

***In vitro* expansion of human basophils by interleukin-3 from granulocyte colony-stimulating factor-mobilized peripheral blood stem cells**

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Summary

Background A number of studies support the belief that human basophils play an important role in allergic inflammation. The exact mechanism of basophil activation at the site of allergic inflammation, however, has not been well understood, mainly due to their low number in blood and difficulty in obtaining a sufficient number of highly purified basophils for investigation.

Objective The purpose of this study is to expand human basophils *in vitro* with high yield and purity by culturing peripheral blood stem cells (PBSCs).

Methods We collected PBSC-rich mononuclear cells containing CD34⁺ cells (0.15–4.9%) by leukapheresis from patients with malignant lymphoma and lung cancer during haematopoietic recovery after chemotherapy plus granulocyte colony-stimulating factor-induced mobilization. PBSC-rich mononuclear cells were cultured in the presence of IL-3.

Results When PBSC-rich mononuclear cells containing more than 1% of CD34⁺ cells were cultured, 20.0–83.3% of the cells, mostly with a yield of >10%, were metachromatic cells after 3 weeks of culture. These cells resembled mature peripheral blood basophils morphologically when examined by light and electron microscopy. Flow cytometric analysis showed that they expressed both FcεRI and FcγRII. FcεRI cross-linking resulted in intracellular calcium mobilization, histamine release and synthesis of cysteinyl leukotrienes. The intracellular histamine content and the release of these chemical mediators triggered by anti-IgE antibodies were comparable to those of peripheral blood basophils.

Conclusion These findings suggest that PBSC-derived basophils expanded *in vitro* are morphologically and functionally mature and will be a useful tool for the analysis of basophil functions.

Keywords: Fc receptor, human basophil, interleukin-3, peripheral blood stem cell

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Introduction

Human basophils have been implicated through FcεRI-mediated reactions in allergic diseases such as bronchial asthma. Cross-linking of FcεRI on basophils as well as mast cells evokes histamine release and synthesis of leukotrienes. Recent studies have also shown that human basophils are capable of producing cytokines such as IL-4 and IL-13 [1, 2]. Thus, basophils, in addition to mast cells and T cells, are recognized to be operating in the process of allergic inflammation. To clarify the mechanism of basophil activation at the site of allergic inflammation, many strategies have been introduced to purify human blood basophils, which are the least abundant among circulating granulocytes. These include density gradient centrifugation, counterflow elutriation,

flow cytometry and immunological selection [3–7]. However, yields with these methods are too low for biochemical and functional assays. Cells from a human basophilic leukaemia cell line, KU812, are not suitable for the study of FcεRI-mediated functions because of the low expression of FcεRI receptors [8]. Therefore, several investigators have attempted to generate mature basophils by culturing precursor cells in bone marrow or cord blood with IL-3 as the main growth and differentiation factor [9–13]. Although IL-3 is a critical cytokine for the growth and differentiation of basophils, cultured cells have predominantly differentiated into eosinophils (30–80%). Consequently, an expanded population of basophils is small (about 30%) in most of these studies.

In the present study, we cultured mononuclear cells from peripheral blood stem cell (PBSC) harvests in the presence of IL-3 for inducing the differentiation and proliferation of basophils. By this method, we could obtain a large number of highly purified basophils, which can be used for biochemical and functional assays.

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Materials and methods

Reagents and antibodies

Recombinant human IL-3 (rhIL-3) (R&D Systems, Minneapolis, MN, USA), human IgE (Chemicon International Inc., Temecula, CA, USA), fluorescein isothiocyanate (FITC)-conjugated goat anti-human IgE, mouse IgG1, mouse IgG2a, mouse IgG2b (Sigma, St Louis, MO, USA), phycoerythrin (PE)-conjugated mouse anti-human CD16, PE-conjugated mouse IgG1 (Becton Dickinson, Franklin Lakes, NJ, USA), PE-conjugated mouse anti-human CD64 (Calbiochem, San Diego, CA, USA), 2E1 (anti-CD32; Immunotech, Marseille, France), PE-conjugated goat anti-mouse IgG (Biosource International, Tago, Camarillo, CA, USA), fluo-3 acetoxymethyl ester (fluo-3/AM) (Dojindo, Kumamoto, Japan), affinity purified goat antibody to human IgE (Kirkegaard & Petty Laboratories Inc., Gaithersburg, Germany), CRA-1 (anti-human FcεRIα chain antibody; Kyokuto Pharmaceutical Indust. Co. Ltd, Tokyo, Japan) [14] and goat anti-mouse IgG (Fab')₂ (ICN Biomedicals Inc., Aurora, OH, USA) were purchased.

