Erythroid Colony Formation by Polycythemia Vera Bone Marrow in Vitro

DEPENDENCE ON ERYTHROPOIETIN

ESMAIL D. ZANJANI, JOHN D. LUTTON, RONALD HOFFMAN, and LOUIS R. WASSERMAN

From the Department of Physiology, Mount Sinai School of Medicine of City University of New York, New York 10029

ABSTRACT In the plasma clot culture system, both normal and polycythemia vera (PV) bone marrow cells respond to erythropoietin (Ep), giving rise to large numbers of colonies of erythroid cells. In PV, but not in normal individuals, the marrow produced endogenous erythroid colonies (EEC) in the absence of exogenous Ep. The number of EEC formed varied from patient to patient comprising anywhere from 6 to 29% of the total number of colonies formed in the presence of Ep. Exposure, before use in culture, of fetal calf serum and citrated bovine plasma to the gamma-globulin fraction of rabbit anti-Ep serum followed by treatment with goat anti-rabbit gamma-globulin resulted in a significant decrease in EEC formation. Addition of anti-Ep directly to the culture medium produced similar results. In addition, the production of EEC in response to added Ep was inhibited in the presence of anti-Ep. Addition of very small doses of highly purified Ep to anti-Ep-treated cultures resulted in the reappearance of a significant number of EEC. These results suggest that EEC formation in PV may be due to a population of erythroid-committed precursors that are abnormally sensitive to small concentrations of Ep which may be present in fetal calf serum and citrated plasma. Although the mechanism of formation of these cells is not known, it appears that the final steps in the formation of red cells derived from this clone of precursors is subject to the usual Ep control.

INTRODUCTION

Erythrocyte production in normal animals and man is regulated by the hormone erythropoietin (Ep)[1] (1). Alterations in the rate of production of this hormone can lead to changes in the numbers of circulating erythrocytes (2). It is generally accepted that the increased production of erythrocytes in all cases of secondary polycythemias is associated with increased Ep formation (3). Such an association can not be demonstrated in polycythemia vera (PV). In this regard, the decreased levels of Ep in PV (4), and the failure to elicit a normal response to Ep by bone marrow cells of these patients in vitro (5, 6) have led to the suggestion that the increased production of erythrocytes in PV results from the proliferation and differentiation of hemopoietic precursor cells not under the usual Ep control (2). Studies in vivo (7–9) and in other in vitro systems (10–12) have provided evidence for the existence also of a population of Ep-responsive erythroid precursor cells in PV. The latter cell population, however, was suggested not to contribute significantly to the overall production of erythrocytes during the active phase of the disease (2). Recent studies, utilizing culture procedures conducive to the proliferation and differentiation of erythroid precursor cells in vitro (13), indicated that the latter cell population may represent a greater proportion of the total erythroid precursor cell pool in PV than previously assumed (10, 12). These studies also provided further evidence for the existence of an erythroid stem cell population in PV with altered proliferative characteristics (10–12). Thus, whereas in the plasma clot culture system (13) bone marrow cells from hematologically normal humans exhibit erythroid activity (erythroid colonies, EC) only in the presence of relatively high concentration of Ep, PV bone marrow formed erythroid colonies (endogenous erythroid colonies, EEC) in the absence of such exogenous Ep. Addition of Ep, however, resulted in the formation of significantly greater numbers of colonies by PV bone marrow cells (10, 12). In this report, the mechanism of EEC formation was investigated in patients with PV. The results indicate that like EC, the formation

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[1] *Abbreviations used in this paper*: CBP, citrated bovine plasma; EC, erythroid colony; EEC, endogenous erythroid colony; Ep, erythropoietin; FCS, fetal calf serum; GARGG, goat anti-rabbit gamma globulin; PV, polycythemia vera.

TABLE I

*Summary of Clinical Data on Patients with PV at the Time of the Study**

Patients	Age	Therapy	Duration of disease	Erythrocyte mass	Leukocytes per μl	Platelets per μl
	yr		*yr*	*ml/kg‡*		
1–6	51–57	none	<1	43.3 (36.9–61.9)	11,800 (9,200–19,300)	526,000 (335,000–686,000)
7–19	35–56	Phlebotomy	2–9	39.9 (36.7–43.4)	15,200 (11,500–18,600)	586,000 (490,000–797,000)
20	63	Phlebotomy + ^{32}P	9	38.9	14,500	980,000
21–23	52–61	Phlebotomy + Leukeran§	11–13	38.2 (36.2–41.7)	17,500 (15,300–19,400)	574,000 (480,000–693,000)
24, 25	53, 67	Leukeran + Alkeran§	5, 13	41.6, 38.6	18,600, 10,600	1,320,000, 610,000

* Arterial O_2 saturation for all patients was ≥92%. On physical examination the spleen was palpable in 19 of 25 patients.

