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Purification of a membrane-derived human erythroid growth factor

(burst-promoting activity/erythropoiesis/colony-forming units)

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ABSTRACT **We have purified erythroid burst-promoting activity (BPA) from human lymphocyte plasma membranes by detergent extraction followed by gel-filtration, ion-exchange, and hydroxylapatite chromatography. BPA is a heat-stable integral membrane glycoprotein of M_r 28,000 by gel filtration whose activity is eluted from NaDodSO$_4$/polyacrylamide gels as a broad band at M_r 25,000–29,000. The growth stimulator appears to be erythroid-specific, stimulating proliferation of the human erythroid burst-forming unit (BFU-E) by up to 600% of control values when tested in serum-free bone marrow culture. In contrast, it is devoid of granulocyte/macrophage colony-stimulating factor activity and has a negligible effect on the formation of human megakaryocyte and mixed hematopoietic colonies. Polyclonal anti-lymphocyte membrane IgG, which neutralizes BPA expression in culture, completely absorbs BPA from all lymphocyte-derived sources [solubilized lymphocyte plasma membranes, membrane-containing vesicles shed into lymphocyte conditioned medium (LCM) and soluble vesicle-free LCM supernatants], suggesting that soluble and membrane-derived lymphocyte BPA are antigenically related. This membrane glycoprotein may be an important mediator of proximal cellular interactions that are known to promote erythropoiesis *in vitro*.**

In vitro differentiation of hematopoietic, multipotential stem cells along the erythroid pathway requires that erythropoietin and/or erythroid burst-promoting activity (BPA) be added to the culture medium (1–3). BPA is released spontaneously into liquid culture medium from circulating and bone marrow low-density mononuclear cells (4–7). We have previously demonstrated that BPA is expressed by lymphocyte plasma membranes and by plasma membrane-derived vesicles that are shed into lymphocyte conditioned medium (LCM) (7). In order to study the role of plasma membrane components in growth control as well as to investigate the relationship of soluble and membrane-derived growth factors in erythropoiesis, we have purified BPA from the plasma membranes of normal, unstimulated peripheral blood lymphocytes. BPA is an integral membrane protein of M_r 28,000 on gel filtration whose activity comigrates with a single band on NaDodSO$_4$/PAGE under reducing conditions. BPA stimulates erythroid burst formation in serum-free human bone marrow culture and exhibits no apparent synergistic interactions with other growth factors that we have tested. BPA is devoid of granulocyte/macrophage growth-factor activity (granulocyte/macrophage colony-stimulating factor, GM-CSF) and has negligible stimulatory activity for megakaryocyte or mixed hematopoietic colony formation. Accordingly, this membrane protein appears to be distinct from panspecific or multilineage hematopoietic growth factors that have been described previously (8, 9).

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MATERIALS AND METHODS

Preparation of Lymphocyte Plasma Membranes and Conditioned Medium. Freshly drawn plateletpheresis residues, obtained from the Dana–Farber Cancer Institute, were fractionated by sedimentation in Ficoll-Paque (Pharmacia) as described (7). Mononuclear cells were depleted of residual platelets by the method of Perper *et al.* (10), and monocytes were removed by adherence to plastic tissue culture flasks (Costar, Cambridge, MA) for 90 min (twice). Nonadherent cells (>97% lymphocytes by cytochemical and histochemical identification) were incubated at 5×10^6 per ml in serum-free alpha minimal essential medium (GIBCO) for production of LCM. After overnight incubation at 37°C in humidified 5% CO_2/air, cells were separated from the LCM by centrifugation at $500 \times g$ for 10 min. LCM was stored at 4°C for further use. The resultant lymphocytes (>98% viable by trypan blue exclusion) were washed twice in phosphate-buffered saline (PBS: 5 mM sodium phosphate/150 mM NaCl; pH 7.6 unless otherwise indicated), and plasma membranes were isolated by differential centrifugation and sucrose gradient fractionation according to a modification (11) of the method of Jett *et al.* (12). Purified plasma membranes were stored at −70°C in PBS. LCM was fractionated into supernatants and membrane-vesicle containing pellets by centrifugation at $40,000 \times g$ for 30 min. Pellets were resuspended in PBS and stored at −70°C, while supernatants were first subjected to a 30–50% $(NH_4)_2SO_4$ cut and then stored at −70°C in PBS.

