FACTOR-DEPENDENT IN VITRO GROWTH OF HUMAN NORMAL BONE MARROW-DERIVED BASOPHIL-LIKE CELLS*

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Basophils and mast cells share similar functional roles in immunological reactions. Both release various chemical mediators including histamine after cross-linkage of IgE or other homocytotropic antibodies on the cell surface. Furthermore, the density of basophils and mast cells in blood and tissue seems to be influenced by immunological reactions. In experimental animals, several reports have found that in vitro production of basophils (1, 2) or mast cells (3-9) from bone marrow or lymphoid tissues is dependent on some factor(s) produced by lectin- or antigen-stimulated lymphocytes.

In man, it has been reported that increased numbers of basophils are observed after tissue transplantation or in other immunological disorders such as atopy (10). So far, there have been only a few reports on the in vitro production of human basophils or mast cells, except for the spontaneous production of basophils in liquid cultures of chronic myelogeneous leukemia bone marrow (11-15). In cultures of normal bone marrow cells, two reports (16, 17) have described the occurrence of rare small clusters of basophils or mast cells in semi-solid agar cultures.

Recently, Rasin et al. (18) have reported a spontaneous and selective differentiation of basophils from human fetal liver cells. To our knowledge, no report has yet clearly established the growth or differentiation of human basophils or mast cells in cultures of normal bone marrow cells. In this paper, we observed the development of cells containing metachromatic granules in normal bone marrow cultures supplemented by supernatants from lectin-stimulated peripheral blood mononuclear cells. The cells developing in such cultures resembled human basophils rather than tissue mast cells and their development is dependent on a factor(s) present in the supernatants from lectin-stimulated peripheral blood mononuclear cells. Furthermore, we describe some biological and biochemical characteristics of the basophil-like cell-promoting activity in culture supernatants.

Materials and Methods

Lectins and Other Chemicals. Lectins were purchased as follows: concanavalin A (Con A) (Calbiochem-Behring Corp., La Jolla, CA), phytohemagglutinin (PHA) (HA 16; Well-

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[1] *Abbreviations used in this paper:* BaPA, basophil-like cell-promoting activity; Con A, concanavalin A; CM, conditioned medium; FCS, fetal calf serum; IL-2, interleukin 2; MSC, metachromatically staining cells; PBMNC, peripheral blood mononuclear cell; PHA, phytohemagglutinin; PWM, pokeweed mitogen.

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come Reagent Ltd., Beckenham, Kent, England), pokeweed mitogen (PWM) (Gibco Laboratories, Grand Island, NY), phorbol myristate acetate and 2-mercaptoethanol (Sigma Chemical Co., St. Louis, MO).

Preparation of Conditioned Medium (CM). Peripheral blood mononuclear cells (PBMNC) were isolated by Ficoll-Hypaque density (1.077 g/ml) sedimentation from cell residues after thrombophoresis. Cultures were set up at 5×10^6 cells/ml in RPMI 1640 medium containing 1% fetal calf serum (FCS) (Gibco AG, Basel, Switzerland) and 10 μg/ml Con A. Supernatants were harvested usually after 2 d of culture at 37°C, 5% CO_2. Supernatants were also prepared by stimulating with 10 μg/ml PHA or with a 1:100 dilution of PWM for 3 or 4 d, respectively. Supernatants from the HSB-2 T cell line were prepared by stimulating 1×10^6 cells/ml in RPMI 1640 containing 1% FCS with 10 μg/ml Con A and 5 μg/ml phorbol myristate acetate as described elsewhere (19). Culture supernatants from the Mo cell line were kindly donated by Dr. Golde (20). CM were sterilized with a Millipore filter (0.45 μm; Millipore Corp., Bedford, MA) and stored at −20°C. Pooled Con A-stimulated PBMNC-conditioned medium (Con A CM) was heat treated at 65°C for 30 min if not otherwise stated.

Bone Marrow Culture. Normal bone marrow mononuclear cells were isolated by Ficoll-Hypaque density sedimentation (1.077 g/ml, 400 *g*) from bone marrow aspirates from normal donors. In some experiments, the bone marrow mononuclear cells were further fractionated by low density Ficoll-Hypaque sedimentation (1.065 g/ml, 400 *g*). This low density Ficoll-Hypaque was made by diluting ordinary Ficoll-Hypaque with Hanks' balanced salt solution to a density of 1.065 g/ml. Cultures were set up at 3.3×10^5 cells/ml in α-modification of McCoy's 5A medium supplemented with 20% FCS, 1.5×10^{-5} M 2-mercaptoethanol, and 33% CM, unless the percentage of CM is otherwise indicated, in upright 25-cm^2 tissue culture flasks (Falcon; Beckton, Dickinson France, Grenoble, France) or 24-well tissue culture plates (Costar, Cambridge, MA). Cells were cultured at 37°C, 5% CO_2 and the cultures were split in half once or twice a week.