‡ Except for patients 20, 24, and 25, the values given represent the mean and range, in parentheses, for each group of patients.

§ Burroughs Wellcome & Co. Inc., Tuckahoe, N. Y.

of EEC is also regulated by Ep. The major difference between the two cell types, however, is that the latter is significantly more sensitive to Ep.

METHODS

All bone marrow aspirations were performed at the posterior iliac crest. In all instances, informed consent was obtained from both patients and normal donors. The bone marrow cells obtained were cultured in the plasma clot culture system described by Tepperman et al. (13). In all studies, the indicated concentration of Ep and bone marrow cells refers to the amounts present in 1.1 ml of culture. Dispersed bone marrow cells in concentrations of 2, 4, 6, and 8×10^5 cells/1.1 ml were cultured in duplicates in the presence of 2 IU of Ep (human urinary Ep, 74 IU/mg) at 37°C in a humidified atmosphere of 5% CO_2 in air. At 7 days of incubation, the clots were removed and transferred to glass slides, fixed in gluteraldehyde, and then stained with benzidine and hematoxylin. Under ×100 magnification, each clot was examined and only colonies consisting of eight or more benzidine-positive cells were counted. No attempt was made to distinguish between smaller colonies (8–32 cells) and large colonies (>32 cells) in the calculation of the results. A total of 18 hematologically normal donors and 25 patients with PV were examined. The patients with PV (diagnosis based on criteria adopted by the National Polycythemia Vera Study Group) included 13 who were treated with phlebotomy alone, 6 who, in addition to phlebotomy, had received chemotherapy or radioactive phosphorous, and 6 patients with no prior treatment. The patients on chemotherapy or ^{32}P had not received such therapy for a minimum of 2 yr before these studies. At the time of these studies, all PV patients had active PV (see Table I). Bone marrow cells from five patients with a history of erythroid hyperplasia (three with erythrocytosis secondary to elevated Ep levels, and two with hemolytic anemia) were also studied. Serum and urinary Ep levels for all individuals were determined in exhypoxic polycythemic mice (14). Female CF-1 mice were rendered polycythemic by exposure to 0.4 atm 19 h/day for a total of 216 h. Each mouse received intraperitoneal injections of either 0.5 ml of serum or 1 ml of reconstituted urine concentrate/day on days 5 and 6 posthypoxia. Radioiron (0.5 μCi/mouse) was given intravenously on day 6 and the percent erythrocyte-^{59}Fe uptake was determined on day 9. 6–10 mice were used to test each sample. Urine concentrates were prepared from 24-h urine specimens (two from each patient) by dialysis against Carbowax (Union Carbide Corporation, New York) after the procedure outlined by Adamson et al. (15). None of the PV sera exhibited Ep activity. The daily urinary excretion of Ep was below normal in all PV patients (average 0.7±0.07 vs. 2.8±0.2 IU/day).

Bone marrow cells from five normal and nine patients with PV were cultured in the presence or absence of 2 IU Ep (74 IU/mg protein) and/or anti-Ep for 7 days. Anti-Ep was prepared in rabbits against a preparation of Ep (5 IU/mg protein) obtained from urine of a patient with aplastic anemia after the procedure described by Schooley et al. (16). Another preparation of human urinary Ep with a greater potency (630 IU/mg protein) was used to boost the antibody titers in these animals. 1 ml of the immune serum after absorption against normal human urinary protein was found to neutralize approximately 90 IU of human urinary Ep. Absorption against human urinary protein was achieved by incubating 20 ml of the antiserum with an amount of urinary protein separated by dialysis and ultrafiltration from 3 liters of normal urine for 1 h at 37°C followed by an additional incubation for 5 h at 4°C. The mixture was centrifuged at 20,000 *g* for 15 min and the supernate was used in subsequent studies. In these studies, the IgG fraction of the immune serum separated by DEAE-Sephadex (Pharmacia Fine Chemicals Inc., Piscataway, N. J.) chromatography (17) was used. Similarly prepared gamma-globulin fraction from normal rabbit serum was used as control. 1 mg of the IgG from the immune serum was found to neutralize approximately 6 IU of human urinary Ep. In studies shown in Fig. 1 and Table V, 1 mg of either the normal or immune IgG was added to each cell concentration at the start of the culture.

