# The In Vivo Effects of Recombinant Human Interleukin-3: Demonstration of Basophil Differentiation Factor, Histamine-Producing Activity, and Priming of GM-CSF–Responsive Progenitors in Nonhuman Primates

By P. Mayer, P. Valent, G. Schmidt, E. Liehl, and P. Bettelheim

Recently human interleukin-3 (IL-3) produced by molecular cloning was characterized as a growth factor for basophils and eosinophils in human bone marrow cultures. Since we found a similar activity of the human factor on simian bone marrow cells, we investigated the in vivo effects of recombinant human (rh) IL-3 in healthy rhesus monkeys (n = 10). rh IL-3 was administered subcutaneously (SC) to monkeys at different doses (11, 33, and 100 $\mu$g/kg/d) for 14 days followed by subsequent rh GM-CSF administration (5.5 $\mu$g/kg/d SC) for another two weeks. During the second week of rh IL-3 administration monkeys responded with a twofold to threefold increase of WBCs caused by a dose-dependent elevation of basophils (up to 40% of WBCs) and eosinophils. rh IL-3 also induced a dose-dependent increase of histamine (up to 700-fold above normal values) in monkey blood cells. Administration of rh GM-CSF to rh IL-3 pretreated monkeys resulted in a twofold enhanced increase in WBCs (due mainly to eosinophils and neutrophils) compared with animals treated with rh GM-CSF alone. Simultaneous administration of both cytokines (100 $\mu$g/kg rh IL-3 + 5.5 $\mu$g/kg rh GM-CSF SC) to two separate monkeys for 14 days induced a WBC elevation similar to that observed in monkeys treated with rh GM-CSF alone. In conclusion, our results indicate that rh IL-3 is a differentiation factor for blood basophils and primes the hematopoietic system for subsequent rh GM-CSF actions.

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THE MOST IMPORTANT growth and differentiation factors for myeloid cells are the colony stimulating factors (CSFs). Among these, GM-CSF promotes growth and differentiation of multipotent and unipotent hematopoietic precursor cells and provides a relative growth advantage for neutrophils, eosinophils, and monocytes.[1-3]

Interleukin-3 (IL-3) is another multipotential hematopoietic cytokine that exhibits an even broader spectrum of biological activities within the hematopoietic system.[4,5] Murine IL-3 has been shown (1) to stimulate synthesis of key enzymes of cell metabolism including those involved in histamine biosynthesis in hematopoietic cells,[6] (2) to induce self-renewal of primitive pluripotent stem cells (CFU-blasts),[7,8] and (3) to support the growth of multipotent and unipotent precursor cells[9] and of mast cells.[10] The human equivalent of murine IL-3 has recently been identified by expression cloning as a 25-Kd polypeptide, which shares a 29% sequence homology with murine and a 96% homology with gibbon IL-3.[11] This significant homology between cloned human and simian IL-3 provided a rationale for their application in heterologous systems.[12,13]

Recently recombinant human (rh) IL-3 was found to induce formation of human blast cell and multipotential colonies and of unipotent myeloid colonies including those of the eosinophil type.[14,15] We and others have demonstrated a very similar result in a human bone marrow suspension culture system in which rh IL-3 supported formation of blasts and eosinophils and induced differentiation of basophils and formation of intracellular histamine during a 14-day culture period.[16-18] Thus the in vitro actions of both human recombinant rh GM-CSF and rh IL-3 on growth and differentiation of human stem cells are now well defined. However, whereas the in vivo effects of rh GM-CSF have already been studied and even clinical usefulness has been proven,[19-21] little has so far been reported on the in vivo action of rh IL-3.

The purpose of this study was (1) to analyze the in vivo effects of rh IL-3 on changes of WBC counts in healthy rhesus monkeys, (2) to evaluate the effect of rh IL-3 on basophil differentiation and histamine kinetics of peripheral blood leukocytes, and (3) to investigate the effects of simul-taneous or subsequent rh GM-CSF administration on WBC levels in rh IL-3–treated monkeys.