Fractionation of PBMNC. T cell-depleted PBMNC were prepared by E-rosette formation technique (21). Briefly, 10% (vol/vol) sheep erythrocytes were treated with neuraminidase (*Arthrobacter meafaciens*; Calbiochem-Behring Corp.) at a concentration of 0.5 U/ml at 37°C for 1 h and washed three times with RPMI 1640. 2 vol of 1% (vol/vol) neuraminidase-treated erythrocytes were mixed with 1 vol of PBMNC (2×10^7 cells/ml) and 1 vol of FCS. This mixture was centrifuged at 400 *g* for 2 min and then allowed to stand at room temperature for 15 min. The pellet was gently resuspended, centrifuged again, and allowed to stand at room temperature for 60 min. Then the cell pellet was gently resuspended and the suspension was applied on a Ficoll-Hypaque gradient and centrifuged at 400 *g* for 25 min. Cells collected from the interphase were regarded as T cell-depleted PBMNC. B cells were depleted with anti-human immunoglobulin-coated plastic dishes (22). Briefly, anti-human gamma globulin-coated petri dishes were prepared by incubating 90-mm petri dishes with 10 ml 0.05 M Tris buffer, pH 9.5, containing 50 μg/ml purified rabbit anti-human gamma globulin antibody and 2 mg/ml normal rabbit immunoglobulin at room temperature for 90 min, followed by two washings with phosphate-buffered saline, pH 7.6, containing 1% FCS. 30×10^6 PBMNC in 3 ml RPMI 1640 that contained 5% FCS were incubated at 4°C in an anti-human gamma globulin antibody-coated petri dish. After 40 min of incubation the cells were well mixed and incubated for an additional 30 min at 4°C. After gentle mixing, the floating cells were decanted for experimental use as B cell-depleted PBMNC. Adherent cells were removed by incubating 50×10^6 PBMNC in 5 ml RPMI 1640 containing 20% FCS in a 90-mm petri dish at 37°C for 90 min. Nonadherent cells were used as adherent cell-depleted PBMNC. Cells fractionated with the three methods described above were cultured at 1×10^6 cells/ml in RPMI 1640 containing 1% FCS and 10 μg/ml Con A. Unfractionated cells were also cultured at 1×10^6 cells/ml with or without Con A. Supernatants harvested after 2 d of culture were used for the analysis of basophil-like cell-promoting activity.

Counting of Metachromatically Staining Cells (MSC). The number of viable cells and the percentage of cells containing metachromatic granules were counted in parallel to assess the absolute number of MSC. Viable cells were counted using 0.1% trypan blue. For

assessment of MSC, 0.2–0.5 ml of cell suspensions were centrifuged in a cytocentrifuge (Shandon Scientific Co., London, England). Then the samples were dried and stained for 15 min with 0.1% toluidine blue in acetate buffer, pH 4.0, containing 50% ethanol (23).

Morphological Study. Smears were stained with toluidine blue, May-Grünwald-Giemsa, or 0.05% Alcian blue in sodium citrate buffer, pH 2.5, containing 50% ethanol. For peroxidase staining, smears previously stained with toluidine blue were further stained for 15 min with 0.5 mg/ml 4-chloro-1-naphtol (E. Merck, Darmstadt, Federal Republic of Germany [FRG]) or 0.5 mg/ml 3-3 diaminobenzidine (Fluka AG, Buchs, Switzerland) in Tris-buffered saline, pH 7.4, containing 0.01% (vol/vol) hydrogen peroxide. Nonspecific esterase and chloroacetate esterase staining were performed by the method described by Yam et al. (24).

Histamine Assay. Fluorometric measurements of histamine were performed using a Technicon Autoanalyser (Technicon Instruments Corp., Tarrytown, NY) as described by Siraganian (25). Histamine content in cell pellets were measured as follows. Cultured cell suspensions were centrifuged at 4°C, 200 *g*, for 10 min and washed twice with 20 mM Hepes-buffered balanced salt solution (125 mM NaCl, 5 mM KCl, 20 mM Hepes, 0.5 mM glucose, pH 7.3). Cell pellets were disintegrated with 8% perchloric acid and served for measurement. To prove the specificity of histamine, several cell lysate supernatants were incubated with 0.5 U/ml diamine oxidase (Sigma Chemical Co.) for 60 min at pH 7.2, resulting in >95% reduction of fluorometrically detectable histamine. Histamine levels in culture supernatants were measured as follows. 0.5 ml culture supernatant was mixed with 1 ml 8% perchloric acid and then centrifuged at 600 *g* to remove the precipitate. Since cell-free complete culture medium recorded as high as ~15 ng/ml of histamine, net histamine levels in the culture supernatants were calculated by subtracting this background from the actual measurements.

Histamine Release. Histamine release with synthetic histamine releasers was performed as follows. Cultured cells were washed twice with Hepes-buffered balanced salt solution, and resuspended in the same buffer supplemented with 0.6 mM $CaCl_2$ and 1 mM $MgCl_2$. 1-ml samples containing $1–2 \times 10^5$ cultured cells/ml were challenged by incubation for 40 min at 37°C with different doses of calcium ionophore (A23187) or compound 48/80 (Sigma Chemical Co.). After the challenge, samples were centrifuged at 200 *g* for 10 min at 4°C and the supernatants were removed, acidified with perchloric acid to a final concentration of 4%, and stored at 4°C until assay. To test histamine release upon challenge with anti-IgE, washed cultured cells were resuspended in Hepes-buffered balanced salt solution without calcium and magnesium, incubated with 100 μg/ml purified myeloma IgE at 37°C for 120 min and washed three times with the above buffered solution. Cells were resuspended in Hepes-buffered balanced salt solution supplemented with calcium and magnesium, and challenged by incubation at 37°C for 60 min with different dilutions of rabbit anti-IgE (Behringwerke AG, Marburg, FRG). The supernatants were removed, acidified, and stored as described above. Spontaneous histamine release was estimated without adding any releaser. Total histamine was measured by disintegrating the cells with 8% perchloric acid. Released histamine was expressed as a percentage of total histamine corrected for spontaneous release.