Bone marrow cells (4×10^5 cells) from seven patients with PV were cultured in the presence or absence of 2 IU Ep as follows. Each marrow was cultured in media containing either untreated fetal calf serum (FCS) and citrated bovine plasma (CBP), FCS and CBP which were pretreated with IgG from normal rabbit serum, or FCS and CBP which were treated as follows. To 50 ml of each was added either 3 mg of normal rabbit serum IgG or IgG from immunized rabbits, incubated at 37°C for 2 h with constant shaking and allowed to stand at 4°C for 22 h. To each mixture was then added 6 mg of IgG separated from a commercially prepared goat anti-rabbit gamma-globulin serum (GARGG) (Antibodies Inc., Davis, Calif.), mixed thoroughly, and then allowed to stand for 2 h at 4°C. The mixtures were spun at 10,000 *g* for 20 min, the precipitate discarded, and the supernate was then passed through a Millipore filter (Millipore Corp., Bedford, Mass.) and used in cultures. The untreated FCS and CBP were also subjected to a similar procedure but without the addition of the IgG to insure uniform conditions.

In separate studies, bone marrow cells from three patients with PV were studied as follows. Each marrow (6×10^5 cells) was cultured for 7 days in the presence or absence of either 0.001, 0.01, 0.04, 0.08, and 0.1 IU of a highly purified preparation of Ep (8–10,000 IU/mg protein), or 0.30, 0.50, 1.0, 2.0, 3.0, 5.0, and 10.0 IU of Ep (74 IU/mg protein) in media containing FCS and CBP which were pretreated with normal IgG and GARGG or immune IgG and GARGG.

The effect of increasing concentrations of Ep on EC formation by bone marrow cells was examined in seven normal and seven patients with PV. Bone marrow cells (6×10^5 cells) were cultured in the presence or absence of 0.5, 1.0, 2.0, 3.0, 5.0, and 10.0 IU Ep (74 IU/mg) for 7 days and the numbers of EC were determined for each bone marrow separately. The results shown in Table IV depict the composite data from all donors in each category.

TABLE II

*Ep-Neutralizing Activity of Anti-Ep before and after Treatment with GARGG**

Material assayed	Erythrocyte-^{59}Fe incorporation ± SEM‡
	%
Saline	0.56±0.15
0.5 IU Ep§	16.32±1.73
+ 0.006 ml normal rabbit serum	15.87±2.14
+ 0.006 ml anti-Ep serum	0.19±0.06
+ 0.1 mg normal rabbit IgG	18.30±1.47
+ 0.1 mg anti-Ep serum IgG	0.98±0.26
+ (0.1 mg anti-Ep serum IgG + 0.2 mg GARGG IgG)	17.01±1.96

* See text for details.

‡ Mean of four separate assays ± 1 SEM.

§ Ep used in these studies was collected and concentrated by the Department of Physiology, University of Northeast, Corrientes, Argentina, and further processed and assayed by the Hematology Research Laboratories, Children's Hospital of Los Angeles, Los Angeles, Calif., under grant HE-10880 from the National Heart and Lung Institute of the National Institutes of Health.

TABLE III

EC Formation by Normal Human Bone Marrow Cells in Plasma Clot Cultures in Vitro

Total number of nucleated marrow cells cultured	Total number of EC*	
	No Ep	+Ep
2×10^5	0	182±29
4×10^5	0	263±36
6×10^5	0	527±51
8×10^5	0	792±63

* Each value represents the mean ± 1 SEM of 18 separate studies each involving a different donor.

