Interleukin-5 Is a Human Basophilopoietin: Induction of Histamine Content and Basophilic Differentiation of HL-60 Cells and of Peripheral Blood Basophil-Eosinophil Progenitors

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Cytokine-induced differentiation of basophils may contribute to various inflammatory processes. We examined the effects of recombinant human interleukin-5 (IL-5) and other human cytokines in vitro on myeloid colony formation in methylcellulose and on alkaline passaged HL-60 basophilic cell differentiation. Myeloid colonies (CFU-C) at day 14, formed in the presence of either IL-3, IL-5, granulocyte-macrophage colony-stimulating factor (GM-CSF), or G-CSF included peripheral blood-derived progenitors of the eosinophil/basophil lineage. IL-5 stimulated a greater proportion of basophil-containing, histamine-positive, eosinophil-type colonies compared with GM-CSF, IL-3, or G-CSF. IL-5 also stimulated dose-dependent increases in histamine content of alkaline-passaged, butyrate cotreated HL-60 cells. The concentration of IL-5 required for half-maximal induction of HL-60 histamine content was similar within twofold to that needed for half-maximal stimulation of the multifactor dependent TF-1 erythroleukemic cell line. Neutralizing rat monoclonal antibodies to human IL-5 were developed and used to demonstrate that each of these IL-5 bioactivities could be specifically blocked. We conclude that in addition to its previously described eosinophil differentiation activity, IL-5 may be considered a basophilopoietin.

MATERIALS AND METHODS

IL-5-induced histamine production in HL-60. The human myeloid leukemic cell line HL-60 (American Type Culture Collection [ATCC], Rockville, MD) was passaged in RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (HyClone, Logan, UT; lot no. 1115774), and 25 mmol HEPES (Sigma, St Louis, MO). The pH of the medium was adjusted to 7.8 before sterile filtration, and supplemented with 2 mmol/L L-glutamine (JR Scientific, Woodland, CA) just before use. For antibody inhibition experiments, varying concentrations of rat MoAbs in a 50-μL vol were mixed with an equal volume of purified 2 ng/mL recombinant L cell expressed human IL-5 (rhIL-5) in flat-bottom 96-well microtiter plates (Falcon, Lincoln Park, NJ). The IL-5 was isolated by affinity chromatography using the TRFK5 MoAb® immobilized on Affigel 10 (Biorad, Richmond, CA), and was a kind gift of Robert Coffman (DNAX, Palo Alto, CA). A 50-μL vol of 1.2 mmol/L sodium butyrate was added, and then a final 50-μL addition of 1 × 10^6 HL-60 cells/mL was added to the plates. The plates were incubated for 72 hours. The entire well content (cells and supernatant) was harvested and boiled for 5 minutes. Samples were then frozen at −80°C before histamine content quantification.

Histamine assays. A radioenzymatic conversion assay that is sensitive to 0.1 ng of histamine has been previously described and is a reliable indicator of basophilic lineage expression in both methylcellulose and HL-60 cultures. In addition, a commercial histamine radioimmunoassay (RIA) (AMAC, Inc, Westbrook, ME) was used. When 20 different samples were compared in both assays, there was reasonable correlation (p = 0.7) between the RIA and radioenzyme techniques, with apparent random divergence. For the RIA, samples were thawed, vortexed, microcentrifuged, and a 20-μL vol was diluted 1:10 in phosphate-buffered saline. The sample was then acylated with reagent provided in the kit, and analyzed in duplicate in the RIA according to the manufacturer’s directions. The remaining antibody-bound 1H-acetyl-histamine was determined using a Cobra 5010 γ-counter (Packard, Santa Ana, CA) equipped with RIA standard curve interpolation software.

TF-1 bioassay. TF-1 cells® were passaged in RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum, 2 mmol/L L-glutamine, and 1 ng/mL human granulocyte-macrophage CSF (hGM-CSF). Varying concentrations of rat MoAbs in a volume of 25 μL were added to wells in flat-bottom 96-well microtiter plates. A 25-μL vol of 2 ng/mL recombinant hIL-5 (rhIL-5) was added to each well. A 50 μL/well vol of washed TF-1 cells was added to give a final cell concentration of 1 × 10^6 cell/well in a final assay volume of 100 μL. The plate was incubated for 48 hours. The remaining
cell viability was determined metabolically by the colorimetric method of Mosmann using a VMax microtiter plate reader (Molecular Devices, Menlo Park, CA) set at \( \lambda_1 = 570 \) and \( \lambda_2 = 650 \) nm.

