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 eosinophils and 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 10³/μL [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 < .016]) 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 10³/μL [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 time-dependent (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 10³/μL [P < .01]) and eosinophils (IL-3: 239 ± 5 v IL-3 + IFN-α: 81 ± 4 v IL-3 + IFN-γ: 67 ± 17 x 10³/μL [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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ASOPHILIC and eosinophilic granulocytes are effectors of allergic events. They are replenished throughout life from multipotential, bipotential, and unipotential progenitors. The growth of eosinophilic and basophilic progenitors as well as the terminal differentiation/maturation of both cell types is regulated by a variety of cytokines. Interleukin-3 (IL-3), a product of activated immune cells, has been shown to induce in vitro proliferation of hematopoietic progenitor cells, including those giving rise to eosinophils and/or basophils, and to promote terminal differentiation/maturation of eosinophilic and basophilic granulocytes. A similar response of hematopoietic (progenitor) cells, and, in particular, in the basophil/eosinophil series, has been observed in healthy rhesus monkeys treated with recombinant human IL-3 (rhIL-3). A number of other cytokines are supposed to regulate growth of human basophils and eosinophils as well. Granulocyte-macrophage colony-stimulating factor (GM-CSF), a major regulator of myeloid cell growth, has been shown to promote growth and differentiation of eosinophilic (progenitor) cells. IL-5, a pleiotropic immune modulator, promotes terminal maturation of eosino-

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ASOPHILIC and eosinophilic granulocytes are effectors of allergic events. They are replenished throughout life from multipotential, bipotential, and unipotential progenitors. The growth of eosinophilic and basophilic progenitors as well as the terminal differentiation/maturation of both cell types is regulated by a variety of cytokines. Interleukin-3 (IL-3), a product of activated immune cells, has been shown to induce in vitro proliferation of hematopoietic progenitor cells, including those giving rise to eosinophils and/or basophils, and to promote terminal differentiation/maturation of eosinophilic and basophilic granulocytes. A similar response of hematopoietic (progenitor) cells, and, in particular, in the basophil/eosinophil series, has been observed in healthy rhesus monkeys treated with recombinant human IL-3 (rhIL-3). A number of other cytokines are supposed to regulate growth of human basophils and eosinophils as well. Granulocyte-macrophage colony-stimulating factor (GM-CSF), a major regulator of myeloid cell growth, has been shown to promote growth and differentiation of eosinophilic (progenitor) cells. IL-5, a pleiotropic immune modulator, promotes terminal maturation of eosino-

The factors regulating terminal commitment towards either basophils or eosinophils, however, are poorly defined.

Type β transforming growth factors (TGFs) are polypeptides controlling the proliferation and differentiation of many cell types. These factors have stimulatory or inhibitory activities depending on the target cell type and the presence of other growth factors. We now report that type β TGFs promote IL-3-dependent differentiation of basophils, but inhibit IL-3-dependent differentiation of human eosinophils in a bone marrow (BM) suspension culture system.

MATERIALS AND METHODS

Cytokines

rhIL-3 and rhGM-CSF (both expressed in Escherichia coli) were provided by the Genetics Institute (Cambridge, MA). Purified rhIL-3 showed a specific activity of 4.5 x 10⁶ U/mg protein and purified rhGM-CSF a specific activity of 1.3 x 10⁷ U/mg protein, as determined by a myeloblast bioassay described by Griffith et al. rhTGF-β1 (biologic activity, 8 x 10⁶ U/mg, as determined by a growth inhibition assay using MvLu mink lung epithelial cells) was purchased from Chemicon (Temecula, CA). rhTGF-β2 (biologic activity, 5 x 10⁶ U/mg protein) was purchased from Boehringer Ingelheim (Ingelheim, Germany) and rhIFN-γ (2.5 x 10⁷ U/mg protein) was provided by Boehringer Mannheim (Mannheim, Germany). rhIL-5 (biologic activity, 0.1 x 10⁶ U/mg protein) was purchased from Amersham (Buckinghamshire, UK).

Monoclonal Antibodies (MoAbs)

For isolation of chronic granulocytic leukemia (CGL) basophils by complement-mediated cell lysis and/or for cell typing, the following MoAbs were used: MoAbs VIT-3 (CD3), VIM-12 (CD11b), VIB-C5 (CD24), VIM-2 (CDw65), VIM-13 (CD14), and
BM Culture System

Cultures were established essentially as described previously\(^{10}\) 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% CO\(_2\) at 37°C. The following cytokines were added alone and/or in combination: rhIL-3, rhGM-CSF, rhIL-5, rhTGF-\(\beta_1\), rhIL-3 plus TGF-\(\beta_1\), and a control medium for 30 minutes at 37°C. For inhibition experiments, a polyclonal anti-TGF-\(\beta_1\) antibody, anti–TGF-\(\beta_1\) (Collaborative Research Inc, Bedford, MA), was used.

**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 KCI, pH 7.35). Granulocytes were resuspended in PIPES buffer containing 2.0 mmol/L CaCl\(_2\) and adjusted to a final concentration of 2.5 × 10\(^6\)/mL. After incubation with cytokines (rhIL-3, 100 U/mL; rhTGF-\(\beta_1\), 10 ng/mL; rhIL-3 plus TGF-\(\beta_1\)) 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\(^6\)) 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-
Differentiation of Human Basophils

Histamine Effects of Type 636
considered significantly different when \( P < .05 \).

