Type β Transforming Growth Factors Promote Interleukin-3 (IL-3)–Dependent Differentiation of Human Basophils But Inhibit IL-3–Dependent Differentiation of Human Eosinophils

By Christian Sillaber, Klaus Geissler, Renate Scherrer, Roswitha Kaltenbrunner, Peter Bettelheim, Klaus Lechner, and Peter Valent

Basophils and eosinophils share a common differentiation pathway. Factors regulating terminal commitment toward one cell type, however, have so far not been defined. Interleukin-3 (IL-3) is a potent differentiation factor for both human eosinophils and basophils. In the present study, the effects of various recombinant human (rh) growth regulators on IL-3–dependent growth of eosinophils and basophils were studied in a bone marrow (BM) suspension culture system (normal donors, n = 13). We found that type β transforming growth factors (TGFs) lead to a significant increase in the absolute numbers of basophils in BM cultures grown in the presence of IL-3 (day 14 of culture; IL-3: 133 ± 20 v IL-3 + TGF-β1: 231 ± 28 x 10^3/mL [P < .01]) and to an increase in the total histamine values (IL-3: 72.6 ± 22.2 v IL-3 + TGF-β1: 142.9 ± 37.3 ng/mL [P < .01]). Compared with rhIL-3 alone, in contrast, type β TGFs were found to inhibit the IL-3–dependent growth of eosinophils (IL-3: 170.4 ± 37.2 v IL-3 + TGF-β1: 16.7 ± 5.2 x 10^3/mL [P < .01]) and formation of eosinophil cationic protein in the same culture system. The effect of TGF-β1 (and TGF-β2) on IL-3–dependent differentiation of basophils and eosinophils was dose- and timedependent (maximum effects observed with 1 to 10 ng/mL of rhTGF-β1 or TGF-β2) and could be neutralized by an antibody specific for TGF-β1. In contrast to the TGFs, interferon-α (IFN-α) and IFN-γ were found to downregulate IL-3–dependent formation of both basophils (IL-3: 167 ± 33 v IL-3 + IFN-α: 67 ± 25 v IL-3 + IFN-γ: 65 ± 33 x 10^3/mL [P < .01]) and eosinophils (IL-3: 239 ± 5 v IL-3 + IFN-α: 81 ± 4 v IL-3 + IFN-γ: 67 ± 17 x 10^3/mL [P < .05]) in our culture system. Type β TGFs as well as the IFNs failed to directly induce differentiation of human basophils or eosinophils in the absence of other growth factors. Together, these results show that type β TGFs and IFNs are potent regulators of cytokine-dependent growth and differentiation of human allergic effector cells.

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VIM-D5 (CD15) were produced at the Institute of Immunology, University of Vienna, Austria, and kindly provided by O. Majdic and W. Knapp. MoAbs BMA-022 (anti-HLA-DR) and BMA-0110 (CD2) were purchased from Behringwerke (Marburg, Germany). MoAbs MY-7 (CD13), MY-9 (CD33), MY-10 (CD34), B1 (CD20), and anti-IL-2R1 were purchased from Coulter Immunology (Hialeah, FL) and MoAbs Leu-1 (CD5), Leu-9 (CD7), and Leu-7 (CD57) from Becton Dickinson (Sunnyvale, CA). The anti-stem cell factor (SCF) receptor MoAb YB5.B8.8,39 recognizing human mast cells, was a kind gift from L.K. Ashman (Dept. of Microbiology and Immunology, University of Adelaide, Australia). MoAb CLB-Ery3 (anti-blood group H antigen) was kindly sent by P.T. Tetteroo (Univ. of Amsterdam, The Netherlands). MoAb Bsp-1 was a kind gift from M. Bodger (Christchurch Hospital, Christchurch, New Zealand). MoAbs BA-2 (CD9), 84H-10 (CD54), and E-124-2-8 (anti-IgE) were purchased from Immunotech (Marseille, France). MoAbs G035 (CD17), M1M23 (CD18), Tac (CD25), LB-2 (CD54), and B5F (CD54) were obtained from the III. and IV. International Workshop and Conference on Human Leukocyte Differentiation Antigens, Oxford, UK, 1986, and Vienna, Austria, 1989.31 For inhibition experiments, a polyclonal anti-TGF-β1 antibody, anti-TGF-β (Collaborative Research Inc, Bedford, MA), was used.