Mobilization and collection of PBSCs

Patients with malignant lymphoma and small-cell lung cancer were referred to us for treatment with high-dose chemotherapy and autologous PBSC transplantation. After obtaining informed consent, a fraction of mononuclear cells was selectively harvested during the haematopoietic recovery phase from chemotherapy-induced myelosuppression by leukapheresis using a continuous-flow blood cell separator (Cobe Spectra, Gambro BCT, Tokyo, Japan). Patients received 5 µg/kg/day of recombinant human granulocyte colony-stimulating factor (rhG-CSF) intravenously or subcutaneously during the recovery phase from chemotherapy. Leukapheresis was begun when haematologic recovery was indicated by increases in white blood cell count $>1.5 \times 10^9/L$ and/or platelet count $>5 \times 10^9/L$ after each course of systemic chemotherapy, as previously reported [14–16]. The total number of harvested mononuclear cells was $1\text{--}2 \times 10^{10}$ containing 0.15–4.9% (mean 1.36%) CD34⁺ cells. We actually used $1\text{--}2 \times 10^8$ cells (approximately 1% of total harvested cells) for culture.

Cell cultures

Mononuclear cells ($1\text{--}2 \times 10^8$ cells) were suspended at a concentration of 1.5×10^6 cells/mL in RPMI 1640 (Sigma) supplemented with 10% fetal calf serum (FCS), 2-mercaptoethanol (0.05 mmol/L), penicillin (100 U/mL), gentamicin (25 µg/mL) and rhIL-3 at different concentrations. Non-adherent cells were collected after 1-week culture. These non-adherent cells were cultured in the same medium, and a half of the culture medium was replaced once a week with freshly prepared medium containing the same concentration of rhIL-3. In some experiments, CD34⁺ cells were purified from the harvested mononuclear cells using immunomagnetic beads according to the manufacturer's instructions (CD34 Multi-Sort Kit; Miltenyi Biotec, Bergisch Gladbach, Germany). These CD34⁺ cells (1×10^6 cells/mL) were also cultured under the same conditions as described above. Cultures were

maintained at 37 °C in a humidified atmosphere with 5% CO₂ in air for up to 21 days. Aliquots of the cells were removed at different days of culture for cell counts and differentials, and cell viability by trypan blue dye exclusion. Cytospin preparations of these cells were stained with May–Grünwald–Giemsa and toluidine blue.

Electron microscopy

Cultured cells were centrifuged for 5 min at 250 g to make cell pellets. The cell pellets were fixed with 1.5% glutaraldehyde in sodium cacodylate buffer for 2 h, and then post-fixed with 1% osmic acid for 2 h. They were dehydrated through a graded series of alcohol and embedded in Epon. The blocks were thin-sectioned and stained with uranyl acetate and lead citrate. They were observed under an electron microscope (Nihon Electronic JEM 1010, Tokyo, Japan).

Detection of Fc receptors

Expression of Fc receptors on cultured basophils was determined using flow cytometry. For the detection of FcεRI, cultured cells (5×10^5 cells/mL) were suspended in RPMI 1640 with 10% FCS and passive sensitization by human IgE (20 µg/mL) was carried out for 4 h at 37 °C. After washing twice in phosphate-buffered saline (PBS), the cells were incubated with FITC-conjugated goat anti-human IgE antibody (10 µg/mL) for 30 min at 4 °C. As a negative control, unsensitized cultured cells were used. Analysis of FcγRs on FcεRI-positive cells (basophils) was performed by two-colour flow cytometry. For the detection of FcγRI (CD64) or FcγRIII (CD16), the cells sensitized with IgE were incubated with FITC-conjugated goat anti-human IgE antibody and either PE-conjugated anti-CD64 (20 µg/mL) or PE-conjugated anti-CD16 (20 µg/mL) for 30 min at 4 °C, respectively. For FcγRII (CD32) expression, the cells sensitized with IgE were treated with anti-CD32 (2E1; 20 µg/mL) for 30 min at 4 °C, and then incubated with FITC-conjugated goat anti-human IgE antibody and PE-conjugated goat anti-mouse IgG as a second antibody for 30 min at 4 °C. Isotype-matched mouse IgGs (PE-conjugated mouse IgG1 for anti-CD64 and anti-CD16, mouse IgG2a for 2E1) were used as negative controls. Data were obtained with a FACSCalibur flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA) and analysed using CELLQuest software (Becton Dickinson Immunocytometry Systems).