Assay for Human Erythroid BPA. Approximately 0.5 ml of spiculated bone marrow was aspirated from the posterior iliac crests of normal volunteers. The cells were placed in Eagle's minimal essential medium (GIBCO) containing heparin and were separated by sedimentation in Ficoll-Paque. Mononuclear cells layering at the interface were separated, washed in alpha medium and cultured at 6×10^5 per ml in serum-free fibrin clots as described (13). Cultures were supplemented with Iscove's modified Dulbecco's medium (GIBCO), purified human serum albumin, transferrin, ferric chloride, and either sheep step-III erythropoietin (Connaught Laboratories, Willowdale, ON) or recombinant human erythropoietin (70,000–80,000 units/mg; Amgen Biologicals, Thousand Oaks, CA) at 2.0 units/ml. Test plates contained 10% (vol/vol) LCM or its fractions, lymphocyte plasma membranes or fractions thereof, or NCTC-109 medium (controls). Cultures were maintained for 12 days at 37°C in humidified 5% CO_2/air and, following harvest, the fibrin clots were stained with benzidine and hematoxylin. Erythroid bursts containing ≥50 benzidine-positive cells were scored under a microscope. The amount of BPA present in experimental

Abbreviations: BPA, burst-promoting activity; CFU, colony-forming unit(s); CFU-E, -GM, -GEMM, and -Meg, erythroid, granulocyte/macrophage, multipotential, and megakaryocyte CFU, respectively; BFU-E, erythroid burst-forming unit(s); CSF, colony-stimulating factor; LCM, lymphocyte conditioned medium.

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plates was determined relative to that in control cultures containing NCTC-109 ("100% growth").

The mean ± SEM of quadruplicate determinations for each test point was calculated, and data sets were compared by the two-sample ranks test of Wilcoxon and White (14).

Additional Culture Systems. The capacity of chromatographically purified BPA (see below) to support the proliferation of granulocyte/macrophage progenitors (CFU-GM), granulocyte/erythroid/macrophage/megakaryocyte progenitors (CFU-GEMM), and megakaryocyte progenitors (CFU-Meg) was determined in human marrow cultures maintained for 12–14 days under serum-free conditions (13). Granulocyte/macrophage cultures were established with and without 25 units of human colony-stimulating factor (CSF; Hyclone, Logan, UT) per ml. Megakaryocyte cultures were established with modification of the serum-free conditions according to Hoffman *et al.* (15) in the presence and absence of purified megakaryocyte CSF (16).

Extraction of Membrane-Derived BPA. Lymphocyte plasma membranes or LCM vesicles were suspended to 1 mg/ml in 5 mM Hepes, pH 7.4/2 mM dithiothreitol/0.2 mM phenylmethylsulfonyl fluoride. Aliquots were mixed with equal volumes of 0.2 M NaOH; 0.2 mM EDTA (pH 8.0); 2 M KCl; 5 mM Hepes (pH 7.4); PBS; or 60 mM octyl β-D-glucopyranoside/5 mM Hepes, pH 7.4. Samples were incubated at 0°C for 30 min and then centrifuged at $100,000 \times g$ for 30 min to separate solubilized proteins from residual insoluble membranes. Both supernatants and pellets (resuspended in PBS) were dialyzed extensively against PBS prior to bioassay.

