Characterization of Biologically Active, Platelet-derived Growth Factor–like Molecules Produced by Murine Erythroid Cells In Vitro and In Vivo

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Abstract

Platelet-derived growth factor (PDGF) is an important serum regulator of erythropoiesis in vitro. We have now obtained evidence suggesting that PDGF-like molecules may also modulate erythropoiesis in vivo. Western blot analysis of cytoplasmic extracts from Rauscher murine erythroleukemia cells and phenylhydrazine-treated mouse splenic erythroid cells revealed the presence of several PDGF-like proteins. The presence of PDGF-like proteins in the cytoplasm of these two erythroid cell types was confirmed by immunohistochemical staining. Using a serum-free biologic assay, PDGF-like biological activity was found in cell lysates and conditioned medium of both Rauscher cells and phenylhydrazine-treated mouse erythroid cells. Subcellular localization experiments revealed the biological activity to be concentrated in the cytosolic fraction. Using a series of antibodies to hematopoietic growth factors we demonstrated that PDGF-like biological activity was specifically immunoprecipitated by both monoclonal and polyclonal anti–human PDGF antibodies but not by antibodies to burst-promoting activity, granulocyte-macrophage colony-stimulating factor, IL-3, or erythropoietin. Taken together, the data are consistent with the hypothesis that PDGF-like molecules play a role in the regulation of mammalian erythropoiesis in vivo. (*J. Clin. Invest.* 1990. 85:40–46.) erythropoiesis • platelet-derived growth factor • progenitors • Rauscher

Introduction

The growth of erythroid progenitors in vitro is stimulated by platelet-derived growth factor (PDGF; 1).[1] PDGF may act to stimulate progenitor growth directly by an effect on erythroid progenitors or indirectly by affecting mesenchymal cells which in turn modulate erythropoiesis (2). PDGF-like proteins are produced by a variety of transformed cell types (3–5). These PDGF-like molecules bind to authentic PDGF receptors and exhibit biological activity. Like the B chain of authentic PDGF, many of these PDGF-like molecules are products of the cellular oncogene c-sis. The production of A-A homodimers by cells has also been reported (6).

Work by Pappayannopoulou and co-workers (7) and Leary et al. (8) demonstrated that some transformed cell lines with erythroid characteristics either produce PDGF-like molecules constitutively or can be induced to do so. Unpublished data obtained in our laboratory indicated that the erythropoietin-responsive Rauscher murine erythroleukemia cell line (9, 10) produces PDGF-like molecules constitutively. The observations that PDGF can stimulate erythropoiesis and that PDGF-like molecules are produced by transformed cells with erythroid characteristics prompted us to determine whether there is an in vivo correlate to these in vitro observations. The results of the present study indicate that the production of PDGF-like molecules accompanies erythropoiesis in vivo and that these molecules may be produced by the erythroid cells themselves.

Methods

Cells. The continuous cell lines Rauscher murine erythroleukemia and K562 (11) were maintained in DMEM containing 10% fetal bovine serum in a humidified atmosphere of 95% air/5% CO_2 at 37°C. Normal splenic lymphocytes were obtained from female $B_6C_3F_1$ mice. Erythroid cells were obtained from the spleens of mice treated with phenylhydrazine as described (12). Crude soluble extracts were prepared by homogenizing cells with 70 strokes of a Dounce homogenizer (ball A) in distilled water followed by centrifugation at 27,000 g for 30 min.

SDS-PAGE and immunodetection Western blotting. Crossreacting proteins were detected in cell extracts with monospecific antibodies to homogeneous, authentic human PDGF (Collaborative Research Inc., Lexington, MA). Extracts were subjected to SDS-PAGE in 15% gels and the proteins were electrophoretically transferred to nitrocellulose membranes (13). After transfer the nitrocellulose blots were incubated in the presence of buffer A (20 mM Tris-HCl, 0.5 M NaCl, 3% BSA, pH 7.5) for 16 h at 23°C. The blots were then incubated in the presence of specified concentrations of anti-PDGF antibodies (Collaborative Research Inc.) or irrelevant goat IgG in buffer A for 2 h at room temperature. Anti-PDGF antibodies were an IgG fraction prepared from the serum of goats immunized with highly purified (> 90% pure) authentic human PDGF. The immunogen appeared as a closely spaced doublet of ∼ 30 kD on 15% polyacrylamide SDS gels under nonreducing conditions. The blots were washed three times with buffer B (20 mM Tris-HCl, 0.5 M NaCl, pH 7.5) for 5, 10, and 15 min, and incubated in the presence of 1:500 biotinylated goat anti-rabbit IgG (Cooper Biomedical, Malvern, PA) in buffer A for 2 h. After washing as described above the blots were incubated in the presence of 1:200 horseradish peroxidase–conjugated avidin in buffer A for 30 min followed by washing. Bound complexes were detected with N-chloro-1-naphthol and H_2O_2.