Measurement of intracellular calcium mobilization

Intracellular calcium measurements were performed according to the method of Kawata et al. [8]. Briefly, cultured cells (5×10^5 cells/mL) were sensitized with human IgE (10 µg/mL) for 4 h at 37 °C with 5% CO₂ in air, or incubated with anti-FcεRIα chain (CRA-1; 10 µg/mL) for 30 min at 4 °C. Cells were washed twice in Hanks' balanced salt solution (HBSS) without Ca²⁺ or Mg²⁺, and then loaded with fluo-3/AM (2 µM) in the dark for 40 min at room temperature. After washing twice in PBS, they were resuspended in HBSS containing Ca²⁺ and Mg²⁺ at a concentration of 5×10^5 cells/mL. FcεRI cross-linking was carried out by adding goat anti-human IgE antibody (20 µg/mL) to the cells sensitized with IgE, or goat anti-mouse IgG (10 µg/mL) to the cells incubated

with CRA-1, and subjected to analysis by a FACSCalibur flow cytometer using CELLQuest software. After FcεRI cross-linking, the fluorescence was continuously monitored through a 525-nm band-pass filter using a linear scale with appropriate forward- and side-scattered gating.

Histamine assay

Histamine was measured using an EIA kit (Immunotech, Marseille, France). Cultured cells were lysed by three freeze-thaw cycles to evaluate the cellular histamine content. Histamine release was performed by FcεRI cross-linking. Briefly, cultured cells (5×10^5 cells/mL) were suspended in HBSS without Ca^{2+} or Mg^{2+} and sensitized by human IgE (10 µg/mL) for 4 h at 37 °C with 5% CO_2 in air. Then the cells were washed twice in PBS, and resuspended in HBSS containing Ca^{2+} and Mg^{2+} . After stimulation with anti-human IgE antibody (20 µg/mL) for 30 min, the cell-free supernatants were harvested for the measurement of histamine release. Histamine release was calculated as a percentage of the total cellular histamine after subtracting the spontaneous histamine release in the absence of stimulation.

Measurement of cysteinyl leukotrienes

Cultured cells were stimulated with anti-human IgE antibody (20 µg/mL), at the same condition as the histamine release assay. After 1-h incubation, the cell-free supernatants were harvested for the measurement of cysteinyl leukotrienes (leukotrienes C_4 , D_4 and E_4) using an EIA kit (CAST ELISA, Buhlmann Laboratories AG, Allschwil, Switzerland).

Statistical analysis

Correlations were performed using the Pearson's correlation coefficient. *P* values of less than 0.05 were considered to be significant.

Results

Effect of IL-3 on cultures

Mononuclear cells ($1\text{--}2 \times 10^8$ cells) were cultured with different concentrations of rhIL-3 (0.1, 1, 3, 5, 10, 20 and 30 ng/mL) in order to determine the optimal concentration. It was difficult to maintain cultures at concentrations of rhIL-3 less than 1.0 ng/mL. When more than 10 ng/mL of rhIL-3 was added, the total number of cultured cells was slightly increased at day 21 of culture. However, neither the yield nor purity of metachromatic cells was increased. Therefore, we selected a concentration of 5 ng rhIL-3/mL for expanding basophils. Cytospin preparations of cultured cells were stained with May-Grünwald-Giemsa and toluidine blue. The total number of cultured cells (non-adherent cells) decreased every week. In contrast, the number of metachromatic cells generally increased during the first 2 weeks, and peaked at days 14–21. When PBSC-rich mononuclear cells containing more than 1% of $\text{CD}34^+$ cells were cultured, 20.0–83.3% of the total cells, mostly with a yield of >10% ($1.8\text{--}3.6 \times 10^7$ cells) (Table 1), had basophilic granules in their

Table 1. Characteristics of the study subjects

No.	Sex	Age	CD34 ⁺ cells (%) [*]	Purity of basophils (%) [†]	Yield of basophils (%) [‡]
<i>Non-Hodgkin's lymphoma</i>					
1	F	56	4.90	27.0	32.5
2	M	39	4.22	56.0	7.0
3	M	37	3.37	68.8	27.5
4	M	48	3.24	48.0	53.0
5	M	62	3.12	71.0	11.0
6	M	56	3.08	63.0	40.0
7	M	50	3.00	64.7	16.5
8	F	53	2.97	56.9	26.5
9	M	38	2.37	71.4	27.5
10	M	33	2.25	38.0	13.5
11	M	17	2.23	54.5	21.0
12	M	62	2.21	65.5	19.0
13	M	55	2.20	35.4	17.0
14	F	62	1.94	78.4	14.5
15	M	53	1.89	31.1	7.0
16	M	33	1.77	83.3	12.5
17	F	41	1.73	50.7	18.5
18	M	53	1.69	20.0	2.0
19	M	54	1.20	38.7	6.0
20	M	37	0.97	37.5	16.5
21	F	68	0.93	33.3	7.5
22	M	27	0.90	23.1	12.5
23	F	43	0.90	6.4	1.5
24	M	15	0.88	27.8	2.5
25	F	60	0.85	28.6	6.0
26	F	56	0.80	9.6	3.5
27	M	52	0.76	52.1	12.0
28	M	28	0.74	10.9	6.0
29	F	19	0.63	28.6	2.0
30	M	61	0.50	13.0	2.0
31	F	43	0.50	26.7	6.0
32	F	28	0.48	2.2	0.5
33	F	54	0.35	10.6	9.5
34	M	60	0.31	1.1	0.5
35	M	42	0.31	10.8	2.0
36	M	24	0.30	11.1	2.5
37	M	24	0.30	10.2	2.5
38	F	44	0.29	0.0	0.0
39	M	21	0.27	24.3	9.0
40	M	60	0.26	0.0	0.0
41	F	54	0.24	3.9	1.1
42	F	43	0.20	22.2	4.0
43	F	67	0.15	12.0	2.0
44	F	60	0.15	19.0	2.0
<i>Small-cell lung cancer</i>					
1	M	54	0.59	28.0	0.8
2	M	50	0.57	18.5	0.5
3	M	63	0.54	17.0	0.8