**BM Culture System**

Cultures were established essentially as described previously10 using BM cells of 13 normal donors after informed consent was given. In brief, heparinized BM samples were diluted in RPMI 1640 medium and layered over Ficoll (1.077 density) to separate mononuclear cells (MNCs). After washing the cells in RPMI medium, 0.5 × 10^6 BM MNCs were placed in each well of 24-well microculture plates (Costar, Cambridge, MA) in 1 mL RPMI medium containing 10% fetal calf serum (FCS). Cultures were maintained in a humidified atmosphere of 5% CO2 at 37°C. The following cytokines were added and/or in combination: rhIL-3, rhGM-CSF, rhIL-5, rhTGF-β1, rhTGF-β2, rhIFN-α, and rhTNF-γ. Unless otherwise stated, cultures were harvested on day 14. Morphology and percentage counts were examined after cytoospin preparation and Giemsa staining, by two independent observers. Total cell counts were determined using a hemocytometer (Coulter Immunology). Absolute numbers of basophils, eosinophils, and other cell types in culture were calculated from total cell numbers and differential counts.

IL-3 and TGF-β1 were also tested on BM cells (from three normal donors) in a colony assay system established as described previously.8 In brief, 1 × 10^5 nonadherent BM MNCs were cultured in Iscove’s modified Dulbecco’s medium (IMDM), 20% FCS, 1% bovine serum albumin (BSA), 5 × 10^-4 mol/L 2-mercaptoethanol, 0.8% methyl cellulose (1,500 cp) as viscous support, and IL-3 (100 U/mL) and/or TGF-β1 (10 ng/mL). One milliliter of the assay mixture was placed in 35-mm petri dishes (in triplicates) and incubated at 37°C in 5% CO2 for 14 days. Colonies were then counted, picked individually, and washed. Cells were stained with Giemsa after cytoospin preparation to determine the cell type(s) present in each individual colony.

**Determination of Cellular Histamine and Eosinophil Cationic Protein (ECP) in BM Cell Cultures**

As nonsubjective markers of cellular differentiation, total histamine and ECP values were determined in whole cell suspensions in BM cell cultures. Total histamine was measured after cell lysis in distilled water and freeze thawing. Histamine and ECP were measured using commercial radioimmunoassays (RIA) (Histamine: Immunotech; ECP: Pharmacia Diagnostics, Uppsala, Sweden).

**Histamine Release Assay**

Histamine release from blood basophils of nonallergic donors (n = 4) was performed as described previously.32 Briefly, peripheral blood cells were fractionated by incubation in 1.1% dextran T 70 and 0.008 mol/L EDTA for 90 minutes at room temperature (RT). Cells of the granulocyte-rich upper layer were then centrifuged (180g at RT for 8 minutes) and washed twice in Ca-free PIPES buffer (25 mmol/L PIPES, 110 mmol/L NaCl, and 5 mmol/L KCl, pH 7.35). Granulocytes were resuspended in PIPES buffer containing 2.0 mmol/L CaCl2 and adjusted to a final concentration of 2.5 × 10^9/mL. After incubation with cytokines (rHL-3, 100 U/mL; rTGF-β1, 10 ng/mL; rIL-3 plus TGF-β1) or control medium for 30 minutes at 37°C, histamine release was performed using various concentrations (0.1 μg/mL up to 10 μg/mL) of MoAb E-124-2-8 specific for IgE. Thereafter, histamine was measured in cell-free supernatants after centrifugation (350g for 8 minutes at 4°C). Total histamine in cell suspensions was quantified after cell lysis. Histamine release was expressed as percentage of total histamine.