1. *Abbreviations used in this paper:* BPA, erythroid burst-promoting activity; GM-CSF, granulocyte-macrophage colony-stimulating factor; PDGF, platelet-derived growth factor.

Immunohistochemistry. Cells were harvested, washed in serum-free DME, resuspended at a concentration of 1×10^6/ml, and centrifuged onto glass slides at 1,500 rpm for 5 min (Cytospin 2; Shandon Southern Instruments Inc., Sewickley, PA). The slides were immersed briefly in acetone at room temperature and stored at $-20°C$. After returning to room temperature the cells were fixed in acetone for 10 min at $4°C$, equilibrated with 50 mM Tris-HCl, pH 7.4, for 5 min, and treated with monospecific anti-PDGF antibodies (1:200) for 1 h in a humidified chamber. Irrelevant goat antibody (recognizing hepatitis B virus) and Tris buffer were used as controls. The slides were washed in 50 mM Tris-HCl, 140 mM NaCl, pH 7.4, and treated with biotin-conjugated rabbit anti-goat IgG (1:100; Cappel Laboratories, Cochranville, PA) for 30 min. After washing, the slides were treated with alkaline phosphatase–conjugated avidin-biotin complex (ABC-AP Vectastain kit; Vector Laboratories, Inc., Burlingame, CA), washed with Tris buffer for 15 min, and reacted with an alkaline phosphatase substrate kit I (Vector Red; Vector Laboratories, Inc.) for 15 min. The appearance of a reddish stain was interpreted as positive. The cells were counterstained with Harris' hematoxylin and mounted.

Subcellular fractionation of mouse splenocytes and cell lines. Plasma membranes from normal splenic lymphocytes and from the Rauscher and K562 cell lines were isolated by a modification of the method of Jett et al. (14) as previously described (15). Briefly, cells were suspended in Dulbecco's PBS, loaded with glycerol at a final volume of 30%, and lysed with 20 strokes of a Dounce homogenizer in 5 mM Tris-HCl and 2 mM $MgCl_2$, pH 7.4 (buffer C). The homogenate was centrifuged for 10 min at 700 g to remove the nuclei. The nuclear pellet was washed and the supernatants were combined and centrifuged at 3,000 g for 10 min to pellet the mitochondria. The resultant supernatant was centrifuged at 40,000 g for 45 minutes to separate crude plasma membranes from the soluble fraction (cytosol). The crude membranes were suspended in 50% sucrose (wt/vol) in buffer C, overlayered with equal volumes of 40, 30, and 20% sucrose in buffer C, and centrifuged in an SW 41 rotor (Beckman Instruments, Inc., Palo Alto, CA) at 200,000 g for 18 h. Plasma membranes were harvested from the 30–40% sucrose interface, washed once with PBS, and stored at $-20°C$ until analysis.

In addition, plasma membranes and soluble fractions were prepared from nucleated erythroid precursors isolated from spleens of phenylhydrazine-treated mice. Splenocytes were suspended in a hypotonic buffer consisting of 10 mM Tris-HCl, 10 mM KCl, and 1.5 mM $MgCl_2$, pH 7.5, and disrupted with 40 strokes of a tight-fitting Dounce homogenizer (15). To restore isotonicity, 2 M sucrose was added to achieve a final concentration of 260 mM. The homogenate was layered over a sucrose gradient of 3 vol 28% (wt/vol) sucrose and 2 vol 50% (wt/vol) sucrose. All sucrose solutions were made in 140 mM NaCl, 2.4 mM $MgCl_2$, and 5 mM Tris-HCl, pH 7.4. The sucrose density gradients were centrifuged at 117,000 g in an SW 41 swinging bucket rotor (Beckman Instruments, Inc.) for 40 min. Plasma membranes were harvested from the 28–50% sucrose interface and the soluble fraction (cytosol) was removed from the top of the gradient. The harvested plasma membrane fraction was washed with PBS and pelleted at 15,000 g for 10 min. The pellet was resuspended in a minimal volume of PBS and stored at $-20°C$ until use.

Conditioned medium. Conditioned medium from splenocytes or cell lines was prepared by washing cells three times with serum-free DME followed by incubation in serum-free DME for 16 h at $37°C$ (5–10 $\times 10^5$ cells/ml).