^{*}Percentage of $\text{CD}34^+$ cells in harvested mononuclear cells. [†]Proportion of the number of metachromatic cells to the total number of cultured cells at day 21. [‡]Proportion of the number of metachromatic cells to the number of harvested mononuclear cells.

cytoplasm, and they were metachromatically stained and judged to be basophils at day 21 of culture (Fig. 1). These cultured cells were also observed by transmission electron microscopy. They had a lobulated nucleus and cytoplasmic

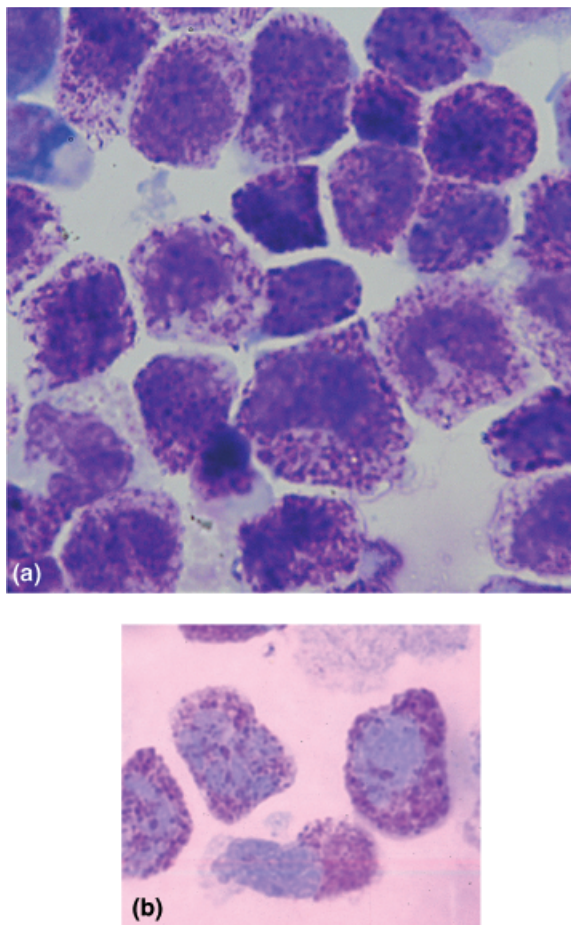


Fig. 1. PBSC-derived human basophils stained at day 21 of culture with (a) May-Grünwald-Giemsa and (b) toluidine blue. Original magnification $\times 1000$.

granules filled with finely granular materials that were characteristic of basophils (Fig. 2). Contaminating cells were mostly lymphoid cells at day 21 of culture, while mast cells were not observed by light and electron microscopy. Eosinophils were occasionally found at a frequency of less than 5% of cultured cells at day 7 or day 14 of cultures (IL-3: 0.1–30 ng/mL), but hardly found at day 21 of culture.

When the percentage of CD34⁺ cells in harvested mononuclear cells was less than 0.3%, cultured cells were differentiated into few basophils. Interestingly, both purity and yield of basophils after 3 weeks of culture were positively correlated with the percentage of CD34⁺ cells (purity, $r = 0.700$, $P < 0.0001$; yield, $r = 0.733$, $P < 0.0001$). In 13 preparations with more than 2.0% of CD34⁺ cells, a relatively high proportion of basophils was obtained (median, 56.9%; range, 71.4–35.4%) (Table 1). In nine of these preparations, the purity of basophils exceeded 50%. We also performed CD34-positive selection from harvested mononuclear cells using a CD34 MultiSort Kit (Miltenyi Biotec). Purified CD34⁺ cells (purity $> 95\%$, $1 \times 10^6/\text{mL}$) were then cultured in the presence of rhIL-3 (5 ng/mL). After 2–3 weeks, 55–84% ($n = 4$) of the cells were differentiated into basophils. Furthermore, we cultured CD34[−] cells negatively selected by the CD34 MultiSort Kit, but few metachromatic cells were observed during cultures.