**IL-5**

rhIL-4 and rhIL-5 were prepared at DNAX. In some experiments, the rhIL-3 (specific activity 2 to 4 \( \times 10^8 \) U/mg) and rhGM-CSF (specific activity 9.3 \( \times 10^7 \) U/mg) were obtained from Genetics Institute (Boston, MA). The rhG-CSF was purchased from Immunotech Corporation (Marseille, France).

**Methylcellulose cultures.** Gradient-separated (density = 1.077) peripheral blood mononuclear cells from six atopic donors were cultured in 0.9% methylcellulose supplemented with Iscove’s modified Dulbecco’s medium, a source of colony-stimulating activity, and 20% vol/vol fetal calf serum. Metachromatic cell- and histamine-containing colonies were identified as previously described, for histamine content, 6 to 10 colonies were randomly picked from plates at day 14 and assayed by a radioenzyme technique.

**Development of neutralizing MoAbs to human IL-5.** Hybridoma cell lines producing MoAbs to hIL-5 were produced from the splenocytes of a male Lewis rat immunized with semi-purified recombinant mammalian-expressed IL-5. These were fused with the P3X63-Ag8.653 nonsecreting mouse myeloma essentially as described. The hybridoma library was screened for indirect enzyme-linked immunosorben assay (ELISA) positive MoAbs on IL-5-coated microtiter plates, and for neutralizing MoAbs using antibody inhibition of IL-5-induced TF-1 target cell proliferation (see above). The most potent blocking MoAbs were selected and cloned by limiting dilution. Production and purification of IgG from serum-free (HB102) defined medium was performed essentially as previously described.

**RESULTS**

**rhILs and CSFs promote basophilic differentiation in methylcellulose cultures.** Myeloid-lineage growth factors such as IL-3, IL-5, G-CSF, and GM-CSF are not absolutely lineage specific for the cell types they can induce to proliferate. In view of our previous results supporting a common lineage between eosinophils and basophils, we became interested in examining the relative ability of each of these factors to support basophilic growth and differentiation from human peripheral blood progenitors. The approach involved assessment of colony-forming units at day 14 in methylcellulose cultures in the presence of T-cell–conditioned media or other sources of colony-stimulating activity. Individual colonies picked from the cultures were assessed for expression of markers of the basophilic/eosinophilic lineage. Cytochemical staining with May-Grünwald-Giemsa and toluidine blue, or alcian blue-safranin was used to assess the presence of basophilic or metachromatic cells, respectively. Toluidine blue staining together with immunofluorescence confirmed the presence of BSP-1, a basophil-specific antigen, on colony-derived metachromatic cells induced by Mo-CM, IL-3, or IL-5 (reference 16 and Denburg JA, Valent P, and Bettelheim P, in preparation). These results were correlated with determination of histamine content of individual colonies.

Table 1 shows the hematopoietic activities of the various cytokines tested in methylcellulose culture. Mo-CM, a supernatant from a hairy T-cell leukemia line known to contain GM-CSF, was used as a positive control. G-CSF, GM-CSF, IL-3, and IL-5 each stimulated colony growth in this assay; the values are expressed for maximal stimulating concentration of each cytokine. Dose-response curves for colony number and type as a function of cytokine concentration for each of these factors demonstrated a log-linear increase in the number of colonies between 0.0001 and 0.1 ng/mL for IL-3 and IL-5 (Fig 1A), and 0.1 and 10 U/mL for GM-CSF and G-CSF (Fig 1B). Maximal or plateau levels were obtained at 0.1 ng/mL (for IL-3 and IL-5) and 100 U/mL (for GM-CSF and G-CSF). The relative proportion of eosinophil/basophil colonies (Eo type) compared with neutrophil/macrophage colonies (GM type) was similar for all cytokines tested (Table 1), apart from IL-4, which had minimal colony-stimulating activity at the concentration tested. However, when analysis of histamine content of single colonies was performed it was found that IL-5 stimulated the highest proportion (0.64) of histamine-positive colonies compared with the other cytokines, and a mean higher level of histamine per colony (Table 2). IL-5 stimulated, as expected, a relatively higher proportion of colonies containing basophilic or metachromatic cells compared with IL-3 and G-CSF (not shown); the maximal mean value for basophilic cells in IL-5–stimulated cultures was 15% (range 6% to 27%).