Gel Filtration of Con A CM. Fresh Con A CM was concentrated 50-fold by ultrafiltration (UM 10 membrane; Amicon Corp., Danvers, MA); 5 ml of this concentrated Con A CM was applied to a Sephacryl S-200 column (2.6 × 90 cm). The column was equilibrated with 0.05 M Tris-HCl buffer, pH 7.8, containing 0.1 M NaCl, 0.01% polyethyleneglycol 6000 (Fluka AG), 20 μg/ml gentamycin (Schering Co., Kenilworth, NJ). The flow rate was maintained at 30 ml/h and fractions of 5 ml were collected. Optical density was monitored at 206 nm. For molecular weight determination, the column was calibrated using bovine serum albumin, ovalbumin, chymotrypsinogen, and ribonuclease (Pharmacia, Uppsala, Sweden). For biological assay, each fraction was diluted 10 times with α-modification of McCoy's 5A medium containing 1% FCS and sterilized with a 0.45 μm filter (Millipore Corp.). Basophil-like cell-promoting activity was assessed by culturing 3.3×10^5 cells/ml normal bone marrow mononuclear cells with 33% diluted fractions. After 14 d, the number of metachromatically staining cells was counted and histamine content

in cell pellets was determined. Interleukin 2 (IL-2) activity was measured using an IL-2-dependent mouse T cell line as described in detail elsewhere (26).

Results

Development of Normal Bone Marrow-derived Metachromatically Staining Cells (MSC). Bone marrow cells from 15 normal donors were cultured with Con A CM. The number of cells staining metachromatically with toluidine blue increased during 2–3 wk of culture in all cases. Fig. 1 shows a typical normal bone marrow culture. The number of MSC increased during the first 2 wk of culture ~100-fold, remained constant for another 2–3 wk, and then decreased. The percentage of MSC in culture increased up to 40–60%, usually after 2 wk of culture, and then decreased rather rapidly due to the increase of other cell types, including macrophages, lymphocytes, or neutrophils (Fig. 1, *top*). Total histamine content in cell pellets and total histamine content in culture supernatants paralleled the increase in the number of MSC (Fig. 1, *bottom*). The estimated histamine content per cell was 0.5–2 pg/cell; it was usually low in the early periods of culture and higher after 2 wk. This correlation between the number of MSC and cell-associated histamine content suggested that the cells with metachromatic granules might contain histamine.

Dose-dependent Development of Basophil-like Cell. Bone marrow cells were cultured with different doses of Con A CM to study whether or not the development

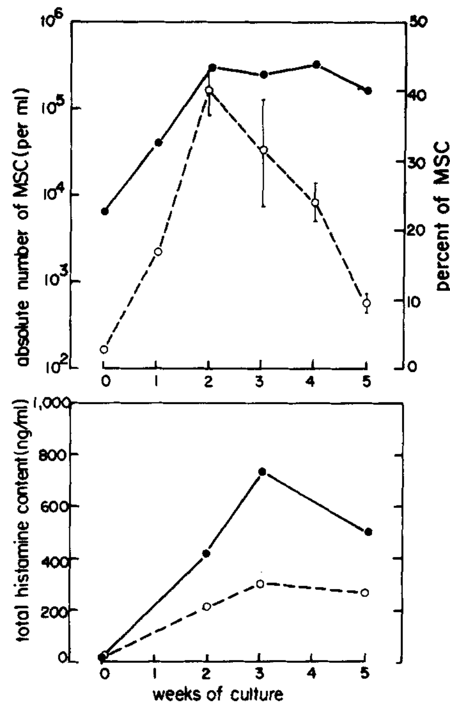

FIGURE 1. Development of MSC and histamine determinations in normal bone marrow cell cultures in the presence of 33% Con A CM. (*Top*) (●) Total number of MSC, (○) percent of MSC in cultures. (*Bottom*) (●) Total histamine content of the culture supernatants, (○) total histamine content in cell pellets.

of basophil-like cells was dependent on Con A CM. Fig. 2 demonstrates that the development of basophil-like cells was positively related to the dose of Con A CM added to the cultures. Furthermore, as shown in Fig. 3, the number of basophil-like cells that developed during culture was linearly related to the total number of bone marrow cells set up in culture.