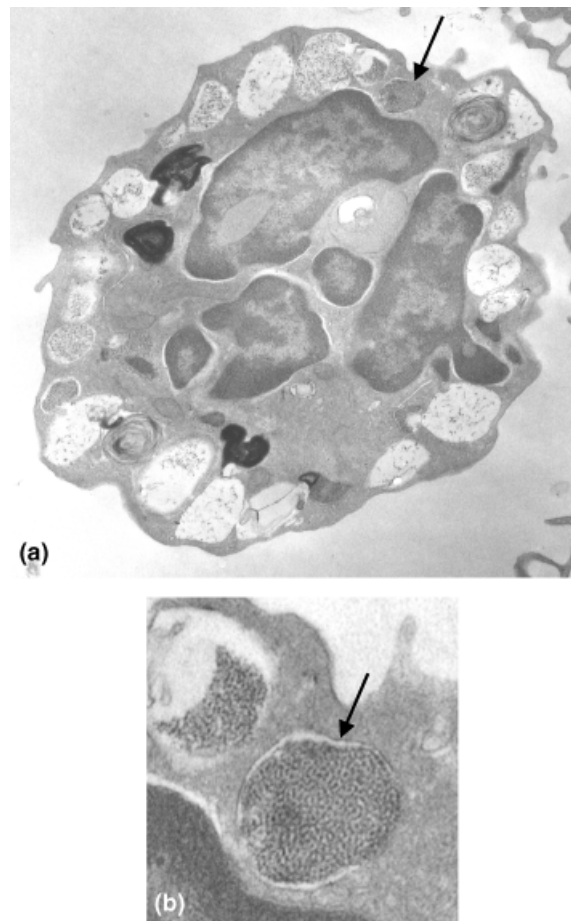


Fig. 2. Ultrastructure of PBSC-derived human basophils at day 21 of culture. (a) Transmission electron micrograph showing a lobulated nucleus and cytoplasmic granules (enlarged in (b)) filled with finely granular materials (arrows) that are characteristic of basophils. Original magnification $\times 8800$.

Expression of Fc receptors on PBSC-derived basophils

The surface expression of FcεRI and FcγRs on PBSC-derived basophils was analysed by flow cytometry. First, we found FcεRI-positive cells in an indicated fraction (gate) (Fig. 3a). A percentage of FcεRI-positive cells in total cells was almost equal to that of metachromatic cells. Two-colour analysis of flow cytometry demonstrated that both FcεRI and FcγRII (CD32) were abundantly expressed on the gated cells (basophil fraction) (Fig. 3b, c). We have also observed FcεRI expression using a monoclonal antibody against the FcεRIα chain (CRA-1). In contrast, either FcγRI (CD64) or FcγRIII (CD16) was not apparently expressed on PBSC-derived basophils (data not shown). Moreover, when we used highly purified basophils (purity $> 95\%$), which were obtained by the effective removal of contaminating lymphocytes using a Basophil Isolation Kit (Miltenyi Biotec) [7], the exclusive expression of FcγRII as well as FcεRI was confirmed.

Intracellular calcium mobilization by FcεRI cross-linking

We evaluated whether PBSC-derived basophils could induce intracellular calcium mobilization in response to FcεRI stimulation. Cross-linking of FcεRI by anti-IgE on the