RESULTS

The potency of the anti-Ep and GARGG preparations used in this study was determined in exhypoxic polycythemic mice and is shown in Table II. Formation of EC by bone marrow cells cultured for 7 days from hematologically normal individuals is demonstrated in Table III. No colonies were formed in the absence of exogenous Ep. This was also true when normal marrow was cultured for as long as 14 days. Addition of Ep to these cultures, however, resulted in the appearance of significant numbers of EC at all cell concentrations examined (Table III). A linear relationship between the numbers of colonies formed and bone marrow cells cultured was seen. Addition of anti-Ep to cultures of bone marrow cells from five normal subjects resulted in the total inhibition of EC formation in response to Ep (Fig. 1). Thus, whereas normal rabbit serum IgG did not exert a significant

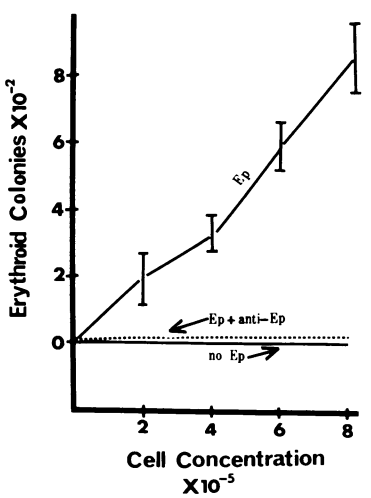

FIGURE 1 Effect of anti-Ep on the formation of EC by normal human bone marrow cells in response to Ep in vitro. Each point represents the mean ± SEM of five separate studies, each involving a different donor. Normal rabbit serum IgG was used as control.

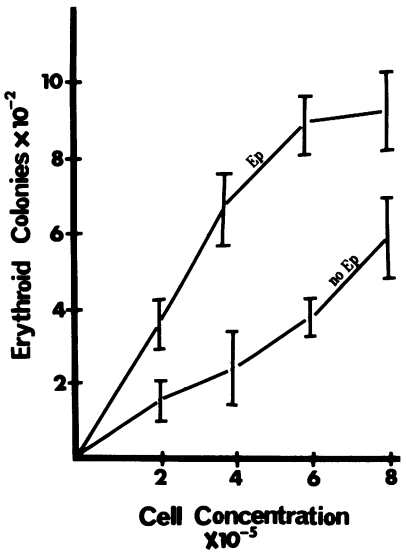

FIGURE 2 EC formation by bone marrow cells from patients with PV. Each point represents the mean±1 SEM of 22 separate studies, each involving a different donor.

effect on EC formation, IgG from immune rabbit serum suppressed EC formation (Fig. 1).

Formation of EC by marrow of patients with PV is depicted in Fig. 2. Unlike normal human bone marrow, cells from all 22 patients with PV formed colonies in the absence of exogenous Ep in the plasma clot cultures. The number of EEC formed was correlated with the numbers of bone marrow cells cultured in a linear fashion (Fig. 2). Addition of 2 IU Ep (75 IU/mg protein) to these cells resulted in the formation of significantly greater numbers of EC than when Ep was absent (Fig. 2). It should be noted that the values given in Fig. 2 represent the mean of all studies, and that considerable variation is seen in the numbers of EEC formed from one patient to another. For example, at a concentration of 6×10^5 bone marrow cells, the number of EECs formed ranged from 75 to 415. On the other hand, significantly less variation was observed when bone marrow from the same patient was examined on separate occasions. Thus, the numbers of EEC formed in three PV patients, each studied on two separate occasions (with no intervening therapy) were 115 vs. 147, 209 vs. 180, and 97 vs. 112 colonies/6 $\times 10^5$ bone marrow cells. The effect of therapy on EEC formation in PV patients remains to be determined.

A comparison between the response of normal and PV bone marrow cells to different concentrations of exogenous Ep is shown in Table IV. Once again, no colonies were formed by normal marrow in the absence of exogenous Ep, whereas significant numbers of colonies were formed by PV bone marrow cells. These composite results also demonstrate that a significant increase in EC formation by both marrows did not occur until an Ep concentration of 1.0 IU Ep/1.1 ml of culture was reached. For both marrows, the optimal dose of Ep was 2.0 IU/1.1 ml of culture. No further increase in colony formation was seen with doses of Ep greater than 2.0 IU. Table IV also shows that for the same Ep and cell concentrations, PV bone marrow produced a significantly greater number of EC. Table V shows that the formation of EC and EEC was significantly decreased when anti-Ep was added to these cultures. No effect was seen in the presence of normal rabbit serum IgG (Table V).