## MATERIALS AND METHODS

*Animals.* Five female and five male adult rhesus monkeys, *Macaca mulatta,* approximately 4 to 8 years old and weighing between 5 and 13 kg, were housed in individual stainless steel cages. Monkeys were provided with ten changes per hour of fresh air conditioned to 23°C ± 2°C with a relative humidity of 60% ± 10%. They were maintained in a 12-hour light/dark cycle and were provided with tap water ad libitum and commercial primate chow and fruit. Research was conducted according to the principles enunciated in the *Guide for the Care and Use of Laboratory Animals,* prepared by the Institute of Laboratory Animal Resources, National Research Council.

*Sources of recombinant cytokines.* rh IL-3 used in this study was provided by Genetics Institute, Cambridge, MA. The nonglycosylated protein was extracted from *Escherichia coli* cells expressing the IL-3 cDNA from a plasmid vector. rh IL-3 accumulating intracellularly was purified to homogeneity by a series of chromatographic steps, including high-performance liquid chromatography (HPLC). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) demonstrated the presence of a single Coomassie blue staining band with a purity of ≥95% and a mol wt of 14 to 15 Kd. The in vitro biological activity was found to be $4.6 \times 10^6$ U/mg protein assayed by thymidine incorporation by chronic myelogenous leukemia (CML) myeloblasts as described.[22] The endotoxin content

was 30 ± 5 pg/mg as determined by the limulus assay[23] (Limulus amebocyte lysate, Whittacker MA Bioproducts, Walkersville, MD).

Glycosylated rh GM-CSF (Sandoz AG, Basle, Switzerland) derived from transfected Chinese Hamster ovary (CHO) cells was purified as described elsewhere.[24] Coomassie blue staining after SDS-PAGE revealed a purity of 98%. The biological activity determined by the CML myeloblast assay[22] was $3.5 \times 10^6$ U/mg protein polypeptide; the endotoxin content 1.4 ng ± 0.3/mg glycoprotein.

*Culture system for monkey bone marrow (BM) cells.* Monkey BM cells were obtained from the posterior iliac crest of three different animals and layered over Ficoll (1.077 density) to isolate mononuclear cells (MNC). MNC were washed three times in RPMI 1640 medium and resuspended in RPMI containing 10% autologous monkey serum (heat inactivated). MNC ($0.5 \times 10^6$/mL) were cultured as triplicates in 1-mL aliquots in 24-well microculture plates (Costar, Cambridge, MA). Cultures were maintained at 37°C and 5% $CO_2$ for 14 days. In vitro application of rh IL-3 and rh GM-CSF to monkey BM cells in cultures was adjusted to the in vivo administration system, with the exception of a 7- instead of a 14-day exposure to individual growth factors. In particular, rh IL-3 was tested at different concentrations (5, 10, 50 and 100 U/mL, respectively). Rh GM-CSF was added to cultures at a final concentration of 100 U/mL. Rh IL-3 and rh GM-CSF were tested simultaneously (a'100 U/mL) as well as in a sequential mode (rh IL-3: days 0 to 7, rh GM-CSF: days 7 to 14). All cultures were analyzed on day 14 for total cell counts (Coulter Counter ZBI), differential cell counts (determined by cytospin preparation and Giemsa staining), and total histamine levels (see below).

*Administration of rh IL-3 and rh GM-CSF to monkeys.* rh IL-3 and rh GM-CSF were prepared for the subcutaneous (SC) treatment by thawing the daily dose and diluting with 6 mL nonpyrogenic saline containing 0.5% monkey serum. Two milliliters of the rh IL-3 or rh GM-CSF solution were administered SC three times daily (at 9 AM, 1 PM, and 6 PM) over a period of 14 days. Control monkeys received SC injections of nonpyrogenic saline supplemented with 0.5% monkey serum.

*Hematologic examinations.* For hematologic examinations, blood was collected in EDTA-coated tubes on day −7 and −5 before treatment, at daily intervals during the treatment periods, and three times a week during the post-treatment period.

Parameters measured included the total counts of RBCs, WBCs, platelets, and determination of hemoglobin and hematocrit (Sysmex 2000, TOA; Tokyo, Japan). Differential blood cell counts were established as normal for rhesus monkeys[25] on the examination of 200 cells of Giemsa-stained blood smears by two independent observers.