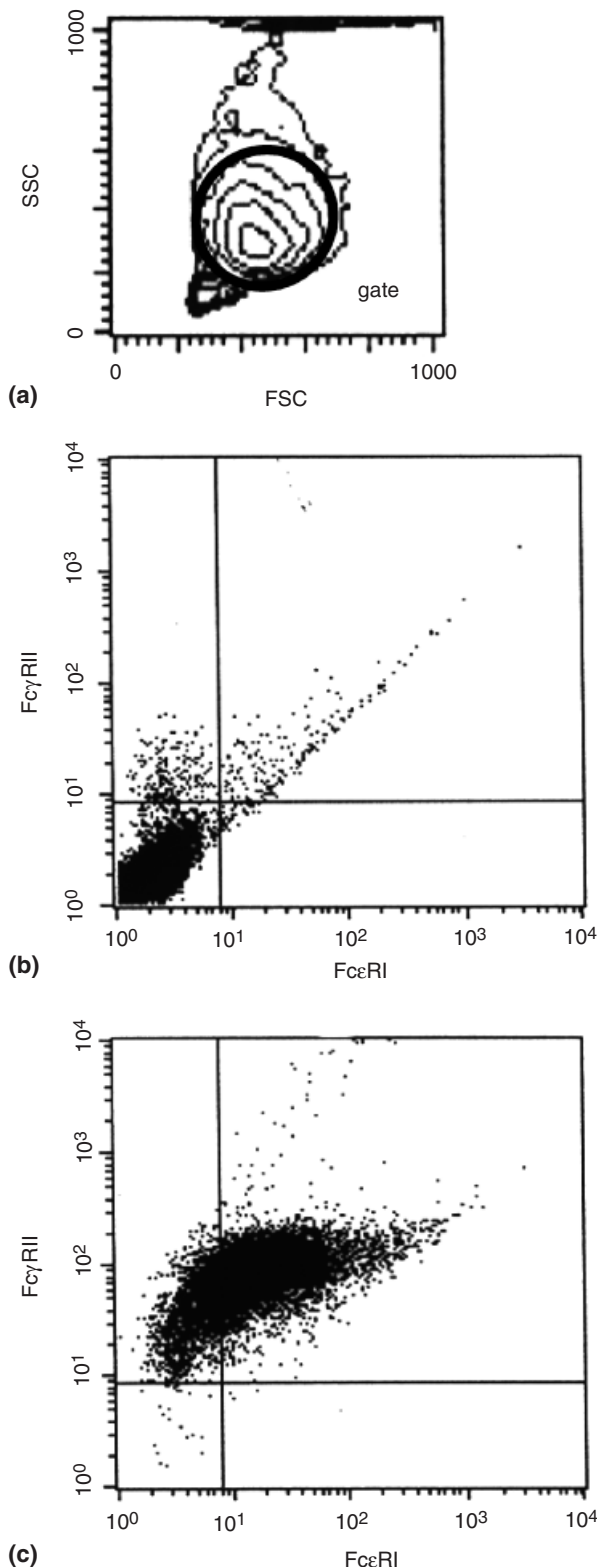


Fig. 3. FcεRI and FcγRII expression on PBSC-derived human basophils. (a) FcεRI-positive cells were in an indicated fraction (gate). Two-colour flow cytometry was performed in gated cells. Cultured cells (5×10^5 cells/mL), (b) unsensitized (negative control) or (c) sensitized with human IgE (20 µg/mL), were treated with (b) mouse IgG2a (negative control) or (c) anti-CD32 (2E1; 20 µg/mL) for 30 min at 4°C, and then incubated with fluorescein-isothiocyanate-conjugated goat anti-human IgE antibody and phycoerythrin-conjugated goat anti-mouse IgG as a second antibody for 30 min at 4°C.

cultured basophils induced intracellular calcium mobilization immediately (Fig. 4a). We have also carried out cross-linking of FcεRI by the anti-FcεRIα chain (CRA-1), and confirmed that PBSC-derived basophils can induce intracellular calcium mobilization in response to FcεRI stimulation (Fig. 4b).

Intracellular histamine content and histamine release triggered by FcεRI cross-linking

We measured the intracellular histamine content of PBSC-derived basophils by using an EIA kit. The histamine content ranged from 0.76 to 2.83 pg/basophil (mean 1.83 pg/cell, $n = 5$) at day 21 of culture. Moreover, the cultured basophils released histamine upon FcεRI cross-linking; the mean percentage of histamine release was 25.8% ranging between 10.2% and 41.0%, while that of spontaneous histamine release was 3.63% ranging between 0.25% and 7.5% ($n = 4$).

Production of cysteinyl leukotrienes by PBSC-derived basophils

The amounts of cysteinyl leukotrienes synthesized and released by PBSC-derived basophils were measured using an EIA kit (CAST ELISA). After cross-linking FcεRI with anti-IgE for 1 h, the supernatants were harvested. The mean production of cysteinyl leukotrienes induced by FcεRI cross-linking was 31.38 ± 2.427 ng/ 10^6 cells, while unstimulated control production was $0.31 \text{ ng} \pm 0.2$ ng/ 10^6 cells ($n = 7$).

Discussion

In this study, we have established a culture method for the *in vitro* expansion of basophils from PBSC harvests. We cultured mononuclear cells from G-CSF-mobilized PBSC harvests, which were collected from patients with malignant lymphoma and lung cancer. After 3 weeks of culture in the presence of rhIL-3, 20–83.3% of the cells were identified as basophils. These cells were similar to peripheral blood basophils morphologically when examined by light and electron microscopy. As peripheral blood basophils [17], they expressed both FcεRI and FcγRII abundantly. Furthermore, FcεRI cross-linking resulted in intracellular calcium mobilization, histamine release and synthesis of leukotrienes. Both the mean histamine content (1.83 pg/cell) and the mean production of cysteinyl leukotrienes upon FcεRI stimulation (31.38 ng/ 10^6 cells) were comparable to those of peripheral blood basophils [18]. Additionally, we have examined a series of surface phenotypes commonly expressed on peripheral blood basophils by flow cytometry. We confirmed the surface expression of receptors for IL-3 and GM-CSF (IL-3Rα and GM-CSFRα, respectively) and adhesion molecules such as CD11b, CD18, CD29 and CD49d (data not shown). These findings indicate that basophils cultured by our method are morphologically and functionally mature and can be used as a tool for their biochemical and functional assays.

