peripheral blood mononuclear cells was reported to enhance BFU-E growth in vitro. However, in both of those studies, BFU-E growth in control cultures of unfractionated mononuclear cells (1–2 BFU-E/10^5 cells) was very poor in comparison with BFU-E growth usually reported (24.5±4.1 in the present study and a 10–30 BFU-E/10^5 cells in other studies reported in the literature). The poor BFU-E growth in the control cultures makes it impossible to interpret accurately the results of their experiments. Furthermore, the present studies and those of Lipton et al. (35) showed no evidence of inhibition of BFU-E even by monocyte concentrations as high as 2×10^5/0.5-ml culture.

The fact that very low numbers of "monocytes" ($\leq 10^4$/culture) could stimulate BFU-E growth suggests strongly that monocytes are a major source of production of BPA. In addition, because the "monocyte" populations used in these studies never were contaminated with >4% lymphocytes, contaminating T lymphocytes present even in cultures containing 2×10^5 "monocytes" always were considerably fewer than even the minimum number of T lymphocytes capable of stimulating BFU-E (Fig. 1).

These experiments and previous reports of BPA in media conditioned by monocytes or macrophages (7, 8, 11, 35) suggest that monocytes are capable of producing all the BPA or other factors necessary for optimal proliferation and differentiation of human BFU-E in vitro. However, the possibility that T lymphocytes also are capable of producing small quantities of BPA has not been excluded. Some prior reports suggested that T lymphocytes were required for growth of primitive human BFU-E derived colonies in vitro (10, 12, 15), but others (7, 13, 14), as well as the present study, showed no dependence of BFU-E on T cells. The reasons for the discrepancies among these studies have not yet been determined.

Several studies have addressed the question of whether T cells could produce BPA. Golde et al. (9) have demonstrated production of BPA by a human T lymphoblast cell line. Nathan et al. (10) reported production of BPA (designated lymphocyte mitogenic factor) by tetanus toxoid-stimulated mononuclear cells. The BPA production in that experiment was attributed by these investigators to T cells. One particular problem with interpretation of the derivation of BPA in the studies of Nathan et al. (10) is that the medium that was

tested for BPA certainly was a mixture of numerous products of both monocytes and lymphocytes (37–39). Even if lymphocyte mitogenic factor can be demonstrated to be a product of a subset of T lymphocytes (40), the cellular source of the factor(s) that stimulates BFU-E remains uncertain. Mangan and Desforges (12) reported the presence of BPA in supernates from mixed leukocyte cultures containing 5×10^6 "T cells" from each of two normal individuals. They attributed the BPA production to the T cells. However, even with as little as 1% monocyte contamination, these cultures contained at least 10^5 monocytes. In addition to the present studies, which demonstrated that very low concentrations of monocytes in the cultures could stimulate BFU-E, previous experiments from this laboratory demonstrated substantial quantities of BPA in media conditioned by only 10^5 monocytes/ml (7). In fact, Mangan and Desforges also noted that BPA production was the same in mixed leukocyte cultures in which both "T cell" populations were irradiated (2,000 rad) as it was in cultures with unirradiated cells. This lends further credence to the explanation that the BPA that stimulated BFU-E in their cultures of null cells could have been derived from the radioresistant contaminating monocytes rather than the T cells. Prior studies from this laboratory demonstrated BPA in media conditioned by $0.5–1 \times 10^6$ unstimulated "T cells"/ml (7); however, the BPA in those cultures also could have been a product of the contaminating monocytes. Thus, although it seems clear that monocytes are a source of BPA, the question of whether or not normal T lymphocytes also are capable of producing BPA remains unanswered.

It is of great interest that concentrations of T lymphocytes, which produced no stimulation of BFU-E when added alone to cultures of monocyte- and T cell-depleted mononuclear cells, were capable of augmenting the stimulating effect of monocytes on BFU-E (Fig. 2). Although the role of cell-cell interaction in the production of regulators of BFU-E has not been demonstrated previously, results of an earlier study from this laboratory suggested that media conditioned by both lymphocytes and monocytes together contained larger quantities of BPA than did media conditioned by comparable numbers of monocytes or lymphocytes alone (7). It is well known that monocytes and macrophages play an important role in the mediation of mitogen- or antigen-stimulated proliferation and activation of lymphocytes (37, 38, 41, 42). Similarly, it has been demonstrated conclusively that products of lymphocytes can activate monocytes and macrophages, resulting in increased production and release of numerous biologically active substances (43, 44). It appears that BPA production by monocytes also can be enhanced by lymphocytes or products of lymphocytes. Although the reverse relationship—monocyte enhancement of BPA production by lymphocytes—probably does not occur, these experiments have not ruled out that possibility completely.

The experiments presented here do not address the question of physiologic significance of BPA in vivo. Unfortunately, there is no good human model for a monocyte and macrophage deficiency state. However, such T lymphocyte deficiency disorders as DiGeorge's syndrome and severe combined immune deficiency help illuminate the importance of T lymphocytes in the maintenance of normal erythropoiesis and responsiveness to erythropoietic stresses. The absence of anemia other than the attributable to chronic and recurrent infections in patients with T lymphocyte deficiency speaks strongly against a major role for T cells in the regulation of BFU-E replication or the initial stages of BFU-E differentiation. Lipton et al. (15) argue that even if T cells are important in the regulation of primitive BFU-E, one would not expect a disturbance of basal erythropoiesis in patients with T lymphocyte deficiency because differentiation of the more mature BFU-E to erythrocytes is responsive to erythropoietin and does not require T cells or T cell products. A major fallacy in their argument is that the more mature BFU-E have a limited proliferative capacity when compared with the more primitive BFU-E (45). Therefore, the lack of a substance necessary for replication and the initial differentiation steps of primitive BFU-E leading up to the more mature BFU-E would lead rather rapidly to a cessation or near cessation of even basal erythropoiesis. Lipton et al. also predicted that T cell deficiency would be expected to limit the ability to respond to erythropoietic stress. However, Schaller et al. (46) demonstrated that a patient with thymic alymphoplasia and autoimmune hemolytic anemia was able to increase erythropoiesis appropriately in response to severe anemia. If T cells actually are required for stimulation of BFU-E, the only way to account for these findings would be to propose that the small number of residual primitive precursors of T cells, which are unable to promote lymphoid differentiation (47, 48), can promote normal erythropoiesis. Although it seems unlikely that these primitive T cell precursors can sustain normal and stress erythropoiesis, this possibility cannot be ruled out entirely.

In conclusion, the studies reported here demonstrate that monocytes, but not T lymphocytes are required for optimal human peripheral blood BFU-E growth in vitro. Small numbers of monocytes, but not T cells, are capable of supporting optimal BFU-E growth in cultures of mononuclear cells depleted of both monocytes and T lymphocytes. It appears that T cells can augment the stimulatory effect of monocytes on human BFU-E proliferation and differentiation in vitro. The possibil-

ity that T cells may produce small quantities of BPA has not been excluded by these experiments, but it is unlikely that such BPA production adds substantially to the stimulatory effect of monocytes on BFU-E. Based upon the information derived from the present series of experiments and previous work done in this laboratory (7), it now seems that BPA can be added to the list of biologically active substances produced by normal human monocytes.

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