*Determination of histamine in WBCs and blood plasma.* Histamine was measured using a commercial radioimmunoassay (RIA; Immunotech, Marseille, France) as described previously.[26] Briefly, standards and samples were prepared in duplicates by exposure to acylating reagent and mixed with ^{125}I-radiolabeled histamine. Solutions were placed in tubes coated with monoclonal antihistamine antibodies. After incubation for 18 hours at 4°C, nonbound iodinated histamine was removed from the tubes by aspiration and surface-bound tracer counted in a gamma counter. Histamine values were calculated by direct comparison with standard curve values. Total histamine (TH) values (extracellular + intracellular histamine) of blood or cell culture suspensions were quantified after cell lysis in distilled water. Extracellular histamine was measured in blood plasma or in cell-free culture supernatants after centrifugation at 4°C. Intracellular histamine was calculated from total minus extracellular histamine.

*Assay for detection of antibodies (Abs) directed against rh IL-3 and rh GM-CSF in rhesus monkey serum.* Serum samples were thawed and tested for antibody content by an enzyme-linked immunosorbent assay (ELISA) as described previously.[27] Briefly, for this analysis, 100 µL of rh IL-3 or rh GM-CSF at a concentration of 10 µg protein/mL in bicarbonate buffer, pH 9.6, was added at 100 µL to each well of micro ELISA plates (Immunoplates II, Nunc, Denmark) and incubated at 37°C for 60 minutes. Plates were washed six times in phosphate-buffered saline supplemented with fetal calf serum (PBS/FCS). One hundred microliters of test sera diluted in PBS/FCS in twofold steps starting with a 1:10 dilution were distributed to each well and incubated again for 30 minutes at

Table 1. Effects of rh IL-3 Singly or in Combination With rh GM-CSF on Total and Differential Cell Counts and Total Histamine Levels in Simian Bone Marrow Suspension Cultures

| Stimulus U/mL Day of Culture (d) | | Total Cell No. ×10⁴ | Cell Types % | | | | | | | Total Histamine ng/mL |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | N | M | EO | B | Meg | BL | L | |
| 0-14 (d) | | | | | | | | | | |
| IL-3 | GM-CSF | | | | | | | | | |
| — | — | 23.4 ± 1.3 | 5 | 70 | 0 | 0 | 0 | 0 | 25 | 1.9 ± 0.4 |
| 5 | — | 37.7 ± 1.3* | 2 | 28 | 3 | 23 | 1 | 16 | 27 | 28.3 ± 6.2 |
| 10 | — | 43.4 ± 1.7* | 2 | 18 | 4 | 43 | 2 | 16 | 15 | 69.1 ± 7.1 |
| 50 | — | 54.4 ± 6.6* | 12 | 5 | 6 | 53 | 1 | 15 | 8 | 131.2 ± 6.3 |
| 100 | — | 54.6 ± 0.9* | 1 | 4 | 5 | 82 | 1 | 3 | 2 | 219.8 ± 6.5 |
| — | 100 | 49.5 ± 3.3 | 21 | 37 | 22 | 8 | 3 | 3 | 6 | 18.7 ± 0.3 |
| 100 | 100 | 54.3 ± 5.5 | 16 | 24 | 11 | 28 | 7 | 8 | 6 | 102.6 ± 18.0 |
| 0-6 (d) | 7-14 (d) | | | | | | | | | |
| IL-3 | GM-CSF | | | | | | | | | |
| — | 100 | 45.3 ± 4.5 | 14 | 52 | 10 | 4 | 3 | 11 | 3 | 3.9 ± 1.0 |
| 5 | 100 | 55.0 ± 0.6 | 18 | 22 | 20 | 14 | 3 | 17 | 6 | 59.7 ± 13.1 |
| 100 | 100 | 70.8 ± 5.6† | 24 | 22 | 17 | 17 | 7 | 8 | 5 | 121.4 ± 14.8 |

Abbreviations: N, neutrophilic granulocyte; M, monocyte-macrophage; Eo, eosinophil; B, basophil; Meg, megakaryocyte; BL, blast cells; L, lymphocyte.

A total of $5 \times 10^5$ simian marrow cells were cultured in 10% autologous serum for 14 days. The mean total cell counts and histamine levels are from triplicate cultures ± SD harvested on day 14. Total histamine levels were assayed after lysis of cells in cultures. Differential cell counts were performed on 100 cells of Wright's stained cytocentrifuge preparation.

*Significantly different to control cultures ($P < .05$).