**Purification of Blood Basophils Using MoAb and Complement**

Basophils were purified to homogeneity from the peripheral blood of two CGL donors, as described,32,33 after informed consent was given. In brief, blood MNCs were obtained by Ficoll gradient centrifugation (30 minutes at 350g) and washed twice in phosphate-buffered saline (PBS). Cells (5 × 10^9) were then incubated with 100 μg of VIM-D5 antibody for 45 minutes at 4°C. Thereafter, cells were washed and incubated with 5 mL of rabbit complement (Behringwerke) at RT for 90 minutes. The remaining cells were exposed to a mixture of MoAbs: VIT-3, VIB-C5, BMA-0110, Leu1, Leu7, Leu9, B1, BMA-022, CLB-ERY3, VIM13, and VIM-D5 (25 μg of each MoAb). After incubation for 45 minutes at 4°C, cells were washed and exposed to 4 mL of complement, washed again, and layered over Ficoll to remove cell ghosts. Thereafter, basophils were washed again and the purity of the cells was determined by morphologic examination after Giemsa staining. Cell viability was assessed by trypan blue exclusion. The purity of basophils was 92% and 96%, respectively.

**Cell Typing With MoAbs**

Combined toluidine blue/immunofluorescence staining technique. Expression of cell surface antigens on cultured metachromatic cells was investigated by a combined toluidine blue/immunofluorescence staining technique using MoAbs as described.33 In brief, cells were incubated with the various MoAbs for 30 minutes at 4°C, washed twice, and then incubated with a second step MoAb [ie, goat F(ab')2: antimouse IgG + IgM antibodies]. Cells were then washed and fixed in glutaraldehyde at RT for 1 minute (0.025% glutaraldehyde in fixation buffer: 0.1 mol/L Tris buffer, 1 vol% glucose, pH 7.8). After washing in PBS, cells were incubated with toluidine blue (Sigma, St Louis, MO) (0.0125 wt/vol in PBS) at RT for 10 minutes. Cells were again washed in PBS and then the toluidine blue-stained cells were identified in bright field; thereafter, cells were examined for reactivity with MoAb under fluorescent light.

**Flow cytometric evaluation.** To quantify expression of cell surface membrane markers on highly enriched human blood basophils (after exposure to rh cytokines), cells were stained with MoAb (see above) and then analyzed by flow cytometry (fluorescence-
activated cell sorter (FACS; Becton Dickinson), as described previously. For evaluation of expression of high-affinity IgE-binding sites, cells (either highly enriched CGL basophils after exposure to cytokines, or metachromatic cells grown in BM cell cultures) were first exposed to monoclonal IgE (Chemicon) (2 μg/mL) for 6 hours, washed twice, and thereafter stained with anti-IgE MoAb E-124-2-8.

Statistical Analyses

The significance of differences was assessed by using standard statistical tests, including the Student’s t-test. The results were considered significantly different when \( P < 0.05 \).

RESULTS

Effects of Type β TGFs and IFNs on Cytokine-Dependent Differentiation of Human Basophils and Synthesis of Histamine

rhIL-3 has recently been shown to induce growth and terminal differentiation of basophils and eosinophils in BM or cord blood cell suspension cultures. In the first step of this study, the effects of various rh growth regulators (TGF-β1, TGF-β2, IFN-α, and IFN-γ) on IL-3-dependent differentiation of basophils and eosinophils in a BM suspension culture system were analyzed. IFN-α and IFN-γ were found to downregulate IL-3-dependent formation of both basophils (day 14; IL-3, 100 U/mL: 167 ± 33 \( \times \) 10 \(^3\) cells/mL) and eosinophils (IL-3: 239 \( \times \) 10 \(^3\) cells/mL) for 6 hours, washed twice, and thereafter stained with anti-IgE MoAb E-124-2-8.