Treatment of the FCS and CBP with anti-Ep before use in culture was found to significantly inhibit EEC formation by bone marrow of all seven patients with PV (Table VI). Substitution with anti-Ep-treated FCS and CBP, however, did not influence EC formation by these marrow cells in the presence of exogenous Ep (Table VI). The effect of the addition of small amounts of highly purified Ep (8–10,000 IU/mg protein) on EC formation by bone marrow cells cultured in media containing anti-Ep-treated FCS and CBP was examined in three patients with PV. Table VII demonstrates once again that the formation of EEC was inhibited by treatment of FCS and CBP with anti-Ep. However, the addition of as little as 0.001 IU of the highly purified Ep caused the reappearance of these colonies with near maximum recovery occurring with 0.04 IU Ep (88±13 vs. 73±11). No further increase in colony formation occurred with doses of 0.08 and 0.10 IU of this Ep preparation. Using a less purified preparation of Ep (74 IU/mg protein), this plateau continued with Ep concentrations of 0.3 and 0.5 IU. However, a second significant increase in colony formation became evident with an Ep

TABLE IV
Comparison between the Response of Normal Human and PV Bone Marrow Cells to Different Doses of Ep in Vitro

Ep concentration	Number of EC/6 × 10⁵ Cells*	
	Normal	PV‡
IU§		
0	0	122±9
0.5	2±0.6	134±6
1.0	98±8	206±11
2.0	436±23	713±24
3.0	449±36	747±30
5.0	413±19	710±12
10.0	387±29	692±44

* Each value represents the mean±1 SEM from seven separate studies each involving a separate donor.
‡ Patients 2, 4, 7–9, 22, and 24 were studied (see Table I).
§ The amount represents Ep concentration/1.1 ml of culture.

TABLE V

*Effect of Immunoglobulins from Either Normal Rabbit Serum (Normal IgG) or Serum from Rabbits Immunized against EP (Anti-Ep IgG) on EC Formation by Bone Marrow Cells from Patients with PV**

	Number of EC/4 × 10^5 cells‡					
Patients§	No Ep	Normal IgG	Anti-Ep IgG	+Ep	Ep + Normal IgG	Ep + Anti-Ep IgG
1	154±42	168±36	0	598±89	—‖	8±6
2	109±31	92±9	10±4	1,132±287	987±169	0
3	183±22	173±43	11±5	632±71	959±106	0
20	139±25	158±39	7±3	446±83	532±43	0
11	132±14	147±29	0	597±62	503±79	12±7
6	140±56	146±20	12±2	875±106	847±88	0
7	44±8	—	0	741±66	603±38	0
5	77±11	63±14	11±4	395±53	474±16	0
9	32±6	42±18	0	449±88	—	14±2

* See text for details.

‡ Each value represents the mean±1 SEM of eight separate clots.

§ See Table I.

‖ Not determined.

concentration of 1.0 IU, reaching a maximum at about 2 IU Ep (Table VII). This was followed by another plateau, which remained unaltered with doses of Ep up to 10.0 IU/1.1 ml of culture medium (Table VII). Bone marrow cells from five patients with a history of extended erythroid hyperplasia produced EC only in the presence of exogenous Ep (0 vs. 649±32 colonies/6 × 10^5 cells). No endogenous colonies were formed under these conditions.

DISCUSSION

The results presented here demonstrate the Ep-dependent nature of EC formation by normal human bone marrow cells in the plasma clot culture system. Thus, no ECs were formed when bone marrow cells of normal individuals were cultured in the absence of exogenous Ep. Moreover, addition of anti-Ep to cultures of normal marrow containing Ep resulted in the total inhibition of EC formation. These in vitro findings are consistent with the observations that neutralization of circulating Ep in normal animals by in vivo administration of anti-Ep resulted in suppression of erythropoiesis (18), and, as such, provide evidence for the suitability of the plasma clot culture system for the study of the regulation of erythropoiesis.

Unlike normal human bone marrow, cells from bone marrow of patients with PV gave rise to significant

TABLE VI

*Effect of Pretreatment of FCS and CBP with Anti-Ep on the Formation of EC by Bone Marrow Cells from Patients with PV in Vitro**

	Number of EC/4 × 10^5 cells‡					
	No Ep			+Ep		
Patients§	Untreated	Normal IgG-treated	Anti-Ep IgG-treated	Untreated	Normal IgG-treated	Anti-Ep IgG-treated
21	175±20	163±23	23±11	472±56	512±37	372±73
22	108±26	119±42	28±9	649±75	493±49	576±67
14	174±31	190±51	9±3	397±45	432±87	412±57
4	186±39	147±48	23±12	843±69	789±39	888±90
8	97±12	122±30	4±2	483±59	563±85	415±62
12	108±13	96±27	12±6	593±70	502±76	631±54
15	86±26	84±10	0	731±86	809±84	780±66

* See text for explanation.