To clarify the roles of human basophils in allergic inflammation such as bronchial asthma and allergic rhinitis, we previously developed a method for the purification of blood basophils by negative selection using flow cytometry [3]. Other methods, including the use of density gradient

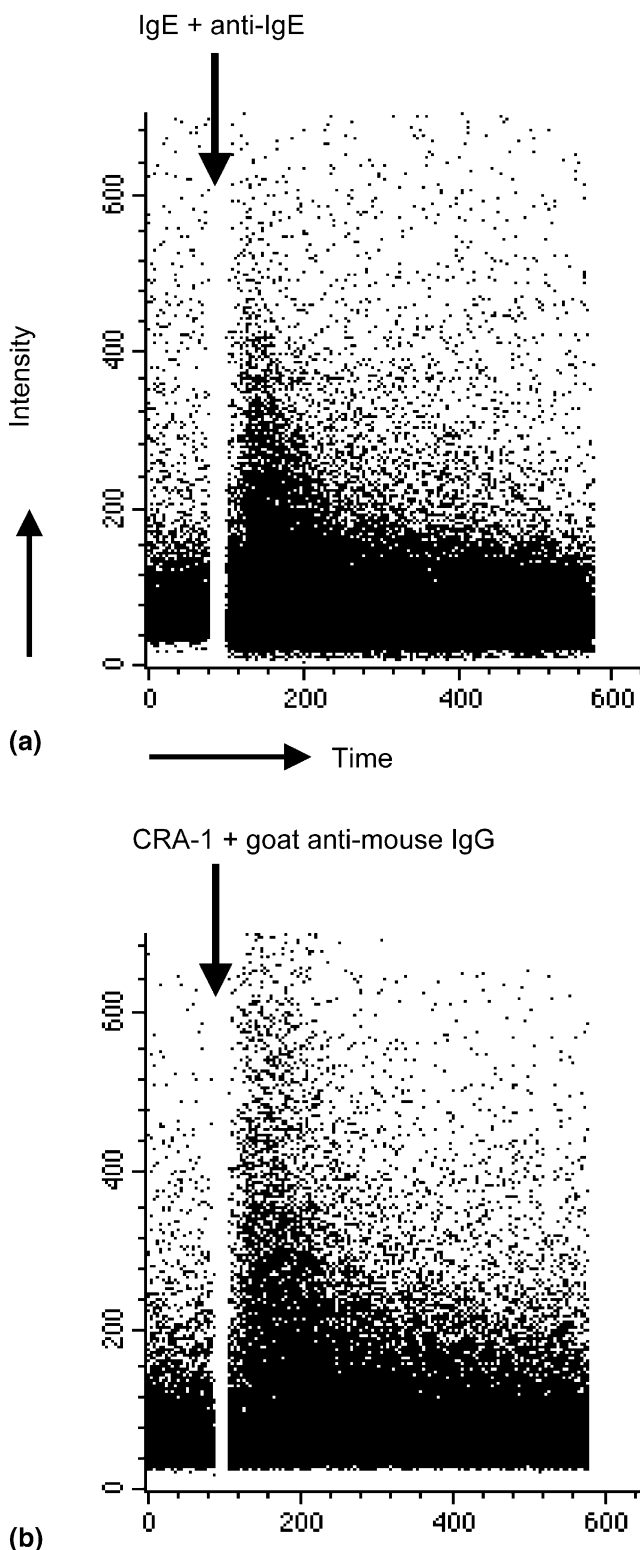


Fig. 4. Kinetics of intracellular calcium mobilization upon Fc ϵ RI cross-linking in peripheral blood stem cell derived human basophils. Cultured cells ($5 \times 10^5/\text{mL}$) were (a) sensitized with human IgE ($10 \mu\text{g}/\text{mL}$) for 4 h at 37°C , or (b) incubated with an anti-Fc ϵ RI α chain (CRA-1; $10 \mu\text{g}/\text{mL}$) for 30 min at 4°C , and then washed and resuspended in Hanks' balanced salt solution (HBSS) without Ca^{2+} or Mg^{2+} . Fluo-3/AM ($2 \mu\text{M}$) was loaded to both suspensions for 40 min at room temperature in the dark. The cells were washed and resuspended in HBSS containing Ca^{2+} and Mg^{2+} , and their fluorescence intensities were recorded after the following stimulations: (a) goat anti-human IgE $20 \mu\text{g}/\text{mL}$ and (b) goat anti-mouse IgG $10 \mu\text{g}/\text{mL}$.