In contrast, TGF-β1 (in the presence of rhIL-3, 100 U/mL) was found to lead to a significant increase in the total number of basophils (day 14 of culture; IL-3: 133 \( \times \) 10 \(^3\) cells/mL + IFN-α: 1,000 U/mL: 67 ± 25 \( [P < 0.01] \) vs IL-3 + IFN-γ, 1,000 U/mL: 65 ± 33 \( \times \) 10 \(^3\)/mL; \( P < 0.01 \); total histamine on day 14; IL-3: 120 ± 29 \( \times \) 10 \(^3\) ng/mL + IFN-α: 52 ± 20 \( [P < 0.02] \) vs IL-3 + IFN-γ: 56 ± 24 ng/mL) and eosinophils (IL-3: 239 \( \times \) 5 \( \times \) 10 \(^3\) cells/mL + IFN-α: 81 ± 4 \( [P < 0.05] \) vs IL-3 + IFN-γ: 67 ± 17 \( \times \) 10 \(^3\)/mL; \( P < 0.02 \)) in our culture system. Figure 1 shows the dose-dependent effects of IFN-α and IFN-γ on IL-3-induced formation of total histamine levels in (day 14) BM cell cultures.

Figure 1. Effects of IFN-α and IFN-γ on IL-3-dependent formation of total histamine in BM cell cultures. BM MNCs were cultured in RPMI 1640 medium with 10% FCS in the presence of various concentrations of rhIFNa and rhIFNγ (as indicated) for 14 days. Thereafter, cells were lysed in distilled water and analyzed for total histamine values by RIA. Results represent the mean ± SD from triplicate cultures obtained from one donor. \( \times \) The medium control (no addition of cytokines).

Recent studies have shown that GM-CSF and IL-5 support differentiation of human basophils under certain conditions. Therefore, we also tested the effects of GM-CSF and IL-5 on basophil growth in the presence of TGF-β in our BM culture system. GM-CSF alone showed no substantial basophil-promoting activity in our BM cell culture system. However, a significant formation of basophils (and cellular histamine) was observed in cultures supplemented with a combination of TGF-β and GM-CSF (Fig 5).

IL-5 induced a slight increase in formation of basophils compared with control (Fig 5). However, no significant increase in basophils could be detected in BM cell cultures...
EFFECTS OF TGF-β ON HUMAN BASOPHILS

![Graphs showing differential cell counts and histamine levels](image)

**Fig 2.** (A) TGF-β-induced changes in differential cell counts in BM cell cultures supplemented with rhlL-3 (100 U/mL) or control medium for 14 days. Cells were cultured and stained as described in the text and analyzed by two independent observers. Cell numbers for each lineage were calculated from total cell numbers (IL-3: 648 ± 75; IL-3 + TGF-β1: 518 ± 48) and differential counts. Values of seven different donors are depicted. Results represent the respective means of duplicate determinations. (B) TGF-β1-induced increase in IL-3-dependent formation of cellular histamine in BM cell cultures. Cellular histamine in cultures supplemented with rhlL-3 (100 U/mL) or rhlL-3 (100 U/mL) + rhTGF-β1 (10 ng/mL) was determined on day 14. The results of seven donors (the same as in A) are depicted. Each value represents the mean from triplicate cultures.

![Histamine levels over time](image)

**Fig 3.** Dose- and time-dependent effects of TGF-β on IL-3-dependent differentiation of human basophils. (A) Dose-dependent effect of TGF-β1 and TGF-β2 on IL-3 (100 U/mL)-induced synthesis of histamine in BM suspension cultures. The effects of various concentrations of rhTGF-β1 and rhTGF-β2 on BM cells grown in the presence of rhIL-3 (100 U/mL) are shown. On day 14 of culture, cells were harvested and total cellular histamine was counted. Results represent the mean ± SD of triplicate cultures. (B) Time dependency. The effect of TGF-β1 (10 ng/mL) on IL-3-dependent growth of basophilic cells from their BM precursors at various days of culture is shown.