‡ Each value represents the mean±1 SEM of eight separate clots.

§ See Table I.

TABLE VII

*Effect of Small Doses of Highly Purified (Lot H4H-35) and Larger Doses of Crude (Lot H-12) Preparations of Ep on EC Formation by Bone Marrow Cells from Normal and Patients with PV Cultured in Media Containing Either Normal IgG- or Anti-Ep-Treated FCS and CBP**

Ep preparations and dose	Number of EC/6 × 10^5 cells‡		
	Type of FCS and CBP used		
	Normal IgG-treated		Anti-Ep treated
	Normal	PV	PV
IU§			
H4H-35			
0	0	88±14	0
0.001	0	—‖	28±4
0.01	0	—	53±8
0.04	0	—	73±11
0.08	0	—	69±9
0.10	—	—	62±12
H-12			
0.30	—	99±8	71±7
0.50	—	85±23	74±10
1.0	—	179±18	157±21
2.0	291±36	359±38	374±32
3.0	—	—	429±38
5.0	—	412±19	398±30
10.0	—	—	408±22

* Bone marrow cells from patients 13, 23, and 24 (see Table I) were used.

‡ Each value represents the mean±1 SEM of three separate studies each involving a different bone marrow donor.

§ Ep used in these studies were lot H4H-35 with a potency of 8–10,000 IU/mg protein and lot H-12 with a potency of 74 IU/mg protein. These preparations were originally prepared and distributed by the National Heart, Lung and Blood Institute (see legend to Table II). We are grateful to Dr. M. J. Murphy, Memorial Sloan-Kettering Cancer Center, New York, for supplying us with the highly pure Ep.

‖ Not determined.

numbers of EEC in the absence of exogenous Ep. These colonies first appeared at day 5 of culture and reached their maximum number and size by day 7. The formation of EEC is most probably due to the presence of a population of erythroid precursor cells with altered proliferative characteristics in PV. That this altered proliferative activity cannot be induced by the extended state of erythroid hyperplasia was demonstrated by the fact that bone marrow cells from patients with a history of extended erythroid hyperplasia did not form EEC. The existence of an abnormal erythroid stem cell population contributing to the overall increase in circulating red cell mass in PV was suggested by the findings that: (a) unlike secondary polycythemias, where increased production of Ep has been shown to accompany and be responsible for the elevated red cell mass (2, 3), increased levels of this hormone cannot be demonstrated in plasma and urine of patients with PV (4, 9). In most cases (as was also true of the patients studied here) urinary excretion of Ep is below normal (4), which is attributable to feedback inhibition by the increased red cell mass on Ep formation (20); and (b) bone marrow cells obtained from donors with PV during relapse, when cultured in suspension, exhibited a significantly reduced response to Ep (5). More recently, Adamson et al. (19) have provided direct eidence for the clonal origin of hemopoietic cell lines in PV. The data presented here, however, does not support the view that the erythroid activity of the abnormal clone is independent of Ep. The formation of EEC by PV marrow was inhibited by the addition of anti-Ep to the cultures. The inhibitory influence exerted by this IgG on EEC formation can be attributed to the neutralization of Ep present in FCS and CBP, and not to a nonspecific effect of the rabbit IgG. Support for this contention is derived from the findings that (*a*) additions of IgG from nonimmunized rabbits to cultures of PV bone marrow failed to affect colony formation, (*b*) treatment of the immune IgG with GARGG before use in culture resulted in the inhibition of anti-Ep effect, and (*c*) small doses of highly purified Ep caused the reappearance of EEC in anti-Ep-treated cultures.