†Significantly different to cultures stimulated with a combination of rh IL-3 + rh GM-CSF (100 U/mL) added simultaneously at day 0 ($P < .05$).

37°C. Washing was repeated six times before 100 μL of peroxidase-labeled antimonkey immunoglobulin (rh mon/IgG [H+L]/PO, Nordic Immunology) diluted 1:500 in PBS was added to each well and incubated for 30 minutes at 37°C. After repeated washing, 100 μL of enzyme substrate solution (Orthophenyl-Diamin in phosphatase citrate buffer containing 3% $H_2O_2$) was distributed to each well and incubated for 10 minutes. After stopping the reaction by adding 100 μL 4 n $H_2SO_4$, the absorbance (OD) at 492 nm was measured in a microtiter spectrophotometer (LST-Reader, Labinstruments, Austria). The antibody response was defined as the serum dilution, which gave an OD of 0.1.

## RESULTS

*Effects of rh IL-3 and rh GM-CSF in a simian bone marrow suspension culture system.* In a monkey BM suspension culture system rh IL-3 and rh GM-CSF had a similar stimulating effect on simian BM precursors as reported for human BM cells with these growth factors.[16-18] In Table 1 the total and differential cell counts and total histamine content of simian BM culture determined by day

14 are summarized. As can be seen, rh IL-3 induced a dose-dependent increase of total cell counts to a maximum of 2.3-fold. Differential counts revealed a rh IL-3 dose-dependent increase in metachromatic cells ranging from 0% to 82% of total cell numbers and an elevation of blasts and eosinophils. Total histamine levels also increased in a dose-dependent manner from 1.9 ± 0.4 ng/mL determined in control cultures to 219.8 ± 6.5 ng/mL in cultures stimulated with 100 U/mL rh IL-3.

rh GM-CSF was also found to stimulate growth of simian BM cells up to 2.1-fold of cell counts obtained in control cultures. However, rh GM-CSF preferentially promoted the growth of neutrophils, macrophages, and eosinophils, whereas basophils and, concomitantly, total histamine were only slightly increased. Simultaneous administration of both growth factors (rh IL-3 + rh GM-CSF) did not result in an enhancement of total cell counts compared with cultures established in the presence of a single cytokine. This combination of both cytokines resulted in a stimulation of almost
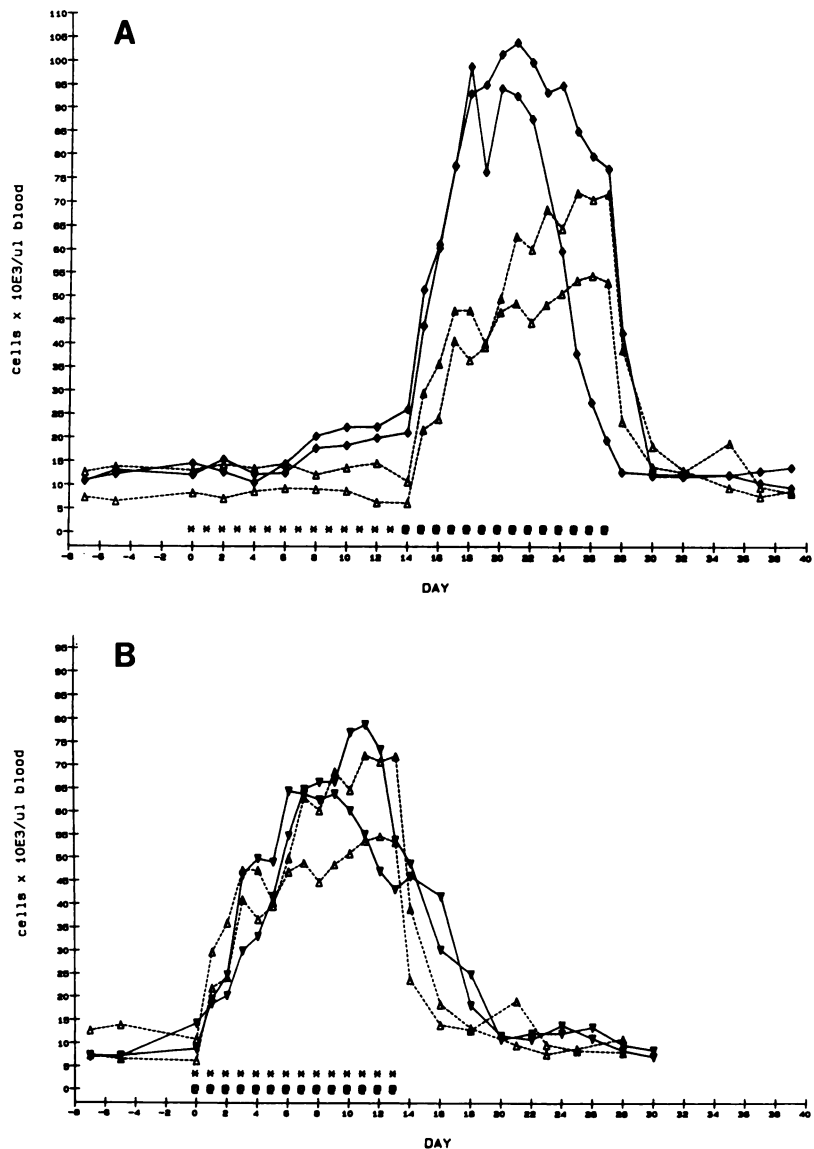