Immunoadsorption of cellular fractions. To determine whether the erythroid growth-promoting activity found in the soluble fraction of Rauscher cells (see Results) was due to the presence of one or more well-characterized hematopoietic growth factors, aliquots of the soluble fractions were preadsorbed with polyclonal anti–erythroid burst-promoting activity (BPA) (16), anti–granulocyte-macrophage colony-stimulating factor (GM-CSF), and anti-IL-3 (courtesy of Steve Clark, Genetics Institute, Cambridge, MA), monoclonal anti-erythropoietin (17), mouse monoclonal anti-PDGF B chain [1-18] antipeptide antibody (Microbiological Associates, Walkersville, MD) (18, 19) and

polyclonal anti-PDGF (Collaborative Research Inc.) as described previously (20). Cytosolic protein (65 μg) was incubated overnight at 4°C with 0–200 μg/ml of the appropriate antibody. Solutions were then incubated for 15 min at room temperature with a quantity of formalin-fixed *Staphylococcus aureus* (Pansorbin; Calbiochem-Behring Corp., La Jolla, CA) sufficient to adsorb a fourfold excess of the highest amount of IgG used. Bound antibody was removed by centrifugation. The adsorbed supernatants were filter sterilized and added to bone marrow culture. Control immunoadsorptions were performed with GM-CSF alone, GM-CSF plus anti–GM-CSF, IL-3 alone, IL-3 plus anti-IL-3, rabbit IgG, and the soluble fractions of Rauscher and K562 cells alone. Immunoadsorption (rather than neutralization) was used since not all antibody preparations were neutralizing and because *S. aureus* immunoadsorption had been shown previously by us to provide a more suitable preparation for incorporation into the serum-free progenitor assay (20, 21) (see below).

Assays for biological activities. Human bone marrow cells were aspirated from the posterior iliac crests of healthy paid volunteers ranging in age from 20 to 30 yr. Informed consent was obtained before aspiration in accordance with Institutional Review Board policy. Cells were placed in Eagle's MEM with Hanks' salts, without L-glutamine (320–1570; Gibco Laboratories, Grand Island, NY) containing 20 U preservative-free heparin/ml. The mononuclear cells appearing at the interface after centrifugation over Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) were separated, washed in alpha medium (Gibco Laboratories), and cultured at 6 $\times 10^4$ marrow cells/ml in serum-free clots (22). The culture mixture contained 9% NCTC-109 (vol/vol) (controls), specified concentrations of subcellular fractions, or medium conditioned by cell lines or splenocytes. Cultures were established with 2 IU/ml recombinant erythropoietin (Amgen Biologicals, Thousand Oaks, CA). After incubation at $37°C$ in humidified air containing 4% CO_2, clots were removed, fixed on glass slides in glutaraldehyde, and stained with benzidine and hematoxylin. The number of burst forming unit–E (BFU-E) derived colonies (appearing as clusters $\geq$ 50 benzidine-positive cells or as $\geq$ 3 aggregates of 8–49 benzidine-positive cells after 12 d of incubation) was scored. The number of CFU-E-derived colonies (appearing as clusters of 8–49 benzidine-positive cells) was scored after 7 d of incubation. The amount of stimulatory/inhibitory activity present in subcellular fractions and conditioned media was determined relative to that in control cultures containing NCTC-109 (100% growth). Cloning efficiency varied between 120 and 400 erythroid bursts, and 160 and 500 erythroid colonies per 6 $\times 10^4$ cells in 1-ml cultures. Data presented in the figures are for erythroid bursts and colonies formed in 125-μl cultures.
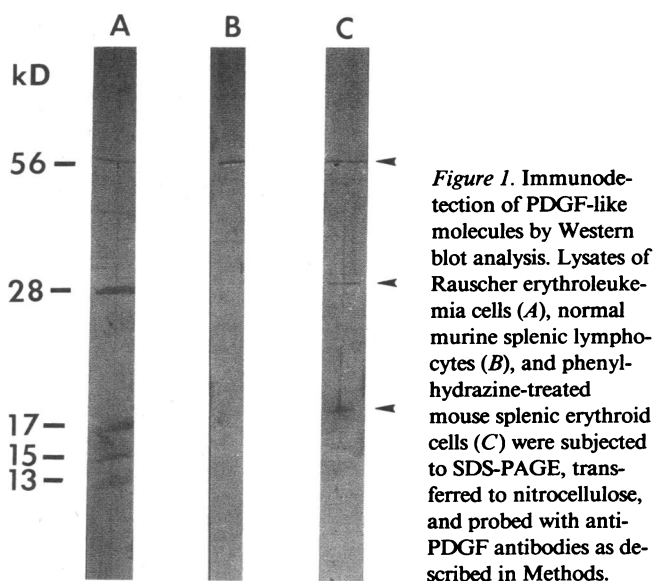