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**Fig 2.** (A) TGF-β1—induced changes in differential cell counts in BM cell cultures supplemented with rhlL-3 (100 U/mL) or control medium for 14 days. Cells were cultured and stained as described in the text and analyzed by two independent observers. Cell numbers for each lineage were calculated from total cell numbers (IL-3: 648 ± 75; IL-3 + TGF-β1: 518 ± 48) and differential counts. Values of seven different donors are depicted. Results represent the respective means of duplicate determinations. (B) TGF-β1—induced increase in IL-3—dependent formation of cellular histamine in BM cell cultures. Cellular histamine in cultures supplemented with rhlL-3 (100 U/mL) or rhlL-3 (100 U/mL) + rhTGF-β1 (10 ng/mL) was determined on day 14. The results of seven donors (the same as in A) are depicted. Each value represents the mean from triplicate cultures.

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**Characterization of Basophilic Cells in BM Cell Cultures**

The calculated amount of histamine per basophil (0.2 to 1.3 pg) assessed on day 14 did not vary in cultures supplemented with different cytokines or combinations of cytokines. As determined by combined toluidine blue/immunofluorescence staining, the cultured metachromatic cells (grown in the presence of IL-3 or in the presence of IL-3 plus TGF-β) exhibited a basophil phenotype (CD11b+, CD13+, CD15−, YB5.B8−, IgE-R−). Mast cells could not be detected under any culture condition. We were also unable to detect any effect of TGF-β1 or a combination of IL-3 and TGF-β1 on the cell surface marker profile of highly enriched human CGL basophils (data not shown).
**TGF-β1 Downregulates IL-3-Dependent Differentiation of Human Eosinophils and Synthesis of ECP**

TGF-β1 was found to downregulate the spontaneous as well as the IL-3-dependent formation of total cell counts in culture (total cell numbers \( \times 10^3/mL \) on day 14: control: 364 ± 119 v TGF-β1: 198 ± 51 v IL-3: 698 ± 288 v IL-3 + TGF-β1: 506 ± 128). TGF-β1 was found to almost completely inhibit IL-3-dependent differentiation of human eosinophils (absolute number of eosinophils on day 14: IL-3: 170.4 ± 37.2 v IL-3 + TGF-β1: 16.7 ± 5.2 \( \times 10^3/mL \) [\( P < .01 \)]) (Figs 2A, 4, and 6) and formation of eosinophil cationic protein compared with rhIL-3 alone (ECP on day 14; control: 4.5 ± 1.3 ng/mL; IL-3: 21.5 ± 0.4; IL-3 + TGF-β1: 4.7 ± 1.8) (Fig 6). As shown in Fig 6, neither GM-CSF nor IL-5 (both potent activators of eosinophil differentiation/maturatation) were found to restore eosinophil growth in cultures supplemented with IL-3 and TGF-β1, although both IL-5 and GM-CSF were found to promote IL-3-dependent differentiation of eosinophils in all donors tested (n = 3) (absolute number of eosinophils \( \times 10^3/mL \) on day 14: IL-3: 504 ± 50 v IL-3 + GM-CSF: 1,508 ± 63 v IL-3 + IL-5: 953 ± 103 v IL-3 + TGF-β1: 70 ± 8). TGF-β was also found to inhibit GM-CSF- and IL-5-dependent formation of eosinophils in (day 14) BM cell cultures (GM-CSF, 100 U/mL: 565 ± 173 v GM-CSF, 100 U/mL + TGF-β1, 10 ng/mL: 81 ± 51 eosinophils \( \times 10^3/mL \) [\( P < .05 \)]; IL-5, 100 U/mL: 266 ± 67 v IL-5, 100 U/mL + TGF, 10 ng/mL: 22 ± 6 eosinophils \( \times 10^3/mL \) [\( P < .05 \)]). Similar results on cytokine-induced differentiation of human eosinophils were obtained with TGF-β2, compared with TGF-β1 (Fig 4). Thus, type β TGFs induce a shift in the eosinophil/basophil differentiation program towards the basophil pathway. A similar shift was seen in a BM colony assay system (Table 2). In particular, TGF-β1 was found to downregulate IL-3-dependent formation of eosinophil-containing colonies (37.8% ± 4.9% colony-forming units [CFU]/10⁶ BM MNCs in cultures grown with IL-3 + TGF-β1; compared with IL-3 alone [100%]) (\( P < .05 \)) and to cause an increase in basophil-containing colonies (127% ± 14%) (Table 2).