Fig 1. Changes in WBC levels in rhesus monkeys treated SC with rh IL-3 (●) and/or rh GM-CSF (@). (A) Filled symbols: Monkeys were treated daily (days 0 to 13) with 100 μg/kg rh IL-3, subsequently receiving daily 5.5 μg/kg rh GM-CSF (days 14 to 27). Open symbols: Monkeys were treated with 5.5 μg/kg rh GM-CSF daily (days 14 to 27). (B) Filled symbols: Monkeys were treated daily with 100 μg/kg rh IL-3 and received 5.5 μg-kg rh GM-CSF simultaneously (days 0 to 13). Open symbols: Monkeys were treated with 5.5 μg/kg rh GM-CSF. The daily doses were divided into three administrations.

Table 2. Maximum Levels of WBC, Neutrophils, Eosinophils, Basophils, Monocytes, and Lymphocytes in Peripheral Blood of Rhesus Monkeys in Response to Treatment With rh IL-3 and rh GM-CSF

| Treatments | Doses of Cytokines µg Protein/kg/d | | WBC 10³/µL | | Seg. Neutrophil 10³/µL Animal | | Eosinophil 10³/µL Animal | | Basophil 10³/µL Animal | | Monocyte 10³/µL Animal | | Lymphocyte 10³/µL Animal | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | rh IL-3 Day 0 to 13 | rh GM-CSF Day 14 to 27 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| Control saline | — | — | 14.7 | 9.3 | 10.0 | 5.0 | 1.2 | 1.0 | 0.2 | 0.1 | 0.8 | 0.5 | 5.9 | 3.8 |
| rh IL-3 day 0 to 13 | 11 | — | 11.4 | 17.3 | 3.3 | 6.4 | 2.6 | 2.6 | 3.5 | 3.9 | 0.5 | 0.7 | 3.4 | 6.6 |
| | 33 | — | 17.6 | 28.1 | 9.8 | 13.0 | 3.2 | 5.2 | 5.8 | 6.0 | 0.8 | 1.7 | 6.7 | 7.0 |
| | 100 | — | 21.2 | 26.1 | 8.7 | 7.2 | 2.9 | 8.4 | 7.0 | 9.7 | 1.2 | 0.9 | 9.8 | 7.3 |
| rh IL-3 day 0 to 13; subsequently rh GM-CSF* day 14 to 27 | 11 | 5.5 | 93.5 | 120.5 | 49.6 | 38.6 | 37.7 | 83.1 | 2.6 | 5.7 | 2.7 | 3.3 | 10.5 | 14.9 |
| | 33 | 5.5 | 94.4 | 110.7 | 47.7 | 71.9 | 34.0 | 19.0 | 3.4 | 12.6 | 6.3 | 5.6 | 16.2 | 13.1 |
| | 100 | 5.5 | 98.4 | 103.4 | 59.0 | 38.2 | 17.5 | 35.6 | 4.6 | 6.9 | 5.6 | 6.5 | 14.4 | 18.2 |
| rh IL-3† simultaneously + rh GM-CSF day 0 to 13 | 100 + 5.5 + | 5.5 | 78.4 | 64.2 | 32.0 | 39.8 | 24.6 | 13.1 | 11.9 | 9.3 | 4.8 | 1.6 | 15.0 | 10.9 |
| rh GM-CSF‡ day 14 to 27 | — | 5.5 | 54.4 | 71.8 | 32.0 | 44.4 | 14.7 | 14.3 | 0.5 | 2.1 | 3.5 | 3.5 | 11.0 | 14.4 |