Chromatographic Fractionation of BPA. Procedures were carried out at 4°C, unless indicated, and all buffers contained 0.1% polyethylene glycol (PEG) 6000 (Sigma) to prevent nonspecific sticking of proteins. Solubilized BPA-containing plasma-membrane or LCM-vesicle fractions or $(NH_4)_2SO_4$-fractionated LCM supernatants were applied to 1.6×90-cm columns of Sephacryl S-300 (Pharmacia) and eluted with PBS (with or without 2 mM dithiothreitol). Individual fractions were sterilized using 0.45-μm filters prior to bioassay. Those fractions demonstrating BPA in culture were pooled and concentrated by lyophilization or by an Amicon Ultrafiltration Stir Cell (Amicon) fitted with a YM10 membrane. Aliquots were dialyzed against 5 mM sodium phosphate (pH 8.0) and applied to 5-ml columns of hydroxylapatite (Calbiochem) or 1-ml columns of DE-52 DEAE-cellulose (Whatman). For hydroxylapatite columns, elution was at room temperature with a step gradient from 5 mM to 100 mM sodium phosphate (pH 8.0). DE-52 columns were flushed at room temperature with 5 mM sodium phosphate (pH 8.0), followed by stepwise elution with 0.01–1.0 M NaCl in 5 mM sodium phosphate (pH 8.0). Active fractions were pooled, concentrated, and dialyzed against PBS for storage. Alternatively, following concentration, material was dialyzed into 5 mM sodium phosphate (pH 8.0) and applied to the opposite column (i.e., active material from DE-52 was applied to hydroxylapatite and vice versa). Finally, in some cases, active fractions were applied to a Bio-Sil TSK-250 HPLC column (Bio-Rad) and eluted at room temperature with PBS (pH 7.0). After chromatographic fractionation, BPA was stored at 4°C in PBS (pH 7.6).

$NaDodSO_4$/PAGE and Electroelution of BPA. Protein samples were electrophoresed in $NaDodSO_4$ polyacrylamide gels according to Laemmli (17), except that slab gels were used. Gels either were silver-stained by the method of Wray *et al.* (18) or, in the case of samples that were iodinated with ^{125}I-labeled Bolton-Hunter reagent (19) (New England Nuclear), were fixed, dried, and exposed for autoradiography. To localize unlabeled BPA in unstained gels, individual lanes were cut from the gel and sliced into 1-cm or 0.5-cm pieces that were then homogenized by forcing them through a 10-ml syringe. Homogenized samples were loaded into the sample wells (cathode) of an ISCO electrophoretic concentrator (ISCO) and electrophoretically eluted onto glycerol cushions in the collection chambers (anode) at 150 V for 2 hr, with 10 mM Tris acetate (pH 8.8) as the electrode buffer. Eluted proteins were dialyzed extensively against PBS containing 0.1% PEG 6000 and were filter-sterilized for use in culture.

Immunochemical Techniques. Antisera were prepared against lymphocyte plasma membranes and against octyl glucoside-solubilized membrane proteins (11). IgG fractions were obtained by $(NH_4)_2SO_4$ fractionation, followed by DEAE-cellulose chromatography. Pre- and postimmune IgG were tested for crossreactivity with the immunizing antigens as well as with LCM fractions as described (11).

For immunoadsorption, BPA-containing samples were incubated overnight at 4°C with increasing amounts of either pre- or postimmune antimembrane IgG. An amount of Pansorbin (Calbiochem) calculated to have an adsorption capacity 4-fold in excess of the maximum amount of IgG used was added and incubated for 15 min at room temperature before bound IgG was removed by centrifugation. Adsorbed supernatants were then filter-sterilized and assayed for BPA in serum-free culture.

Protein Determination. Protein was estimated either by the method of Lowry *et al.* (20) or by the fluorescamine method (21), using bovine serum albumin as a standard.