**Fig 4.** Effects of TGF-β1 (solid symbols) on IL-3–dependent differentiation of eosinophils (○–○) and of basophils (■–■). Comparison with TGF-β1 is also shown (basophils (□–□); eosinophils (○–○)). BM cells were cultured as described in the legend to Fig 1 and analyzed on day 14.

**Fig 5.** Effect of TGF-β1, GM-CSF, and IL-5 on basophilic differentiation in BM cell cultures. BM cells were cultured with TGF-β1 (10 ng/mL) in the presence or absence of either rhGM-CSF (100 U/mL), rhIL-5 (100 U/mL), or control medium for 14 days and thereafter analyzed as described in the text. Results represent the mean ± SD of triplicate determinations. One of two experiments (with almost identical results) is depicted. (■) Basophils; (□) histamine.

**Fig 6.** Effect of eosinopoietic growth factors (GM-CSF and IL-5) on TGF-β1–induced suppression of IL-3–dependent formation of eosinophils and cellular ECP in BM cell cultures. BM cells were cultured in the presence of various growth factors (as indicated) for 14 days and then analyzed as described in the text. Results represent the mean of triplicate determinations.
TABLE 2. Effect of TGF-β1 on IL-3-Induced Formation of Eosinophil-Containing, Basophil-Containing, and Mixed Colonies From BM Progenitor Cells

<table>
<thead>
<tr>
<th>Donor</th>
<th>Cytokine</th>
<th>No. of Colonies/10^6 MNC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>eo</td>
</tr>
<tr>
<td>1</td>
<td>None</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>IL-3</td>
<td>49 ± 11</td>
</tr>
<tr>
<td></td>
<td>IL-3 + TGF-β1</td>
<td>17 ± 1</td>
</tr>
<tr>
<td>2</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>IL-3</td>
<td>34 ± 4</td>
</tr>
<tr>
<td></td>
<td>IL-3 + TGF-β1</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>3</td>
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<td>NT</td>
</tr>
<tr>
<td></td>
<td>IL-3</td>
<td>26 ± 3</td>
</tr>
<tr>
<td></td>
<td>IL-3 + TGF-β1</td>
<td>11 ± 3</td>
</tr>
</tbody>
</table>

BM progenitor cells of three normal donors were cultured in a colony assay as described in the text. On day 14 of culture, the number of CFU ± SD was determined.

Abbreviations: eo, eosinophil; ba, basophil; eo/ba, eosinophil/basophil; NT, not tested.

**Effect of TGF-β1 on IL-3-Dependent Increase in Releasability of Normal Human Peripheral Blood Basophils**

To investigate the influence of TGF-β1 on the function of normal mature human basophils, anti-IgE-induced histamine release (from basophils of four nonallergic donors) was analyzed after incubation of basophils with rhIL-3 (100 U/mL), TGF-β1 (10 ng/mL), or a combination of both. IL-3 increased the capacity of the basophils to release histamine compared with control. However, no effect of TGF-β1 on IL-3-induced changes in basophil histamine releasability was found in four different donors. TGF-β1 also failed to enhance or suppress IgE-dependent release in the absence of rhIL-3, or to induce histamine release from human basophils directly (results not shown).

**DISCUSSION**

IL-3 promotes the growth and differentiation of human basophils and eosinophils.7-9,14 Both cell types share a similar differentiation pathway and have been described to share a common progenitor.5,6 Factors regulating terminal commitment to one cell type (either basophil or eosinophil) have not been characterized in detail so far. TGFs promote or inhibit hematopoietic cell growth depending on the target cell type and/or presence of other growth factors.34-36 The results of this study show that type β TGFs differentially regulate IL-3-dependent differentiation of human eosinophils and basophils.