*Figure 1.* Immunodetection of PDGF-like molecules by Western blot analysis. Lysates of Rauscher erythroleukemia cells (*A*), normal murine splenic lymphocytes (*B*), and phenylhydrazine-treated mouse splenic erythroid cells (*C*) were subjected to SDS-PAGE, transferred to nitrocellulose, and probed with anti-PDGF antibodies as described in Methods.
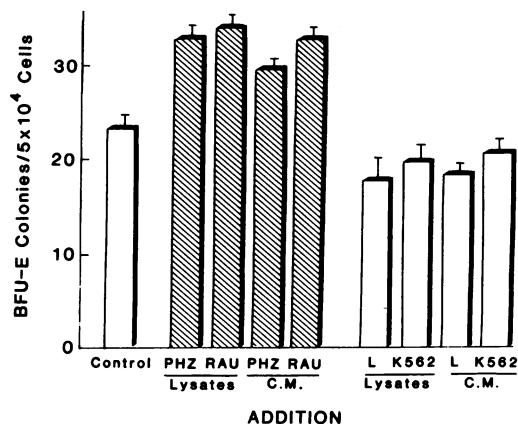
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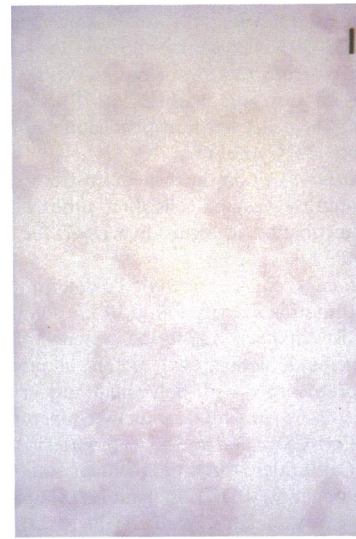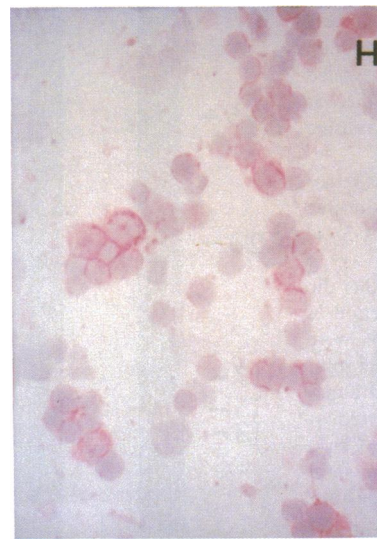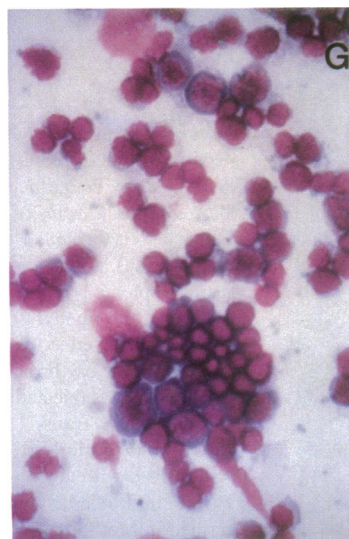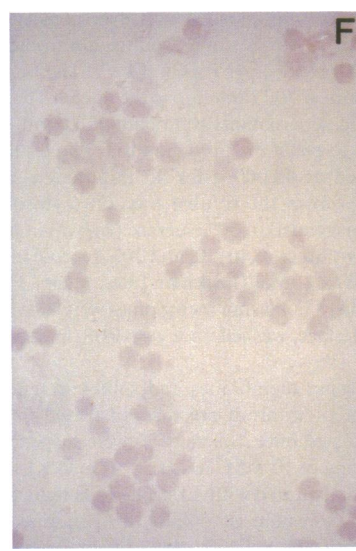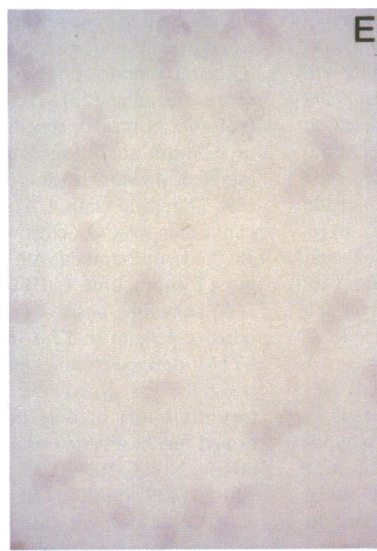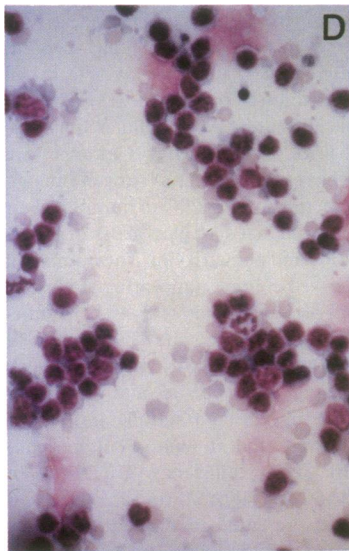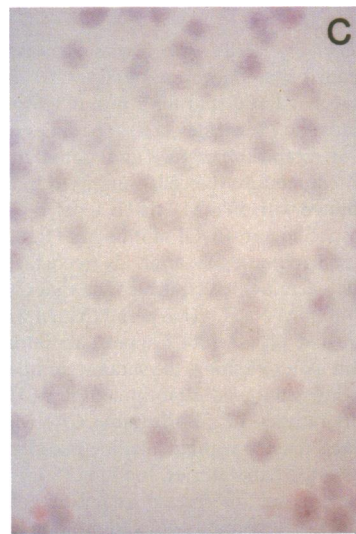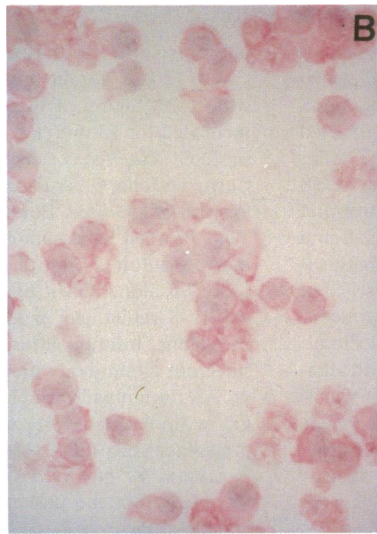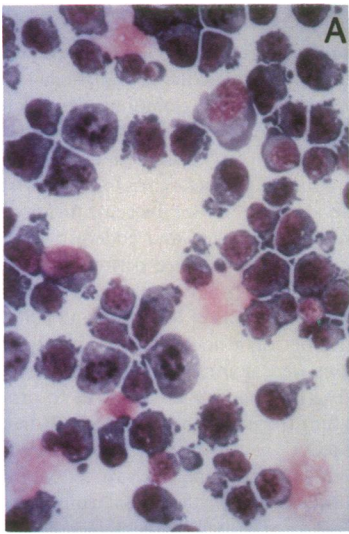
Figure 3. Extracts and conditioned medium of phenylhydrazine-treated mouse erythroid cells (*PHZ*) and Rauscher cells (*RAU*) stimulate BFU-E growth in serum-free, PDGF-deficient culture. Human marrow cells were grown serum-free in the presence of 2 IU erythropoietin/ml (see Methods). The addition of lysates or conditioned medium (*C.M.*) of PHZ cells or RAU cells stimulated BFU-E growth (*hatched bars*). Lysates or conditioned medium of normal splenic lymphocytes (*L*) or K562 cells had no effect. Mean±SE burst formation is displayed for quadruplicate cultures. Similar results were obtained in one additional study.