**Comparison of in vitro IL-5 bioactivities using two different human target cell lines.** We have used cultures of the human myeloid leukemic cell line, HL-60, as an alternative to performing 14-day colony assays in semisolid medium to assess basophil differentiating activity. HL-60 cells can also be induced preferentially to the eosinophilic lineage through the use of alkaline conditions in the presence of sodium butyrate alone. In the presence of both sodium butyrate and Mo-CM from the hairy cell leukemia line, increases in metachromatic cells, surface IgE receptors, and histamine

| Table 1. Effect of Cytokines on Myeloid Colony Growth in Methylcellulose |
|---------------------------|-----------------|-------------|
| Condition               | Eo Type | GM Type | Total |
| Mo-CM, 5% vol/vol        | 34.7 ± 8.4 | 8.8 ± 3.8 | 43.3 ± 11.4 |
| Medium control           | 6.9 ± 3.1  | 3.1 ± 1.7 | 10.0 ± 5.0  |
| rhIL-3, 0.1 ng/mL        | 52.2 ± 22.1 | 13.3 ± 5.2 | 65.5 ± 25.8 |
| rhIL-4, 3 U/mL           | 6.8 ± 6.8  | 4.5 ± 4.5 | 11.3 ± 11.3 |
| rhIL-5, 0.1 ng/mL        | 33.8 ± 16.3 | 6.3 ± 2.6 | 40.0 ± 17.8 |
| rhGM-CSF, 10 U/mL       | 39.5 ± 2.5  | 14.6 ± 4.4 | 50.4 ± 4.7  |
| rhG-CSF, 100 U/mL        | 42.8 ± 22.8 | 11.9 ± 6.1 | 54.8 ± 27.4 |

Mean ± SE; results of six experiments in duplicate. Staining for cyanide-resistant peroxidase was used to distinguish neutrophilic from eosinophilic/basophilic cells in methylcellulose cultures.
content can be observed at 3 to 5 days in suspension-cultured HL-60 cells, indicative of basophilic lineage differentiation. Recent studies we have performed confirm the presence of high-affinity IgE receptors on 80% to 90% of the HL-60 cells cultured under alkaline conditions in the presence of sodium butyrate, and either rhGM-CSF, rhIL-3, or rhIL-5; each of these cytokines induced an equivalent quantity of high-affinity IgE receptors. This finding is consistent with our original observations using Mo-CM.

Because IL-5 induced the greatest proportion of histamine-positive colonies in methylcellulose, the highest levels of histamine per colony (Table 2), and the greatest proportion of basophilic cells per colony, relative to the other cytokines tested, we examined its ability to induce histamine by day 3 in alkaline-passaged HL-60 cells cultured in the presence of sodium butyrate. This assay used a commercially available RIA kit to determine histamine content. A representative dose-response curve of the differing histamine content in HL-60 cells induced by varying amounts of IL-5 cocultured with 0.3 mmol/L sodium butyrate is shown in Fig 2.

Recently, a multifactor-dependent erythroleukemic cell line, TF-1, which demonstrates a strict growth dependency on either GM-CSF, IL-3, erythropoietin, or IL-5, has been described (Kitamura T, Takaku F, Miyajima A, submitted). We tested the same preparation of purified rhIL-5 (L-cell derived) in the TF-1 cell proliferation assay (Fig 2) to compare the relative activity of IL-5 in the TF-1 and HL-60 in vitro systems. The specific activity of purified IL-5 in each in vitro assay system, as defined by half-maximal stimulation, was determined by nonlinear regression. Half-maximal IL-5 concentration for the TF-1 assay was 208 ± 7 pg/mL and agreed within twofold with the value of 94 ± 22 pg/mL obtained in the HL-60 system. The TF-1 bioassay system appeared to be more precise. We have determined the specific activity of L-cell derived, recombinant IL-5 to be in the range of 5 to 10 x 10^6 U/mg in the TF-1 and HL-60 systems, based on estimates of protein content obtained from densitometry of the silver-stained protein band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (M. Bond, personal communication, July 1989). This value correlated well with the half-maximal concentration of recombinant, COS7-expressed mouse IL-5 (approximately 225 pg/mL) necessary to stimulate the factor-responsive BCL, mouse lymphoma.

Neutralizing MoAbs to IL-5 block its basophilopoietin activity. We have recently developed a panel of neutraliz-