RESULTS

Purification of Lymphocyte Plasma Membrane-Associated BPA. Lymphocyte plasma membranes or plasma membrane-derived LCM vesicles were extracted with a variety of reagents as described above. Of the reagents tested, only the detergent octyl glucoside solubilized BPA from the membrane, suggesting that BPA is an integral membrane protein. Sequential extraction of membranes with 0.1 M NaOH, which extracts ≈50% of total protein while leaving BPA associated with the insoluble membrane fraction, followed by 30 mM octyl glucoside resulted in >5-fold enrichment of BPA in the octyl glucoside supernatant.

Fractionation of octyl glucoside-solubilized proteins on Sephacryl S-300 resulted in a chromatographic profile wherein the bulk of UV-absorbing protein was eluted in the void volume, and BPA, as measured by the activity of eluted fractions in serum-free marrow culture, was eluted with a M_r of 28,000 (Fig. 1). Although the inclusion of 2 mM dithiothreitol in the column buffer decreased slightly the size of the void-volume protein peak, it was without apparent effect on the activity profile of the column.

When BPA-containing fractions from the gel-filtration column were applied to DE-52 in 5 mM sodium phosphate (pH 8.0) and eluted with a salt gradient, all growth-promoting activity was eluted between 0.15 M and 0.25 M NaCl. When this activity was concentrated, applied to a hydroxylapatite column, and eluted with increasing phosphate concentrations, BPA was eluted at 20 mM phosphate (Fig. 2*a*). Reapplication of the active fractions to DE-52, under conditions identical to those used previously, resulted in elution of BPA sharply at 0.2 M NaCl (Fig. 2*b*). Finally, when the 0.2 M NaCl eluant from DE-52 chromatography was concentrated and applied to a TSK-250 HPLC column in PBS (pH 7.0), BPA was eluted with a M_r of 28,000 (Fig. 2*c*). The chromatographic behavior of membrane-derived BPA was indistinguishable from that of BPA derived from membrane vesicle-free LCM supernatant.

$NaDodSO_4$/PAGE. After electrophoresis of column-purified BPA, silver staining failed to reveal any stained bands. However, when proteins were prelabeled with ^{125}I Bolton–Hunter reagent, subsequent autoradiography of the dried gel revealed that the majority of radiolabel migrated in the

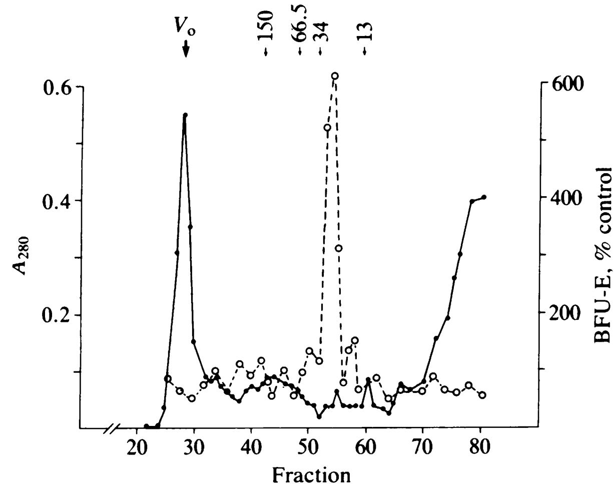

FIG. 1. Sephacryl S-300 fractionation of octyl glucoside-extracted lymphocyte plasma membranes. Solubilized membrane protein (2 ml) was applied to a 1.6 × 90-cm column and eluted with PBS (pH 7.6). Absorbance at 280 nm was measured (●), and individual fractions were assayed for growth-promoting activity on erythroid progenitors (erythroid burst-forming units, BFU-E) in serum-free marrow culture (○). Control growth (100%) represents that in plates containing NCTC-109 medium. Column calibration [void volume (V_o) and elution positions of molecular weight standards ($M_r \times 10^{-3}$)] is shown by arrows.