Statistical analysis. The mean±SEM of colonies and bursts formed in quadruplicates was determined and data sets were compared by the two-sample ranks test of Wilcoxon-White (23).

Results

PDGF-like molecules were detected in Rauscher murine erythroleukemia cells by Western blotting cytoplasmic extracts and probing with monospecific antibody to authentic human PDGF (Fig. 1). As seen in lane *A,* the antibody identified proteins with approximate molecular masses of 56, 28, 17, 15, and 13 kD. The 17-, 15-, and 13-kD proteins have molecular weights that are virtually identical to those reported for the A and B chains of human PDGF purified from platelets (24). Interestingly, the 28-kD protein is identical in size to p28^{v-sis}, the transforming protein encoded by the v-*sis* oncogene (25), suggesting that it may represent the c-*sis* gene product before amino- and carboxy-terminal cleavage. Probing with irrelevant goat IgG instead of anti-PDGF antibodies resulted in no visualization of these bands (not shown). As seen in lane *B,* all PDGF-like proteins were absent from the control splenic lymphocytes except for the 56-kD species. However, the cytoplasm of splenic erythroid cells, shown in lane *C,* which were under erythropoietin stimulation in vivo when harvested, contained the 28- and 17-kD proteins. These results suggest that the production of PDGF-like molecules may accompany erythropoiesis in vivo.