Table 2. Histamine-Positive Colonies Induced by Various Cytokines

<table>
<thead>
<tr>
<th>Condition</th>
<th>Proportion</th>
<th>Histamine-Positive Colonies*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mo-CM, 5% vol/vol</td>
<td>.27</td>
<td>0.16 ± 0.05</td>
</tr>
<tr>
<td>Medium control</td>
<td>&lt;.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>rhIL-3, 0.1 ng/mL</td>
<td>.14</td>
<td>0.22 ± 0.05</td>
</tr>
<tr>
<td>rhIL-5, 0.1 ng/mL</td>
<td>.645</td>
<td>0.36 ± 0.035</td>
</tr>
<tr>
<td>rhGM-CSF, 10 U/mL</td>
<td>.35</td>
<td>0.11 ± 0.06</td>
</tr>
<tr>
<td>rhG-CSF, 100 U/mL</td>
<td>&lt;.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
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*Containing ≥ 0.2 ng/colony (Eo type).
†Number positive/number of picked, mean of two to three experiments.
‡Mean ± SE for two experiments in which at least six colonies were picked per condition.
§P < .05 compared with other cytokines by unpaired t-test.
The 39D10 MoAb was also evaluated for its ability to neutralize the IL-5-dependent growth of eosinophil-basophil colonies (ie, both histamine-positive and histamine-negative Eo-type) in methylcellulose culture (Table 3). A 100% inhibition of colony growth was observed when 0.05 ng/mL of rIL-5 was preincubated with a 1:1,000 dilution of the 39D10 purified MoAb (1.4 μg/mL) for 2 hours at 37°C before culture in methylcellulose. Colony growth was again observed when a 1:5,000 dilution of MoAb was used. The HL-60 and methylcellulose results confirmed the ability of IL-5 to specifically induce basophilic differentiation from circulating normal or leukemic human myeloid progenitors, and this activity could be specifically blocked with neutralizing antihuman IL-5.

IL-5 is not responsible for any Mo T-cell-conditioned medium biologic activity. We have recently observed that there is a residual histamine inducing biologic activity remaining in Mo-CM after addition of polyclonal neutralizing antiserum specific for GM-CSF. We reproduced this observation in this study with a specific neutralizing anti-GM-CSF MoAb, BVD2-23B6, present in an approximately 400-fold molar excess over the amount of immunoreactive GM-CSF (2 ng/mL final concentration by immunoenzymometric assay) in the batch of Mo-CM. In light of IL-5 basophilopoietic activity, we attempted to inhibit the remaining non-neutralizable histamine inducing activity of Mo-CM with JES1-39D10 anti-IL-5 MoAb. In these experiments, purified anti-IL-5 MoAb was added to cultures containing Mo-CM or Mo-CM in combination with excess anti-GM-CSF MoAb, 23B6. In Fig 4 it can be observed that no inhibition was obtained with Mo-CM plus blocking anti-IL-5, and no additional inhibition was provided by the anti-IL-5 MoAb over that already provided by the anti-GM-CSF reagent, whereas in control cultures incubated with IL-5, MoAb 39D10 completely inhibited the exogenous IL-5 bioactivity. In addition, when a neutralizing anti-IL-3 MoAb was tested analogously, no inhibition of Mo-CM bioactivity was obtained nor was there any additional inhibition over that furnished by anti-GM-CSF, or anti-GM-CSF + anti-IL-5. Therefore, neither IL-5 nor IL-3 can be implicated as the butyrate-synergizing, histamine-inducing bioactivity present in Mo-CM or remaining after addition of...
DISCUSSION

Cytokines influencing inflammatory processes, especially those involving basophils, mast cells, and eosinophils in allergic reactions, include GM-CSF, IL-3, and IL-5.9 IL-5 in particular has been hypothesized to play an important role in allergic inflammation because it can functionally activate, increase the survival, and promote the differentiation of eosinophils.3,4,22 IL-3 is a murine mast cell growth factor as well as a hematopoietic growth factor,24 and has been shown in rodents to play an important role in reactions involving mast cell hyperplasia in response to parasitic infection.23,24 In addition, IL-3 can induce human basophil differentiation in vitro38 and basophilia in nonhuman primates in vivo.26,27 IL-4 has been shown to promote the in vitro phenotypic switch of murine mast cells,7 evidenced by the induction of metachromatic cells and histamine content of individual colonies. In addition, IL-6 can synergize with IL-6 in promoting hematopoietic stem cell differentiation.29