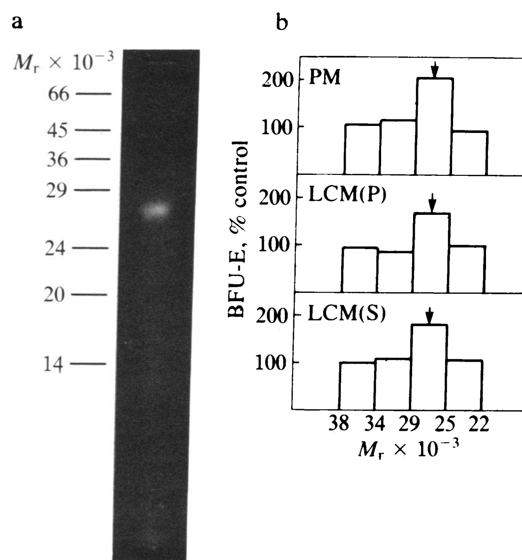

FIG. 3. NaDodSO$_4$/PAGE and electroelution of column-purified BPA. (*a*) Column-purified BPA was iodinated with ^{125}I Bolton–Hunter reagent (19) and applied directly to an 11% polyacrylamide gel (17). The dried gel was exposed for autoradiography using Kodak X-Omat paper. (*b*) After electrophoresis of unlabeled samples of BPA from lymphocyte plasma membranes (PM) or from particulate or soluble fractions of LCM [LCM(P) and LCM(S), respectively], the unfixed gel was sliced into 1-cm slices and proteins were electroeluted as described in *Materials and Methods*. Aliquots of eluted protein were assayed in serum-free marrow culture.

molecular weight range 25,000–29,000 (Fig. 3*a*). The broad nature of the BPA "band" even on a tightly crosslinked gel (25:1 acrylamide/*N,N'*-methylenebisacrylamide weight ratio) is consistent with the charge heterogeneity of glycoproteins (22).

Electroelution of unlabeled BPA from 1-cm slices of unstained gels indicated that all BPA was eluted at M_r 25,000–29,000 (Fig. 3*b*), corresponding to the position of the labeled material (Fig. 3*a*). For simplicity, only the molecular weight range(s) of interest are depicted in Fig. 3*b*; however, the entire gel was assayed for bioactivity. Those fractions not shown were totally devoid of BPA. Similar results were obtained when unlabeled gels, composed of 10%, 12.5%, or 15% acrylamide, were electroeluted (0.5- or 1-cm slices) and subjected to bioassay.

Properties of Purified BPA. Column-purified BPA was stable to heat treatment at 100°C for 10 min. Because this result was inconsistent with our earlier published results (7) suggesting that LCM subjected to 56°C for 2 hr lost BPA, we investigated the heat stability of the growth-factor activity more systematically. Samples of unfractionated LCM super-