Immunohistochemical localization of PDGF-like molecules. To confirm the findings obtained with Western blotting, we stained the same three cell populations immunohisto-

chemically (Fig. 2). When stained with Wright's stain, Rauscher cells resemble malignant pronormoblasts (Fig. 2 *A*). Essentially all of the cells stained positively with anti-PDGF antibodies (Fig. 2 *B*), whereas none of the cells were stained by control goat IgG (Fig. 2 *C*). Wright's stain of normal spleen cells revealed a population of virtually 100% lymphoid cells (Fig. 2 *D*). These cells did not stain positively with anti-PDGF antibodies (Fig. 2 *E*) or with the control IgG (Fig. 2 *F*), supporting the results shown in Fig. 1, lanes *A* and *B,* that normal splenocytes do not contain PDGF. In contrast, spleen cells from phenylhydrazine-treated mice were a heterogeneous population consisting of lymphocytes and erythroid cells at various stages of differentiation (Fig. 2 *G*). The early erythroid precursors were relatively larger than lymphoid elements and often appeared in clusters. Staining of these spleen cells with anti-PDGF antibodies demonstrated PDGF-like crossreactivity in the cytoplasm of the larger erythroid cells (Fig. 2 *H*) but not in that of the smaller lymphoid cells. Finally, control IgG did not stain any cells in this population (Fig. 2 *I*).

Production of PDGF-like biological activity by Rauscher cells and splenic erythroblasts. To determine whether PDGF-like biological activity was present in and secreted by mouse splenocytes and/or cell lines, we prepared and assayed cell lysates and serum-free medium conditioned by these cells. As shown in Fig. 3, both lysates and conditioned medium (*C.M.*) of phenylhydrazine-induced erythroid cells (*PHZ*) and Rauscher cells (*RAU*) stimulated BFU-E growth (relative to control, *P* < 0.05 for each). The degree of stimulation in these studies was comparable to that obtained with authentic human PDGF (1). In contrast, lysates and conditioned medium from normal splenic lymphocytes (*L*) and K562 cells had no effect. Similar results were obtained when CFU-E proliferation was assessed (not shown). The data indicate that bioactive molecules are produced by Rauscher cells and murine splenic erythroblasts, but not by K562 cells or murine splenic lymphocytes.

Subcellular localization of hematopoietic growth factors. We next asked whether a particular subcellular fraction of Rauscher cells or splenic erythroblasts contained the erythroid growth-promoting activity. We observed that on a per microgram protein basis, erythroid burst stimulatory activity was localized to the cytosolic fraction of Rauscher cells (Fig. 4 *A, closed circles*). Dose-dependent stimulation was observed over a range of 12.5–50 μg cytosolic protein. A smaller but nonetheless significant (*P* < 0.05) increase in the formation of CFU-E–derived colonies was also observed (Fig. 4 *B, open circles*). In comparison, nucleus, mitochondria, endoplasmic reticulum, and membrane fractions expressed less (*P* < 0.05) or no stimulating activity (Fig. 4).

The results of adding subcellular fractions derived from splenocytes of phenylhydrazine-treated and normal mice are shown in Fig. 5. The cytosolic fraction from phenylhydrazine-treated mouse splenocytes strongly enhanced erythroid burst (Fig. 5 *A, closed circles*) and CFU-E–derived colony formation (Fig. 5 *C, open circles*) in a concentration-dependent manner. The degree of stimulation was similar to that seen with

Figure 2. Immunohistochemical identification of PDGF-like molecules in the cytoplasm of Rauscher cells and phenylhydrazine-treated mouse erythroid cells. *A–C,* Rauscher cells; *D–F,* normal splenic lymphocytes; *G–I,* phenylhydrazine-treated mouse splenocytes. Stains are as follows: *A, D, G,* Wright's stain; *B, E, H,* anti-PDGF antibodies; *C, F, I,* control goat IgG. Note positive staining for PDGF-like proteins in Rauscher cells (*B*) and splenic erythroid cells (*H*). See Methods and Results.
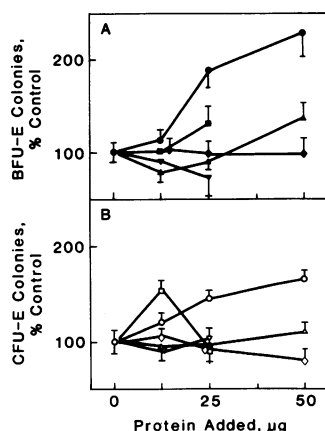
Figure 4. Effects of subcellular fractions from Rauscher cells on BFU-E (A) and CFU-E (B) derived colony formation. Aliquots of cytosolic (soluble) fraction (●, ○), nuclear pellet (♦, ◊), mitochondrial pellet (▲, △), endoplasmic reticulum (■, □) or plasma membranes (▼, ▽) were added at the indicated protein concentrations to serum-free bone marrow cultures containing 2 IU erythropoietin/ml. Mean±SE percent colony formation is shown. Control cultures prepared with medium alone contain 35±1 bursts and 58±1 colonies in each of four 125-μl clots. Similar results were obtained in another study.