Development of MSC Is Dependent on Cell Proliferation As Well As Differentiation. Granulomonocytic colony-forming cells are enriched by density gradient sedimentation in low density fractions (27–30). Therefore, we isolated a low density fraction of normal bone marrow by further Ficoll-Hypaque (1.065 g/ml) density sedimentation after the usual Ficoll-Hypaque (1.077 g/ml) density sedimentation. These low density bone marrow cells recovered from the inter-

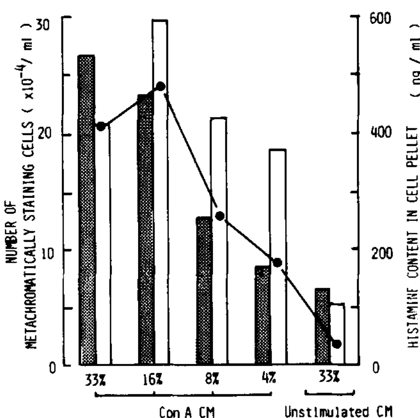

FIGURE 2. Dose-dependent development of MSC. 3.3×10^5 normal bone marrow cells were cultured with different doses of Con A CM. Numbers of MSC after 2 wk of culture (hatched columns) and 3 wk of culture (open columns) as well as histamine content in cell pellets after 3 wk of culture (●) are shown.

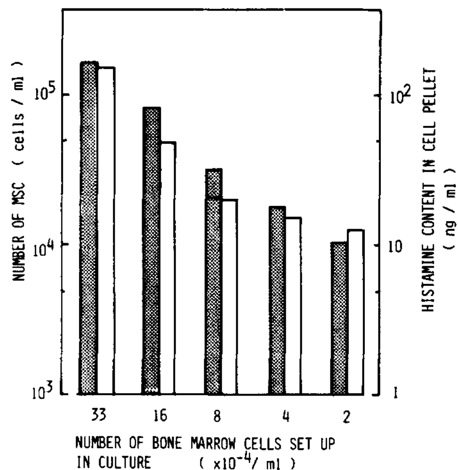

FIGURE 3. Development of MSC according to the number of bone marrow cells set up in culture. Different numbers of bone marrow cells were cultured with 33% Con A CM. The number of MSC (hatched columns) and histamine content in cell pellets (open column) after 2 wk of culture are shown.

phase were cultured with Con A CM. Although there were no MSC at the onset of the culture, their development was observed (Fig. 4). This suggested that MSC were differentiating from precursor cells not detectable with toluidine blue staining. To study whether the development of MSC requires cell proliferation or merely occurs after proliferation-independent differentiation from precursor cells, cultures were irradiated or treated with hydroxyurea. Fig. 5 demonstrates that the blocking of cell proliferation with either treatment inhibited the initial as well as further development of MSC. These data suggested that the development of MSC was dependent on cell proliferation.

Morphology of MSC. MSC contained round to lobulated nuclei; the cytoplasmic granules stained metachromatically with toluidine blue, light bluish green with Alcian blue, and azurophilic with May-Grünwald-Giemsa. The size of metachromatic granules as well as their density varied within individual cells. The granules showed some tendency to be fewer and finer in MSC with round nuclei, and

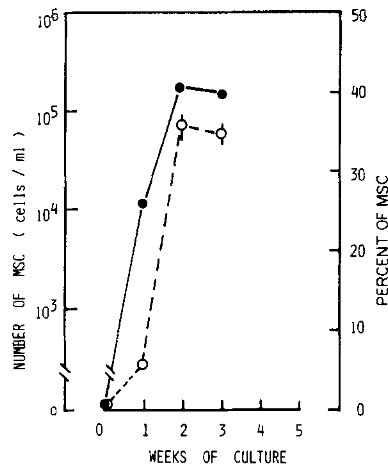

FIGURE 4. Development of MSC from low density fractions of normal bone marrow cells lacking detectable MSC. Low density fractions (1.065 g/ml) of normal bone marrow cells were cultured with 33% Con A CM. (○) Means ± SD percent of MSC in the total cultured cells (●) number of MSC per ml.

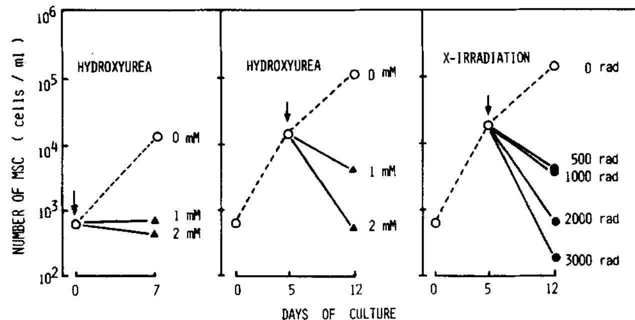

FIGURE 5. Cell proliferation-dependent development of MSC. 3.3×10^5/ml normal bone marrow cells were cultured with 33% Con A CM with or without treatment with hydroxyurea or X irradiation on the day of culture indicated by arrows.

denser and larger in MSC with lobulated nuclei. Some cells with vacuoles or in mitosis were observed. ~20% of the MSC stained positive for peroxidase. No cells except large macrophages stained positive for nonspecific esterase. 21% of the total cells stained positive for naphtol chloroacetate esterase and 40% stained with toluidine blue (Fig. 6).

Histamine Release. As shown in Fig. 7, histamine release was induced with optimal concentrations of anti-IgE, using cultured cells passively sensitized with purified myeloma IgE, but not with unsensitized cells. This result, together with the parallel increase of MSC and histamine, suggested that the MSC have receptors for IgE. Furthermore, histamine release was also induced with noncytotoxic doses of calcium ionophore A23187, but not with compound 48/80, whereas both agents induced histamine release from rat peritoneal mast cells. Thus, the morphology and the histamine release reactions of MSC were similar to those of human peripheral blood basophils.