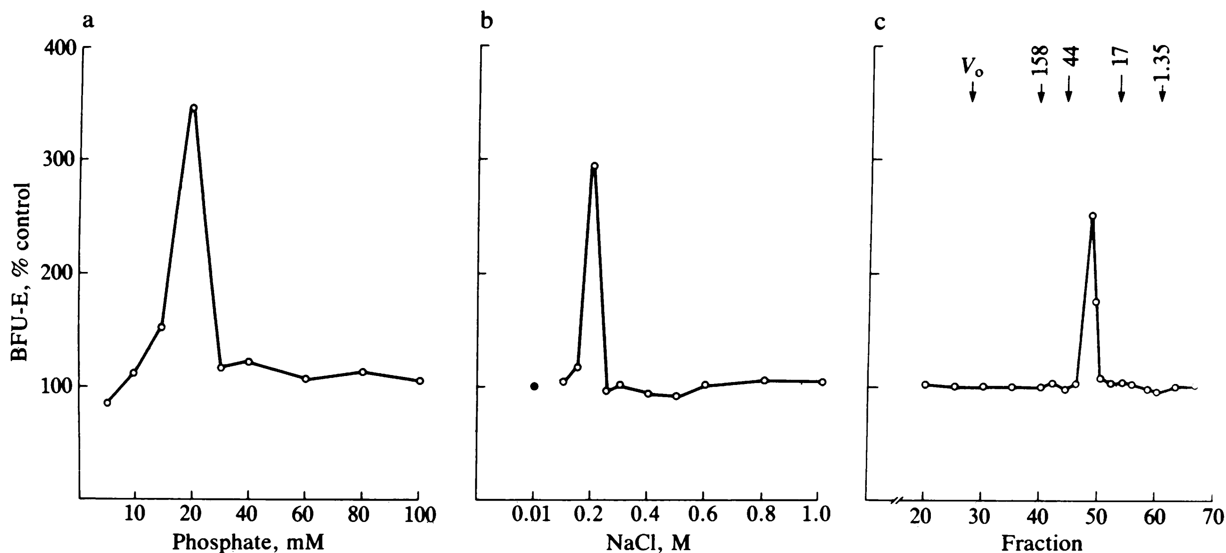

FIG. 2. Chromatographic fractionation of BPA. (*a*) Hydroxylapatite chromatography. Active fractions from Sephacryl S-300 (Fig. 1), concentrated and dialyzed against 5 mM sodium phosphate (pH 8.0), were applied to a 5-ml column of hydroxylapatite and eluted stepwise with 5–100 mM sodium phosphate (pH 8.0). BPA was eluted with 20 mM phosphate. (*b*) Anion-exchange chromatography. Active fraction from *a*, concentrated and dialyzed against 5 mM sodium phosphate (pH 8.0), was applied to a 1-ml column of DE-52. Column was flushed with the same buffer (●), and bound material was eluted stepwise with 0.01–1.0 M NaCl in 5 mM sodium phosphate (pH 8.0). BPA was eluted with 0.2 M NaCl. (*c*) HPLC. Active fraction from *b* was concentrated and dialyzed against PBS (pH 7.0). A 200-μl sample was applied to a Bio-Sil TSK-250 column and eluted with the same buffer. BPA was eluted in a position corresponding to M_r 28,000. Column calibration is shown by arrows.

natant or of partially purified BPA from either LCM supernatant or extracted lymphocyte plasma membranes (all with and without 2 mM dithiothreitol) were heated to either 56°C for 2 hr or 100°C for 10 min, cooled on ice, and tested for BPA. The results in Table 1 indicate that regardless of the presence or absence of dithiothreitol in the sample, the activity of column-purified BPA was stable to treatment at either 56°C or 100°C. In contrast, in the absence of dithiothreitol, LCM lost virtually all activity when heated to either experimental temperature for the times indicated. The findings are consistent with the possibility that heat inactivation of BPA in unfractionated LCM is mediated by contaminating proteases or oxidants in the conditioned medium, and that fractionation on Sephacryl S-300 is sufficient to separate these contaminants from BPA.

Stability of BPA is further demonstrated by the ability to recover biologically active growth factor following NaDod-SO_4/PAGE and by the ability to store growth-factor preparations for periods of up to a year at 4°C or −70°C.

Throughout chromatographic purification, membrane-derived BPA can be adsorbed from solution by anti-lymphocyte membrane IgG. Therefore, although present on the lymphocyte surface in minute quantities, membrane-associated BPA is highly immunogenic. The data in Fig. 4 indicate that BPA from octyl glucoside solubilization or from Sephacryl S-300 or hydroxylapatite fractionation of membranes is completely adsorbed to anti-membrane IgG. Additionally, Sephacryl S-300-fractionated BPA from LCM supernatant is also adsorbed by anti-membrane IgG, indicating that soluble and membrane-derived BPA are immunologically related. In contrast, our previous work indicates that BFU-E-directed growth-promoting activities were not removed from monocyte conditioned medium (23), serum-free bovine aorta endothelial cell conditioned medium, serum-free Mo cell conditioned medium, or purified human CSF even at high antibody concentration, suggesting that these erythroid growth-regulatory factors are immunologically distinct from lymphocyte-derived BPA.