The increase in the absolute number of basophils observed in BM cell cultures grown in the presence of rhIL-3 + TGF-β1 compared with rhIL-3 alone might be explained by a shift in the program of commitment of IL-3-induced progenitors (giving rise to either eosinophils or basophils). This would explain the simultaneous loss of eosinophils in these cultures and the decrease in eosinophil-containing colonies upon stimulation with TGF-β1 + IL-3, compared with colonies grown in the presence of IL-3 alone. In this regard, it is also noteworthy that in cultures maintained in the presence of IL-3 and TGF-β1, no significant amounts of so-called “eosinobasophils” (probably representing an advanced stage of a common differentiation pathway of eosinophils and basophils49) could be detected in BM cultures, whereas these cells were seen in larger amounts on day 14 in cultures containing IL-3 alone. However, TGF-β apparently acts on many progenitor cell classes and the origin of eosinophils and basophils from a common progenitor cell may only hold true for a subset of these cells. Therefore, the regulation of growth of basophils and eosinophils in our culture system may have involved a number of progenitor cell types. We also observed a decrease (in the number) of various types of (IL-3-induced) eosinophil-containing colonies upon stimulation with TGF-β1 (including CFU eosinophil/basophil).

Substantial evidence exists that IL-3-dependent differentiation of eosinophils in BM cultures depends (in part) on the presence of (IL-3-stimulated) accessory cells, eg, macrophages or fibroblasts and/or their products (such as GM-CSF known to be eosinophilopoietic).10,35-37 TGF-β1, in turn, is well known to inhibit growth of macrophages and to deactivate these cells in culture.38 Therefore, one may speculate that the downregulatory effect of TGF-β1 on IL-3-dependent differentiation of eosinophils was in part an indirect effect and caused by accessory cell depletion and/or deactivation. On the other hand, eosinophils by themselves express IL-3-binding sites and have prolonged survival in the presence of rhIL-3.39,40 Moreover, in the present study we were unable to restore eosinophilopoiesis in cultures supplemented with TGF-β1 and IL-3 by adding eosinophil growth factors (ie, GM-CSF and IL-5). Therefore, we believe that the effects of TGF-β1 on eosinophilopoiesis in our culture system were mostly due to direct inhibition of eosinophilic (but not basophilic) precursor cell growth. This would be in agreement with the recent observation that TGF-β affects hematopoietic precursor cell growth and commitment41 and would be in agreement with our colony assay data (in which no significant amounts of stroma cells are present). Interestingly, the IFNs tested (ie, IFN-α and IFN-γ) were found to inhibit IL-3-dependent growth of both eosinophils and basophils in our culture system.

IL-3 has recently been shown to upregulate the capacity of normal human basophils to respond to releasing compounds.32,42,43 In the present study, IL-3-dependent changes in the releasability of human basophils were neither increased nor suppressed by TGF-β1. TGF-β also failed to induce histamine release from human basophils directly. In mice, TGF-β1 has been shown to inhibit IL-3-dependent proliferation and differentiation of mast cells in BM cell cultures.44 An effect of human TGF-β1 on mast cell proliferation or differentiation in IL-3-induced BM cell cultures could not be shown in our culture system, because all metachromatic cells cultured in the presence of either rhIL-3 or in the presence of a combination of rhIL-3 and...
TGF-β₁ resembled human basophils and not mast cells, as assessed by cell surface marker analyses.

Recent studies have shown that TGFs are formed in larger amounts in human BM as well as at sites of allergic inflammation, such as the bronchial airway system, and that TGF-β₁ may play a role as a local growth factor. In addition, studies by Denburg et al have shown that local differentiation of basophils and eosinophils from myeloid progenitors may play a role in allergic processes. Our results show that cytokine (ie, IL-3)-dependent differentiation of basophils and eosinophils in vitro may thus play an important role in the regulation of cytokine-dependent growth and differentiation of granulocytic effector cells in allergic and/or inflammatory processes associated with the production of cytokines.

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