Basophil-like Cell-promoting Activity (BaPA). In addition to Con A, PHA and PWM were used to prepare CM. PHA CM harvested after 3 d of culture and PWM CM harvested after 4 d of culture promoted MSC basophil-like cells as well as Con A CM (data not shown). These results suggested that it was not a specific lectin that promoted the MSC. However, it remained to be elucidated whether or not the development of basophil-like cells might be directly dependent on the presence of lectins. Since the final concentration of Con A remaining in 33% Con A CM is not precisely known, different concentrations of Con A were added to normal bone marrow cultures, having assumed that the concentration of Con A is 3.3 μg/ml at the maximum. Furthermore, α-methylmannoside, a competitive inhibitor of Con A (31), was added to the normal bone marrow culture containing Con A CM. As shown in Table I, 50 mM α-methylmannoside did not significantly inhibit the development of basophil-like cells. On the other hand, Con A alone had far less effect on the development of basophil-like cells. These data suggested that the promotion of basophil-like cells was not directly dependent on a lectin but rather on a factor(s) contained in the conditioned medium. This factor(s) will be called basophil-like cell promoting activity (BaPA).

T Cell-dependent Production of BaPA. PBMNC were fractionated and then stimulated with Con A to investigate the cellular source producing the BaPA. Table II demonstrates that CM produced by Con A-stimulated and T cell-depleted PBMNC promoted significantly less basophil-like cells than CM obtained from Con A-stimulated unfractionated PBMNC. In contrast, CM obtained from Con A-stimulated B cell-depleted or adherent cell-depleted PBMNC promoted almost the same numbers of basophil-like cells as did CM obtained from Con A-stimulated unfractionated PBMNC. The number of basophil-like cells induced by CM from unstimulated unfractionated PBMNC was far less than that induced by CM from Con A-stimulated unfractionated PBMNC and almost the same as that without CM. These results indicate that the production of BaPA is dependent on the presence of activated T cells.

BaPA Is Biologically Distinct from IL-2. Con A CM contained the T cell growth factor, IL-2 which is known to be produced also by activated T cells. Therefore, the possible relationship between BaPA and IL-2 activity was investigated. According to the report by Schrader et al. (5), CM from Con A-stimulated mouse