Erythroid Specificity of BPA. Samples of column-purified or gel-eluted BPA were assayed in culture for their ability to stimulate the proliferation of granulocyte/monocyte (GM), megakaryocyte (Meg), or mixed (GEMM) progenitors. In contrast to purified CSF, which stimulated CFU-GM to 866 ± 10% of control values, BPA failed to stimulate CFU-GM proliferation relative to cultures without added growth factor (Table 2). Multiple samples of BPA also failed to stimulate CFU-GEMM proliferation, and BPA did not stimulate CFU-Meg proliferation under conditions where 1 nM megakaryocyte CSF stimulated growth to 280 ± 5% of control values.

Table 1. Temperature stability of BPA

Sample	DTT	BFU-E, % control* Untreated	56°C, 2 hr	100°C, 10 min
LCM(S)	−	253 ± 10	110 ± 8	87 ± 2
	+	247 ± 9	213 ± 8	247 ± 12
S-300				
LCM(S)	−	263 ± 3	260 ± 6	274 ± 4
	+	256 ± 4	254 ± 2	246 ± 2
S-300				
PM	−	230 ± 7	220 ± 10	206 ± 5
	+	230 ± 5	200 ± 6	210 ± 7

Samples of LCM supernatant [LCM(S)], or of Sephacryl S-300-fractionated LCM(S) or extracted lymphocyte plasma membranes (PM), were treated in the presence or absence of 2 mM dithiothreitol (DTT). All samples were then cooled on ice and assayed in serum-free marrow culture.

*Data are for quadruplicate determinations (±SEM). Control cultures (100% growth) contained 10% (vol/vol) NCTC-109 medium in place of growth factor.

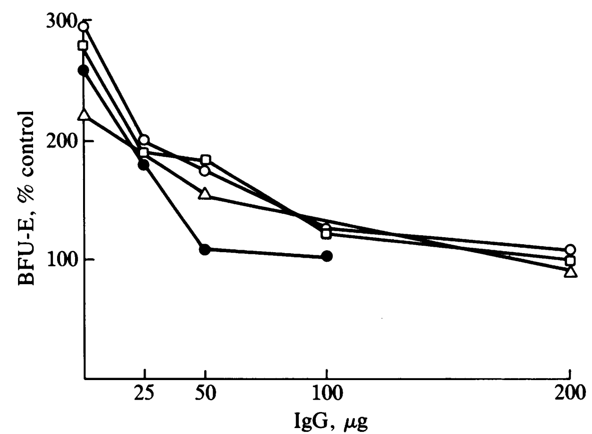

FIG. 4. Immunoadsorption of BPA by anti-membrane IgG. Samples (200 μl) of lymphocyte-derived BPA at various stages of purification were incubated with various amounts of anti-membrane IgG for 16 hr at 4°C prior to immunoprecipitation with excess Pansorbin. Preadsorbed supernatants were then assayed in serum-free marrow culture for residual growth-factor activity. ●, Sephacryl S-300-fractionated LCM supernatant; □, octyl glucoside-solubilized lymphocyte plasma membranes; ○, Sephacryl S-300-fractionated plasma membranes; △, plasma membranes fractionated over S-300, hydroxylapatite, and DE-52.

DISCUSSION

We have chromatographically purified human erythroid BPA from lymphocyte plasma membranes and have shown that this activity copurifies with a protein of M_r 28,000. BPA is exceptionally stable, resisting denaturation even after heat treatment at 100°C for 10 min. This behavior is consistent with published data regarding heat stability of BPA (24) and other glycoprotein growth factors (25–27). Heat stability of BPA has been used as a criterion for separating this activity from the colony-stimulating activity (CSA) with which it copurifies from concanavalin A-stimulated T cells (24).