Human peripheral blood contains progenitor cells capable of differentiating into eosinophils and basophils; these progenitors are especially increased in patients with various atopic disorders.5,30,31 Fluctuations in these progenitors occur during the course of natural and provoked allergic exposure.30,31 Because IL-5 is an important regulator of eosinophil differentiation and function4,10,22 and because eosinophils are important components of allergic inflammation, including the cellular component in bronchial asthma, a role for IL-5 in human allergy in vivo has been postulated.21 Our current results indicate that IL-5 induces a progenitor of both eosinophils and basophils in atopic blood to differentiate in vitro. In particular, the effects of IL-5 on differentiation of the basophilic lineage are pronounced, as evidenced by morphologic examination of colonies derived from peripheral blood progenitors and the histamine content of individual colonies. In addition, IL-5 can foster basophilic differentiation in the HL-60 cells, as evidenced by the induction of metachromatic cells and histamine synthesis. In previous studies, we have shown that these HL-60 cells stained metachromatically with toluidine blue7 or alcian blue, and were negative for the specific human mast cell protease, tryptase.2,23 These cells were also shown to bear surface IgE receptors.33 More recently, analysis by fluorescence-activated cell sorter using a variety of MoAbs to myeloid and basophil lineage-specific markers41 of HL-60 cells induced by IL-3, IL-5, G-CSF, and GM-CSF to basophilic differentiation demonstrated that these cells, under conditions of alkaline passage and in the presence of sodium butyrate, bore basophil antigen surface markers and surface IgE receptors.33 Cultured as described, these alkaline-passaged HL-60 cells, which produce histamine, may represent an atypical basophil with some eosinophilic characteristics. Recent results have indicated that GM-CSF is probably the principal active moiety in Mo-CM promoting histamine increases as well as morphologic differentiation to the basophilic lineage in HL-60 cells.37 This effect, observed with Mo-CM alone, can be augmented by the presence of nerve growth factor.3,34 In the present study, we show that some histamine content-inducing factor in addition to (normal) GM-CSF, and which is not IL-3 or IL-5, must be present in Mo-CM.

In support of these findings regarding IL-5 basophil-inducing activity, Yokota et al have also reported the generation of mixed eosinophil/basophil colonies obtained from normal human bone marrow progenitors cultured in semisolid medium in the presence of COS7 conditioned medium containing rhIL-5. The recent conclusion by Clutterbuck et al,30 that hIL-5 is exclusively an eosinophilopoietin, was based on agar cultures of bone marrow wherein they detected only Eo-type colonies, and is not in agreement with ours. Also, others have found that under certain conditions some basophils can differentiate from cord blood cultures in the presence of IL-5.3,36,38 Differences in degree of response compared with our findings probably relate to differences in cell populations used for progenitors; the peripheral blood progenitors or leukemic HL-60 used in our study may represent cells with higher responsiveness to IL-5 than those derived from marrow or cord blood.

In this study, we have used two different human target cell lines to measure human IL-5 biologic activity. We found IL-5 to have similar potency within a factor of 2, as defined by half-maximal stimulation of the alkaline-passaged HL-60 myeloid leukemic cell line, as well as the TF-1 erythroleukemic cell line. This quantity of factor may also be comparable with the half-maximal amount of IL-5 needed to enhance secretory IgA-induced degranulation of purified human normodense eosinophils.39 Also, in preliminary studies, 1 U of HL-60/TF-1 IL-5 bioactivity corresponded to an amount of IL-5 equivalent to approximately 2 U as defined by the dilution of factor providing half-maximal induction of eosinophil colonies from normal human bone marrow30 (Sanderson C, personal communication, January 1990). Therefore, bioassays using these human target cell lines may provide the appropriate means for future standardization of hIL-5 specific activity.

Recently we have demonstrated that hematopoietic growth factors, including GM-CSF, can be derived from cell populations such as nasal fibroblasts and epithelial cells, which are in close proximity to surfaces where allergic reactions occur.3,30 In particular, nasal polyp epithelial cell and fibroblast-conditioned media constitutively produce GM-CSF and possibly other growth and differentiation factors for eosinophilic and basophilic cells.30 Most of these activities are significantly increased in epithelial or fibroblast-conditioned media from atopic individuals or those with nasal polyposis and/or asthma.30 Thus, we have hypothesized that microenvironmental differentiation may play an important role in the local ingress of the effector cells of allergy and that this process is mediated by the elaboration of and interactions among hematopoietic cytokines in mucosal tissues of the respiratory tract. Indeed, there has been growing evidence for the existence of a cascade of cytokine effects inducing hematopoietic growth factors from accessory (“structural”) cell populations such as vascular endothelial cells, fibroblasts, and epithelium.32

anti–GM-CSF; this activity may be due to the presence of a novel entity.
IL-5 is a Basophilopoietin

Nerve growth factor (NGF) may also be a cofactor in the growth and differentiation of human basophilic cells. NGF synergizes with T cells or GM-CSF to produce this effect in vitro.3,6,15,16 NGF also synergizes with IL-5 in promoting differentiation of the basophilic lineage, using the HL-60 assay.3,6 Thus, IL-5 may be involved in cytokine interactions which promote local ingress, activation, and differentiation of eosinophilic as well as basophilic cells in allergic and related nonallergic tissue inflammation.

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