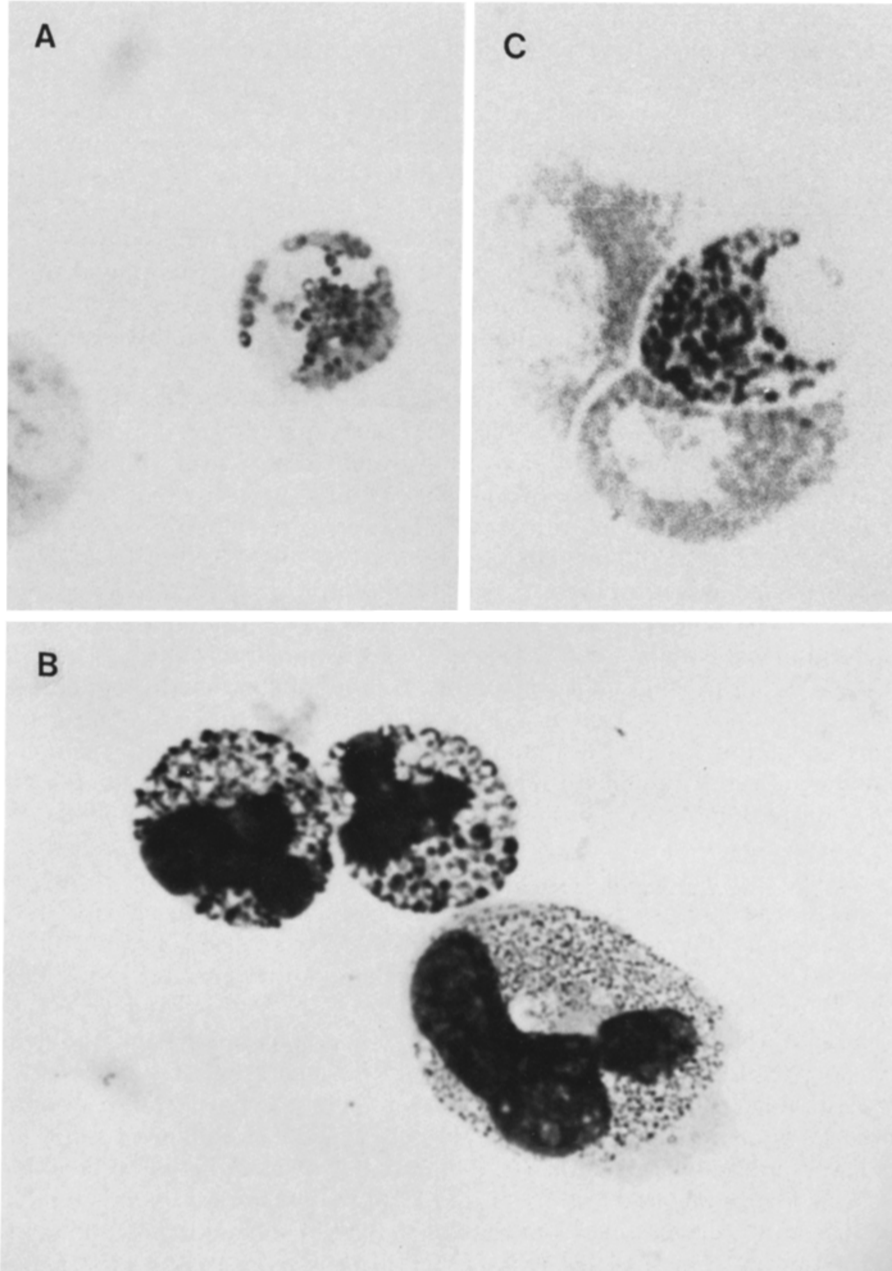

FIGURE 6. Morphology of cultured basophil-like cells (day 14 of culture; × 520). (A) Toluidine blue staining of MSC. (*B*) Staining with May-Grünwald-Giemsa, showing cells with azurophilic granules and lobulated nuclei. (*C*) Double staining for metachromasia (toluidine blue) and for peroxidase. Greyish granules are peroxidase positive. A basophil-like cell (toluidine blue-positive dark granules) is shown with greyish peroxidase-positive granules.

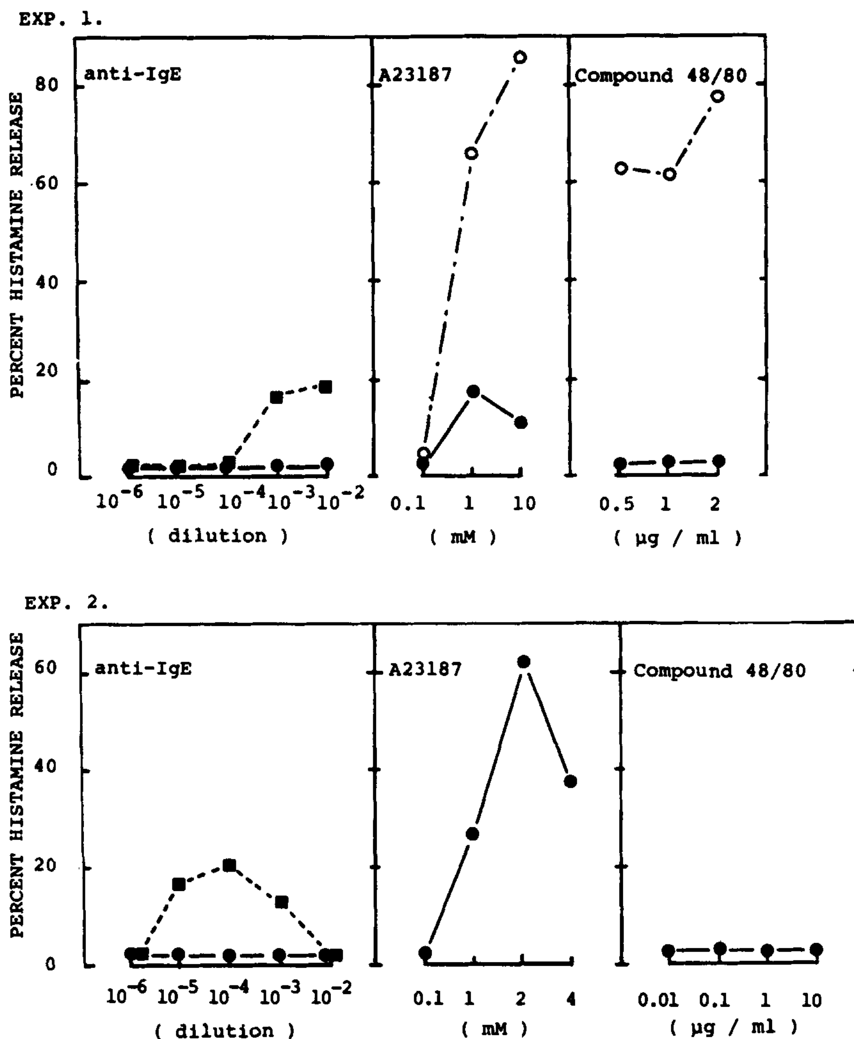

FIGURE 7. Histamine release from cultured normal bone marrow cells after challenge with anti-IgE, calcium ionophore A23187, or compound 48/80. Normal bone marrow cells cultured for 15 d (experiment 1) or 28 d (experiment 2) were used for assay. Histamine release from cultured bone marrow cells (●), from cultured bone marrow cells passively sensitized with purified myeloma IgE (■), and from rat peritoneal mast cells (○) are shown.

spleen cells had more mast cell-like cell growing activity when heat-treated at 65°C for 30 min than the original CM. Therefore, our Con A CM was also heat-treated at 65°C for 30 min. Although this procedure abrogated detectable IL-2 activity, the promotion of basophil-like cells was not inhibited. Furthermore, supernatants from the HSB-2 T cell line, containing high IL-2 activity (19), did not promote basophil-like cells, although they supported the growth of T cells in five different normal bone marrow cultures (data not shown). Thus, these results suggested that BaPA is biologically distinct from the IL-2 activity.