Rauscher cell cytosolic protein (Fig. 4 *A*). In contrast, there was no effect of plasma membranes from these cells on either BFU-E or CFU-E proliferation (*inverted triangles; P* > 0.10 for each). Importantly, there was no effect of the cytosolic fraction from normal splenic lymphocytes on early or late progenitor cell proliferation (Fig. 5, *B* and *D*). The data indicate that one or more soluble factors in murine erythroblasts augment erythroid colony formation and that this activity is absent from the cytosol of normal mouse splenic lymphocytes.

Immunologic crossreactivity of the cytosolic growth factor with PDGF. To characterize the nature of the cytosolic bioactive molecules, an attempt was made to immunoadsorb the activity from Rauscher cell cytosolic proteins with antibodies to several hematopoietic growth factors. Both polyclonal anti-human PDGF antibodies and monoclonal anti–PDGF B chain [1–18] antibody immunoadsorbed the stimulatory activity in a concentration-dependent manner (Fig. 6, *circles* and *squares*). The results were comparable for both BFU-E and CFU-E stimulatory activities. In contrast, none of the other antibodies tested (anti-BPA, anti-GM-CSF, anti-erythropoietin, and anti–IL-3) immunoadsorbed the activity from Rauscher cell cytosol. The results indicate that factors found in
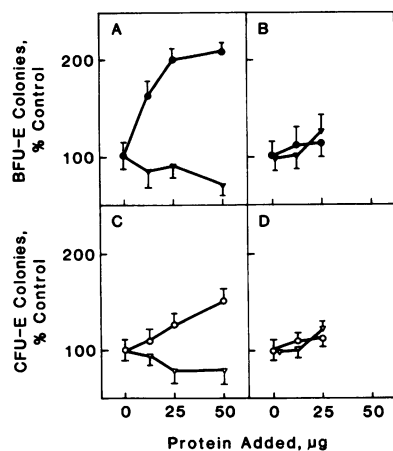
Figure 5. Effects of subcellular fractions from splenocytes on BFU-E (*top*) and CFU-E (*bottom*) proliferation. *A* and *C*, Aliquots of cytosol (●, ○) or purified plasma membranes (▼, ▽) from splenocytes of phenylhydrazine-treated mice were added at the indicated protein concentrations. *B* and *D*, Aliquots of cytosol or plasma membranes from normal lymphocytes were added to culture. Mean±SE percent erythroid bursts and colonies are recorded for cultures established with 2 IU erythropoietin/ml. Control cultures prepared with medium alone contained 31±2 bursts and 50±4 colonies in each of four 125-μl clots. Similar results were obtained in three additional studies.
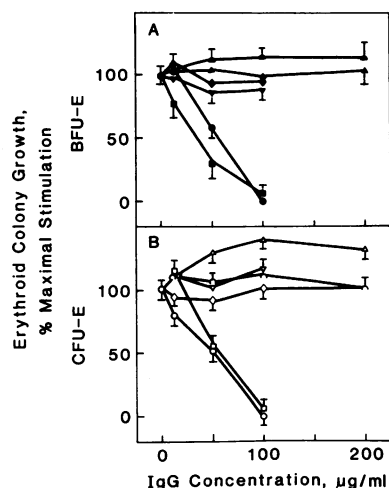
Figure 6. Immunoadsorption of growth factors from Rauscher cell cytosolic protein. Cells were homogenized and the fraction containing soluble cytoplasmic proteins was prepared. Aliquots of soluble fraction (each containing 65 μg protein) were incubated with antibodies to BPA (♦, ◊), GM-CSF (■, □), IL-3 (▲, △), monoclonal PDGF (●, ○), polyclonal PDGF (■, □), or erythropoietin (▼, ▽), and immunoprecipitated as described in Methods. Supernatants were assayed for residual growth-promoting activity for BFU-E (*A*) and CFU-E (*B*) progenitors. Maximum stimulation = [(number of colonies or bursts in presence of immunoadsorbed cytosolic fraction) − (number of colonies or bursts in absence of fraction)] ÷ (number of colonies or bursts in absence of fraction). All cultures contained 2 IU erythropoietin/ml. Mean±SE percent colonies for quadruplicates are displayed. Control cultures prepared with cytosol alone contained 58±1 bursts and 74±1 colonies in each 125-μl clot, representing 185 and 150% stimulation, respectively, above baseline burst and colony formation in cultures prepared with NCTC-109 alone. Similar results were obtained in three additional studies.