These results imply that sufficient amounts of Ep must have been present in FCS and CBP to intiate the proliferation and differentiation of these cells, despite the fact that repeated bioassays of the FCS and CBP used in these studies failed to demonstrate Ep activity. It is generally accepted that the polycythemic mouse assay system is not sensitive to doses of Ep below 0.05 IU/ml of test material (14). There is little doubt that sera from normal adult animals and man (21) and normal mammalian fetus (22) contains Ep. The concentration of Ep in normal serum has been reported to range from 0.0003 to 0.003 IU/ml. The amount may reach approximately 0.03–0.05 IU/ml in fetal serum at the time of delivery (22). Since the combined volume of FCS and CBP used to culture a given concentration of marrow cells in these studies was about 0.4 ml, the total amount of Ep present in these cultures could not have exceeded 0.025 IU. This concentration of Ep is not an effective dose for EC formation by normal bone marrow cells; the optimal number of colonies being produced with 2–3 IU of Ep (Table IV). Therefore, these precursor elements in PV bone marrow appear to be far more sensitive to Ep than normal erythroid precursors. Additional support for this contention is derived from the finding that

after the inhibition of EEC formation by neutralization of Ep present in FCS and CBP, addition of very small amounts of highly pure Ep to cultures of bone marrow cells from patients with PV resulted in the reappearance of these colonies. Thus, a significant number of ECs were formed with as little as 0.001 IU Ep. A linear increase in colony formation was noted with increasing doses of this Ep up to 0.04 IU. No further increase occurred with concentrations of pure Ep greater than 0.04 IU. Similar concentrations of the highly purified Ep were without effect in cultures of normal human bone marrow cells (Table VII). The limited availability of pure Ep prevented us from examining the effect of progressively larger doses of this Ep on EC formation in this system. However, using a more crude preparation of Ep, we found that this initial plateau persisted through 0.5 IU Ep. A further increase in EC formation occurred with 1.0 IU Ep with maximum numbers of EC formed at 2 IU Ep/6 × 10^5 bone marrow cells. Larger doses of Ep (3, 5, and 10 IU) failed to induce a significant increase in EC formation beyond that produced by 2 IU.

These results also demonstrate the presence of a population of cells in PV with a normal Ep response. Addition of Ep to cultures of PV bone marrow cells resulted in a significant increase in the numbers of EC formed. Several possibilities exist as to the nature of this increase in EC formation. One possibility is that the major portion of the erythroid precursor pool is composed of the highly sensitive type, but the amount of Ep present in FCS and CBP is not sufficient to trigger all these cells to undergo proliferation and differentiation. Therefore, the exogenous Ep provides the additional amounts needed. If this were indeed the case, it would be possible to achieve near maximal EC formation with relatively low doses of Ep. However, we were not able to increase the numbers of EC significantly above the EEC level by doses of Ep as high as 0.5 IU. A more likely explanation appears to be that normally responsive populations of erythroid precursor cells are also present in PV which give rise to the EC in the presence of exogenous Ep. Moreover, the results suggest that the number of normal precursor cells exceeds those with altered characteristics. Prchal and Axelrad (10) have reported similar findings. The existence of these two precursor cell populations is further illustrated by the observation that two distinct areas of activity were seen when the effect of different concentrations of Ep on EC formation by PV bone marrow was studied. The first region of erythroid activity was detected with small doses of Ep, whereas the second region required relatively large amounts of Ep. The two areas were clearly separated, as was demonstrated by the finding that a significant rise in EC formation above the first plateau (induced by 0.04 IU Ep) did not occur until 1.0 IU Ep was employed. Moreover, the numbers of EC formed with larger Ep doses represented nearly 70% of the total numbers of colonies formed by PV bone marrow.

These observations, combined with those previously published (10–12), lead us to conclude that Ep plays an important role in the regulation of erythropoiesis not only in normal but in individuals with PV as well. A regulatory role of Ep in erythropoiesis in PV has also been demonstrated by in vivo studies. Patients with PV responded to anemia (8) and hypoxia (7) by increased red cell production. This increase occurred only in those patients in whom a rise in Ep production was detected (9). The presence of a population of cells highly sensitive to Ep would be compatible with increased erythropoiesis in the face of low levels of circulating Ep in these patients. PV is a panmyelopathy, probably clonal in nature (19), involving all three formed elements of the blood. Thus, any consideration of the regulation of erythropoiesis in this disorder must also include an explanation for the increased production of leukocytes and platelets, since it is unlikely that the basic defect is different for the three cell types. The results presented here do not provide evidence for or against the clonal nature of this disease. However, evidence is provided to indicate that the final steps in the formation of erythrocytes in PV are subject to the usual Ep control. It is conceivable that a similar mechanism could be involved in the regulation of platelet and leukocyte formation. The role of the myelostimulatory factor suggested to be present in some patients with PV (12, 23, 24) in the regulation of hematopoiesis in this disorder remains to be determined.

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