Biochemical Characteristics of BaPA. BaPA could not be dialyzed; it was stable

TABLE I
Effect of Con A on Normal Bone Marrow Cultures

Stimulation	Results of treatment after:*					
	12 d			14 d		
	MSC‡	Histamine§	Total cells¶	MSC‡	Histamine§	Total cells∥
	× 10^{-4}/ml	ng/ml	× 10^{-4}/ml	× 10^{-4}/ml	ng/ml	× 10^{-4}/ml
33% Con A CM	18.2	84	57	21.6	234	112
33% Con A CM + 50 mM α-MM¶	—	—	—	21.2	163	96
33% Con A CM + 25 mM α-MM	—	—	—	29.2	243	103
5 μg/ml Con A	0.9	10	35	—	—	—
2 μg/ml Con A	—	—	—	5.2	33	29
1 μg/ml Con A	3.8	22	29	2.2	15	28
0.5 μg/ml Con A	—	—	—	1.4	5	27
0.25 μg/ml Con A	—	—	—	1.5	8	33
33% unstimulated CM	1.4	13	12	1.8	23	36
Medium control	—	—	—	1.8	18	44

* 3.3 × 10^5/ml normal bone marrow cells were cultured with listed stimulants.
‡ Total number of MSC in culture.
§ Histamine content in cell pellets.
∥ Numbers of total cells in the culture.
¶ α-methylmannoside.

TABLE II
T Cell-dependent Production of BaPA

Source of Con A CM	MSC*		Histamine content in cell pellets‡	
	Percent control	*t* test	Percent control	*t* test
Unfractionated PBMNC	100.0		100.0	
T cell-depleted PBMNC	45.7 ± 17.7	$P < 0.05$	57.3 ± 14.9	$P < 0.05$
B cell-depleted PBMNC	98.7 ± 12.1	NS‡	106.3 ± 9.0	NS
Nonadherent PBMNC	81.7 ± 23.2	NS	90.0 ± 8.7	NS
Unstimulated PBMNC	7.3 ± 0.8	$P < 0.001$	11.0 ± 1.9	$P < 0.001$

* 3.3 × 10^5/ml normal bone marrow cells from three different donors were cultured with 33% CM for 12, 14, or 17 d, respectively. Data are given as mean ± SE percent of the control culture with unfractionated Con A CM (100% MSC = 16.5 × 10^4 cells/ml; 100% histamine = 142 ng/ml).
‡ Not significant.

at 4°C for >4 mo and at −20°C for >12 mo. As described above, it was also stable at 65°C for 30 min. For further biochemical analysis, Con A CM was concentrated 50 times and fractionated by gel filtration using a Sephacryl S-200 column. Because there was no BaPA in the CM fractions below 80 and beyond 120 in a preliminary experiment using chronic myelogeneous leukemia bone marrow cells, only fractions between 70 and 130 were tested. As shown in Fig. 8, the highest peaks in the number of MSC and in the cell-associated histamine content were both observed in the fractions corresponding to a globular protein of mol wt 25,000–40,000. Fig. 8 also demonstrates that the IL-2 activity is biochemically separable from BaPA. The molecular weight of IL-2 is in agreement with earlier reports (32–34).

Discussion

Human mature basophils have lobulated nuclei, relatively larger metachromatic granules, and a relatively low content (1–2 pg/cell) of histamine (35),

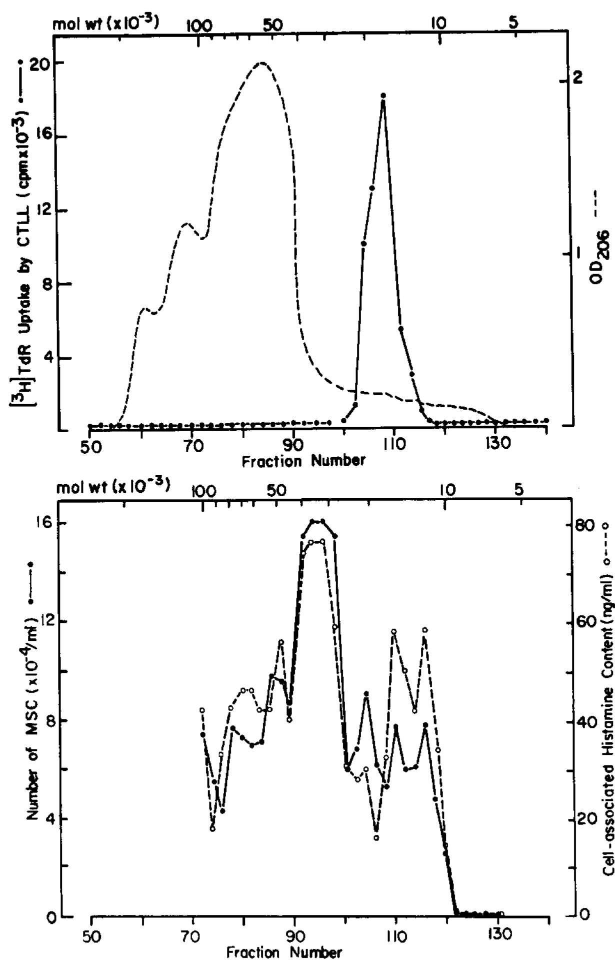

FIGURE 8. Gel filtration of Con A CM on a Sephacryl S-200 column. 50-fold concentrated Con A CM was applied on a Sephacryl S-200 column, and optic density at 206 nm (---) was monitored. Each column fraction was 10 times diluted with culture medium and filter sterilized. 3.3×10^5/ml normal bone marrow cells were cultured with 33% of the diluted fractions. The number of MSC (●, *bottom*) and histamine content in cell pellets (○, *bottom*) after 14 d of culture are shown. IL-2 activity (●, *top*) was measured by [^{3}H]thymidine incorporation by murine cytotoxic T lymphocytes.