centrifugation, counterflow elutriation, flow cytometry and immunological selection, have also been described to purify blood basophils [4–7]. However, the main limitation of these methods is a low yield, which is not sufficient for a series of functional assays. Therefore, several investigators have attempted to culture basophils from precursor cells in bone marrow or cord blood, using several haematopoietic growth factors including IL-3, IL-5 and GM-CSF [9–13]. IL-3 is known to be a critical cytokine for the growth and differentiation of basophils in humans. In most of these studies, however, cells cultured in the presence of IL-3 were predominantly differentiated into eosinophils (30–80%), and basophils were obtained as a minor population (about 30%). We also cultured cord blood mononuclear cells under the same conditions (IL-3: $5 \text{ ng}/\text{mL}$) as PBSC-rich mononuclear cells. Our data showed that approximately 30% of the cells were differentiated into eosinophils and the yield of eosinophils was dependent on the concentration of IL-3 (unpublished observations). Besides, both histamine content and Fc ϵ RI expression on basophils derived from cord blood mononuclear cells have been reported to be lower than those of blood basophils [9]. More recently, Kepley et al. [19] have reported the usefulness of basophils derived from IL-3-pulsed cord blood cells. We consider that there is not much difference between our culture method and theirs in the characteristics of cultured basophils such as morphologic features and histamine content. The advantage of our culture system is the capability of obtaining a larger amount of basophils than their system. The maximum yield of cultured basophils is 3×10^6 cells per donor in their culture system with IL-3-pulsed cord blood cells, while our system easily yields more than 1×10^7 basophils.

We harvested mononuclear cells containing 0.15–4.9% $\text{CD}34^+$ cells after chemotherapy plus G-CSF-induced mobilization. These cells were then cultured in the presence of IL-3 alone. Interestingly, they have been predominantly differentiated not into eosinophils but into basophils. At day 21 of culture, 20–83.3% of the cells were identified as basophils. Especially, in nine of 13 preparations with more than 2.0% of $\text{CD}34^+$ cells, over 50% of the cultured cells were basophils. We found a positive correlation between the percentage of $\text{CD}34^+$ cells and yield as well as purity of basophils after 3 weeks of culture. We also cultured PBSCs obtained from patients with other malignancies, including ovarian cancer and breast cancer. The yield of basophils tended to depend on the percentage of $\text{CD}34^+$ cells in these cases. Moreover, we could culture basophils from the PBSCs of normal donors for allogeneic PBSC transplantation. These basophils resembled those from patients with malignancies, in terms of morphology, expression of Fc receptors and intracellular calcium mobilization. Cultures of $\text{CD}34^-$ cells failed to yield a large number of metachromatic cells. Therefore, we suppose that precursors of the metachromatic cells (basophils) are mainly $\text{CD}34^+$ cells and cultured basophils are not neoplastic. These findings suggest that $\text{CD}34^+$ cell-rich mononuclear cells may contain a large number of basophil progenitor cells, and we can predict a yield of cultured basophils by counting the number of $\text{CD}34^+$ cells in harvested mononuclear cells. Thus, our culture method could provide more than 1×10^7 cells of basophils from 1×10^8 harvested mononuclear cells, when the percentage of $\text{CD}34^+$ cells was $>1\%$. In addition, cultured

basophils were similar to blood basophils in terms of intracellular histamine content, ability of histamine release and synthesis of cysteinyl leukotrienes upon FcεRI stimulation. It is not well known how selective differentiation and proliferation of basophils could be induced when chemotherapy plus G-CSF-mobilized PBSCs were cultured with IL-3 alone. Pederson et al. [20] have reported that rhG-CSF could mobilize immature basophils or their precursors from the bone marrow. We speculate that G-CSF-mobilized PBSCs may promote early commitment to the basophil lineage preferentially as compared with the eosinophil lineage. It is also necessary to investigate the effect of other haematopoietic growth factors such as IL-5, GM-CSF and SCF on the growth and differentiation of basophils in mononuclear cells from G-CSF-mobilized PBSC harvests.

Taken together, our culture system can easily provide more than 1×10^7 basophils with a purity of 20–83.3% from 1×10^8 PBSC-derived mononuclear cells containing 1% or more CD34⁺ cells. Moreover, we could obtain highly purified basophils (purity > 95%) by the effective removal of contaminating lymphocytes using the Basophil Isolation Kit (Miltenyi Biotec) (data not shown). Therefore, our culture method enables us to get a sufficient number of highly purified human basophils for a series of biochemical and functional assays.

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