Rauscher cell cytoplasm that express erythropoietic activity share antigenic determinants with PDGF.

Discussion

We and others have reported previously that PDGF stimulates the proliferation of early and late erythroid progenitors in vitro (1, 2). Here we present data suggesting that PDGF-like molecules may also modulate erythropoiesis in vivo. This hypothesis is supported by our findings that (*a*) like Rauscher erythroleukemia cells, erythroid cells from spleens of phenylhydrazine-treated mice produced PDGF-like proteins (Figs. 1, 2, and 6), and (*b*) these proteins stimulated the proliferation and/or differentiation of erythroid progenitors (Figs. 3–5).[2]

The data presented in Fig. 1 are notable for the presence of PDGF-like molecules of several molecular weights. These differences are presumably due to differential amino- and carboxy-terminal processing of the nascent protein, a well-established posttranslational modification that has been shown to result in conversion of the 28-kD c-*sis* gene product to the 14-kD B chain of authentic PDGF. Since the cytoplasmic mRNA of c-*sis* found in a variety of cells is 4.3–4.6 kb (thus encoding a theoretical 158–169-kD protein), it seems clear that other processing events are also likely. Niman et al. (18) found a wide variety of PDGF-like molecules, including several > 30 kD, in both simian sarcoma virus–infected (v-*sis*) and noninfected cells. Proteins of 50–60 kD were common and may be related to the 56-kD species shown in Fig. 1. The absence of the 13- and 15-kD species in splenic erythroid cells (Fig. 1, lane *C*) versus their presence in the transformed

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2. The relationship of these PDGF-like effects to those mediated by other intracellular growth factors (27) is unknown.
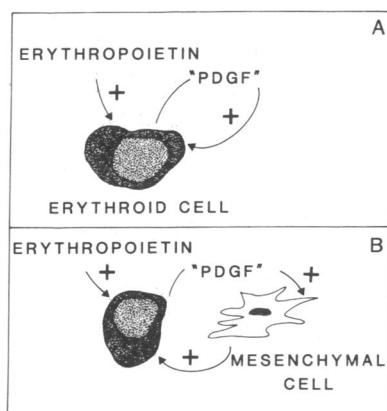
Figure 7. Two possible mechanisms for the participation of PDGF-like proteins in the regulation of erythropoiesis (see Discussion).

Rauscher cells (Fig. 1, lane *A*) presumably reflects different processing events in these two cells types, a finding that is not unexpected (18).

Phenylhydrazine-induced hemolysis in mice is accompanied by elevated circulating levels of erythropoietin, which in turn induce an expansion of the erythroid compartment in the bone marrow and spleen. In this regard, the action of erythropoietin in regulating red cell production can be considered to be twofold. First, the hormone induces DNA synthesis in and proliferation of erythroid progenitor and precursor cells. Second, erythropoietin induces terminal differentiation of erythroid cells that is characterized by hemoglobin accumulation. The mechanism(s) through which the hormone-receptor interaction triggers these distinct cellular responses is unknown. One possibility is that multiple signals may be induced by a single hormone-receptor event. This motif has been demonstrated with the hormone glucagon, the action of which involves alterations in cyclic nucleotides as well as in phosphoinositol intermediates (26). Our results with PDGF raise the possibility that the proliferative effects of erythropoietin may be mediated in part by the production of biologically active PDGF-like molecules in erythroid cells themselves. Since PDGF has not been reported to have an effect on hemoglobinization of committed erythroid cells, erythropoietin-induced production of PDGF-like molecules may serve as one regulatory pathway involved in erythropoietin-dependent cell proliferation. Although this need not be the only means through which erythropoietin exerts a stimulatory effect, it could prove to be an especially effective one since one means of controlling this stimulus would be via regulating the expression of the gene or genes involved. Additional studies with highly purified populations of erythropoietin-responsive progenitor cells will be required to explore this possibility.

The modulation of erythropoiesis by PDGF-like molecules in vivo might be effected by several possible mechanisms, two of which are depicted in Fig. 7. The first possibility, shown in Fig. 7 *A,* suggests that erythropoietin induction of the erythroid progenitor stimulates the synthesis and release of one or more PDGF-like proteins. These proteins then modulate growth of the erythroid progenitor directly by an autocrine mechanism. The second possibility, depicted in Fig. 7 *B,* would have the PDGF-like proteins released from the erythroid progenitor acting on an intermediate cell in the bone marrow microenvironment. Subsequently, one or more factors produced by this intermediate cell would feed back upon the erythroid progenitor to stimulate its growth. Although our data do not address the operative mechanism(s) directly, they do suggest that PDGF-like molecules may play an important role in the regulation of mammalian erythropoiesis in vivo.

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