which is released upon challenge with calcium ionophore A23187 but not with compound 48/80 (36, 37). Immature basophils that are recognized after an early myelocyte stage have round nuclei and fewer granules (38). On the other hand, although rat peritoneal mast cells are often considered as typical mast cells, it is being increasingly recognized that mast cells are morphologically and functionally heterogeneous among different species as well as among different organs and tissues within the same animal (39). Mast cells are now roughly classified into two types, tissue and mucosal. Tissue mast cells, as exemplified by rat peritoneal mast cells, have round or ovoid nuclei, the contour of which is obscured by abundant granules; they have a relative high content (10–40 pg/cell) of histamine

(40) that is released both by calcium ionophore and compound 48/80 (37). On the other hand, mucosal mast cells as exemplified by rat intestinal mast cells, have round or lobulated nuclei, and an intermediate content (1–5 pg/cell) of histamine that is released with calcium ionophore but not with compound 48/80, similar to basophils (41–43). Cytochemically, basophils are peroxidase positive, while tissue mast cells are negative. Tissue mast cells and immature basophils are chloroacetate esterase positive, while mature basophils are negative (38). According to these criteria, the human MSC grown in culture from human bone marrow are clearly different from tissue mast cells since they possess lobulated nuclei, do not respond to compound 48/80, and show positive peroxidase activity. Therefore, it is likely that the MSC obtained by us in culture are basophils and/or mucosal mast cells. So far, all the characteristics determined in bone marrow-derived MSC are compatible with those of human basophils. Human mucosal mast cells have not yet been well studied except for some functional aspects of dispersed lung mast cells (44, 45) that also appear similar to those of basophils. Because of these functional and morphological similarities between mucosal mast cells and basophils, our bone marrow-derived MSC in culture are tentatively called "basophil-like cells". Electron microscopic examinations will be undertaken for further characterization of these MSC.

In any case, our results have clearly shown that at least one and possibly several factors in the supernatants from lectin-stimulated PBMNC promotes the in vitro production of basophil-like cells derived from normal human bone marrow. The production of this basophil-like cell promoting factor (BaPA) was dependent on the presence of activated T cells. Furthermore, a supernatant from the Mo cell line, derived from a human T cell leukemia, also enhanced the development of basophil-like cells (unpublished data, collaboration with Dr. Golde). Thus, it is likely that activated T cells are the cellular source of BaPA. These results also suggested that the modest development of basophil-like cells induced by Con A alone might have been induced by a factor(s) produced in situ by Con A-stimulated T cells present in the bone marrow cultures. The gel filtration elution profile of Con A CM suggested that the fractions corresponding to a globular protein of mol wt 25,000–40,000 contained the activity-promoting basophil-like cells from normal bone marrow. However, it is at present not known whether BaPA is a single factor or a mixture of factors. IL-2 was biologically and biochemically distinct from BaPA. On the other hand, BaPA was similar to granulocyte-macrophage colony-stimulating factors in its heat stability and its apparent molecular weight (20, 46). Therefore, it will be of interest to further study whether BaPA is different from other myeloid colony-stimulating factors. Both the high percentage of MSC on day 14 of culture and the absolute increase in the number of MSC in culture suggest that BaPA preferentially promotes basophil-like cells.

If basophil-like cells are actually related to normal basophils, it might be possible that in vivo basophilopoiesis is modulated by activated T cells similarly to the regulation of other hematopoietic cells (47). Finally it can be stressed that our system enables a quantitative analysis of basophil-like cell production. This system could eventually be useful for studies on the mechanism of basophilia in chronic myelogeneous leukemia, atopy, or tissue transplantation.

Summary

A factor(s) present in supernatants from lectin-stimulated peripheral blood mononuclear cells promoted the production of basophil-like cells in liquid cultures of normal human bone marrow cells. The cultured basophil-like cells had lobulated or round nuclei, and the cytoplasmic granules stained metachromatically with toluidine blue and azurophilic with Giemsa. 20% of the metachromatically staining cells were peroxidase positive but not positive for nonspecific esterase. The histamine content was 0.5–2 pg/cell. The basophil-like cells released histamine upon challenge with calcium ionophore A23187 but not with compound 48/80. They also released histamine with anti-IgE when passively sensitized with human myeloma IgE. The development of basophil-like cells was promoted in a dose-dependent fashion by a factor(s) in the conditioned medium. Blocking of cell proliferation with hydroxyurea or X irradiation inhibited the development of basophil-like cells. The production of the factor was dependent on the presence of T cells. The factor was different from interleukin 2 and its molecular weight was estimated to be 25,000–40,000 by gel filtration on a Sephacryl S-200 column. Thus, human basophil-like cells derived from normal bone marrow cells can grow and differentiate in vitro under the regulation of T cells.

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