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 \( P < .05 \).

RESULTS
Effects of Type \( \beta \) 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.9-13 In the first step of this study, the effects of various rh growth regulators (TGF-\( \beta_1 \), TGF-\( \beta_2 \), IFN-\( \alpha \), and IFN-\( \gamma \)) on IL-3-dependent differentiation of basophils and eosinophils in a BM suspension culture system were analyzed. IFN-\( \alpha \) and IFN-\( \gamma \) were found to downregulate IL-3-dependent formation of both basophils (day 14; IL-3, 100 U/mL: 167 ± 33 v IL-3, 100 U/mL + IFN-\( \alpha \), 1,000 U/mL: 67 ± 25 \([P < .01]\) v IL-3 + IFN-\( \gamma \), 1,000 U/mL: 65 ± 33 \(\times 10^3\)/mL \([P < .01]\); total histamine on day 14: IL-3: 120 ± 29 v IL-3 + IFN-\( \alpha \): 52 ± 20 \([P < .02]\) v IL-3 + IFN-\( \gamma \): 56 ± 24 ng/mL) and eosinophils (IL-3: 239 ± 5 v IL-3 + IFN-\( \alpha \): 81 ± 4 \([P < .05]\) v IL-3 + IFN-\( \gamma \): 67 ± 17 \(\times 10^3\)/mL \([P < .02]\)) in our culture system. Figure 1 shows the dose-dependent effects of IFN-\( \alpha \) and IFN-\( \gamma \) on IL-3-induced formation of total histamine levels in (day 14) BM cell cultures.

In contrast, TGF-\( \beta_1 \) (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 ± 20 v IL-3 + TGF-\( \beta_1 \): 231 ± 28 \(\times 10^3\)/mL \([P < .01]\)) and to an increase in the total histamine values (IL-3: 72.6 ± 22.2 v IL-3 + TGF-\( \beta_1 \): 142.9 ± 37.3 ng/mL \([P < .015]\)) compared with rhIL-3 alone (Fig 2). This effect of TGF on IL-3-induced basophil differentiation was found to be dose-dependent with maximum stimulation using 1 to 10 ng/mL of recombinant TGF-\( \beta_1 \) (Fig 3A). Figure 3B shows the time-dependent effect of TGF-\( \beta_1 \) on the synthesis of histamine in cultures supplemented with IL-3. The effect of TGF-\( \beta_1 \) on IL-3-dependent differentiation of human basophils could be neutralized in all donors (n = 3) tested by using anti-TGF-\( \beta_1 \) antibody (90% to 100% inhibition of TGF-\( \beta_1 \) effects, \( P < .03 \)) (Table 1). TGF-\( \beta_1 \) failed to induce differentiation of basophils in BM cell cultures in the absence of rhIL-3. Almost identical effects of TGF-\( \beta_1 \) and TGF-\( \beta_2 \) on IL-3-dependent growth of basophils and synthesis of histamine were observed (\( P > .05 \)) (Figs 3A and 4). No significant effects of TGF-\( \beta_2 \) on the formation of neutrophils, monocytes/macrophages, or lymphocytes (in IL-3-induced BM cell cultures) were observed (Fig 2).

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-\( \beta_5 \) 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-\( \beta_1 \) 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.
Fig 2. (A) TGF-β-induced changes in differential cell counts in BM cell cultures supplemented with rhIL-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-β: 518 ± 48) and differential counts. Values of seven different donors are depicted. Results represent the respective means of duplicate determinations. (B) TGF-β-induced increase in IL-3-dependent formation of cellular histamine in BM cell cultures. Cellular histamine in cultures supplemented with rhIL-3 (100 U/mL) or rhIL-3 (100 U/mL) + rhTGF-β (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.

grown in the presence of TGF-β1 plus IL-5, compared with IL-5 alone (Fig 5).

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).

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Fig 3. Dose- and time-dependent effects of TGF-βs 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.
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 × 10⁷/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 × 10⁴/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/maturation) 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 × 10⁷/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 × 10⁷/mL [P < .05]; IL-5, 100 U/mL: 266 ± 67 v IL-5, 100 U/mL + TGF-β₁, 10 ng/mL: 22 ± 6 eosinophils × 10⁷/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).
was analyzed after incubation of basophils with rhIL-3 (100 pg/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-β 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).

**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 ng/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-β 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).

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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.

**DISCUSSION**

IL-3 promotes the growth and differentiation of human basophils and eosinophils. Both cell types share a similar differentiation pathway and have been described to share a common progenitor. 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. 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 basophils) 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). TGF-β1, in turn, is well known to inhibit growth of macrophages and to deactivate these cells in culture. 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. 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 commitment 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. 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. 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,⁴ 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.⁵ Our results show that cytokine (ie, IL-3)-dependent differentiation of human basophils and eosinophils in vitro by BM-derived precursor cells is regulated by TGF-β₁ and TGF-β₂, as well as by the IFNs. Type β TGFs and the IFNs 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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REFERENCES

19. Clutterbuck EJ, Hirst EMA, Sanderson CJ: Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: Comparison and interaction with IL-1, IL-3, IL-6, and GM-CSF. Blood 73:1504, 1989