Our purified BPA appears to be hematopoietic-lineage-specific, failing to enhance the formation of granulocyte/macrophage, megakaryocyte, or mixed colonies. Furthermore, it exhibits no apparent synergy with other growth factors that we have tested. A more exhaustive survey using highly purified and/or recombinant growth factors will be necessary before we can conclude with confidence that BPA is indeed a unique growth factor. However, the data suggest that our growth factor is a reasonable candidate for the

Table 2. Erythroid specificity of BPA

Purification step	LCM fraction	% Control CFU-GM*	CFU-GEMM	CFU-Meg†
S-300	Supernatant	115 ± 6	ND	ND
	Pellet	95 ± 3	100 ± 8	89 ± 10.5
Hydroxylapatite	Supernatant	118 ± 6	115 ± 7.5	ND
	Pellet	94 ± 4	100 ± 1	120 ± 6
PAGE	Supernatant	97 ± 1	108 ± 6	ND
	Pellet	107 ± 2	108 ± 3.5	82 ± 13

BPA from LCM supernatants or extracted vesicle-containing LCM pellets, purified through the steps indicated, was assayed in serum-free marrow culture for their ability to support the proliferation of granulocyte/macrophage (GM), mixed (GEMM), or megakaryocyte (Meg) precursors. Data represent the mean ± SEM for quadruplicate determinations in comparison to control cultures (100% growth) lacking added growth factor(s). ND, not done.

*Value for CSF (from Hyclone; 25 units/ml) was 866 ± 10%.

†Value for megakaryocyte CSF (from R. Hoffmann; 1 nM) was 280 ± 5%.

erythroid BPA that acts on (one of) the earliest committed erythroid progenitor and is distinct from the factor(s) known to act on multi- or pluripotential stem cells. The bone marrow target-cell population used in these studies is heterogeneous and undoubtedly contains a spectrum of erythroid progenitors ranging from early (BFU-E) to late (CFU-E). It is likely that erythroid progenitors bear different growth-factor receptors and, hence, have a differential response to BPA. Experiments performed with highly enriched bone marrow stem-cell populations should help to clarify this issue.

It was important to distinguish our growth factor from erythroid potentiating activity (EPA), previously purified by Westbrook *et al.* (28). Both BPA and EPA have M_r of 28,000 by gel filtration, are stable to heat treatment at 100°C, and stimulate proliferation of BFU-E progenitors. However, we have observed that EPA is not absorbed from solution by antibodies (data not shown) that completely remove BPA from all lymphocyte-derived sources (see Fig. 4). Additionally, we have found that EPA is inactivated from crude or partially purified Mo cell conditioned medium by the addition of 2 mM dithiothreitol to the growth factor at 25°, 56°, or 100°C, suggesting that disulfide bonds are required for maintenance of activity. This is in contrast to BPA, which appears unaffected by the presence of 2 mM dithiothreitol. Most important, whereas EPA is produced by a T-lymphoblast cell line (26), our preliminary evidence indicates that when normal unstimulated peripheral blood lymphocytes are fractionated on nylon wool columns, BPA is found exclusively associated with the non-T-cell (i.e., nylon wool-adherent) fraction (29).

Although it is strongly suspected that cellular interactions are critical for hematopoietic cell proliferation, little is known about precisely how such communication occurs. Our finding that cells exfoliate surface membrane components that express BPA (7) raises the possibility that proximity to, but not necessarily contact with, target progenitor cells may be sufficient to exchange growth-regulatory signals. Here, we report that the active factor expressed by plasma membranes is biochemically and immunologically similar to that associated with shed membrane-derived vesicles as well as to that recovered in vesicle-free LCM supernatants. This raises intriguing questions not only regarding extracellular trafficking of shed surface components but also regarding the origin of soluble BPA. The role of the plasma membranes either directly or as a potential generator of soluble messengers in cell–cell communication and growth control remains an area of intense interest.

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