*Monkeys were treated daily SC with various doses of rh IL-3 over a period of 14 days, thereafter receiving rh GM-CSF 5.5 µg/kg/d/SC for additional 2 weeks.
†Monkeys were treated daily with 100 µg/kg rh IL-3 and 5.5 µg/kg rh GM-CSF simultaneously for 14 days.
‡Monkeys received a daily dose of 5.5 µg/kg rh GM-CSF over a period of 14 days.

all myeloid lineages. Interestingly, the rh IL-3 effect on total histamine was reduced to about 50% by the simultaneous administration of rh GM-CSF. However, when rh GM-CSF was added to cultures after a seven-day rh IL-3 prestimulation period, cell numbers were found to be elevated compared with responses to rh GM-CSF after preincubation with control medium (days 0 to 6 rh IL-3, days 7 to 14 rh GM-CSF: 70.8 ± 5.6 × $10^4$ cell/mL, v days 0 to 6 control medium, days 6 to 14 rh GM-CSF: 45.3 ± 4.5 × $10^4$ cells/mL [P < .05]). Subsequent addition of rh GM-CSF to rh IL-3–pretreated BM cells was found to result in expansion of all myeloid lineages.

*Effect of rh IL-3 treatment on peripheral blood cell counts.* Daily SC injections of 11, 33, or 100 μg rh IL-3/kg body weight over 14 days induced a maximum of a twofold to threefold increase in WBC counts. WBCs began to rise and reached maximum levels in the range of 11.4 to 28.1 × $10^3$ cells/μL blood during the second week of rh IL-3 treatment (Fig 1A, Table 1). Differential WBC counts revealed a dose-dependent increase in basophils resulting in an approximately 50-fold increase at a daily dose of 11 μg, 80-fold elevation at 33 μg, and 110-fold rise at 100 μg rh IL-3/kg body weight. Whereas in control animals 0% to 1% of WBCs were classified as basophils, about 10% to 40% of WBCs represented basophils in rh IL-3–treated monkeys by days 8 to 10. Beside the marked increase in basophils, a dose-dependent increase in eosinophils (twofold increase at 11 μg/kg, 7.5-fold at 33 μg/kg, and tenfold at 100 μg/kg) was observed in monkeys treated with rh IL-3. No significant changes were observed in neutrophil and lymphocyte counts and levels of RBCs and platelets (Table 2, Fig 2A).

*Effect of pretreatment with rh IL-3 and subsequent rh GM-CSF application on hematologic parameters.* A prompt rise in circulating WBC levels was found in monkeys receiving a daily dose of 5.5 μg/kg body weight of rh GM-CSF (Fig 1A, 1B). However, animals pretreated with rh IL-3 for 14 days showed higher maximum WBCs ranging from 93.5 to 120.5 × $10^3$ cells per μL blood by days 4 to 6 of rh GM-CSF treatment, compared with monkeys pretreated with saline (54.4 and 71.8 × $10^3$/μL cells blood, respectively). All monkeys pretreated with the various doses of rh IL-3 showed a similar WBC response to the subsequent rh GM-CSF administration. In Fig 1A a representative pattern of WBC changes in monkeys receiving 100 μg/kg rh IL-3 for 14 days and subsequently treated with rh GM-CSF for an additional 2 weeks is depicted. Maximum WBC counts were found not to be related to the dose of rh IL-3 (Table 1). Interestingly, in almost all monkeys (five out of six) receiving rh IL-3 over a period of 14 days followed by a 2-week rh GM-CSF treatment, WBC counts began to decline between days 6 and 8 of the rh GM-CSF administration period. In contrast to animals pretreated with rh IL-3, monkeys receiving saline showed a more delayed WBC rise in response to rh GM-CSF. In these control animals WBC counts increased steadily during the rh GM-CSF application, reaching maximum WBC levels by the last day of rh GM-CSF treatment (Fig 1A).

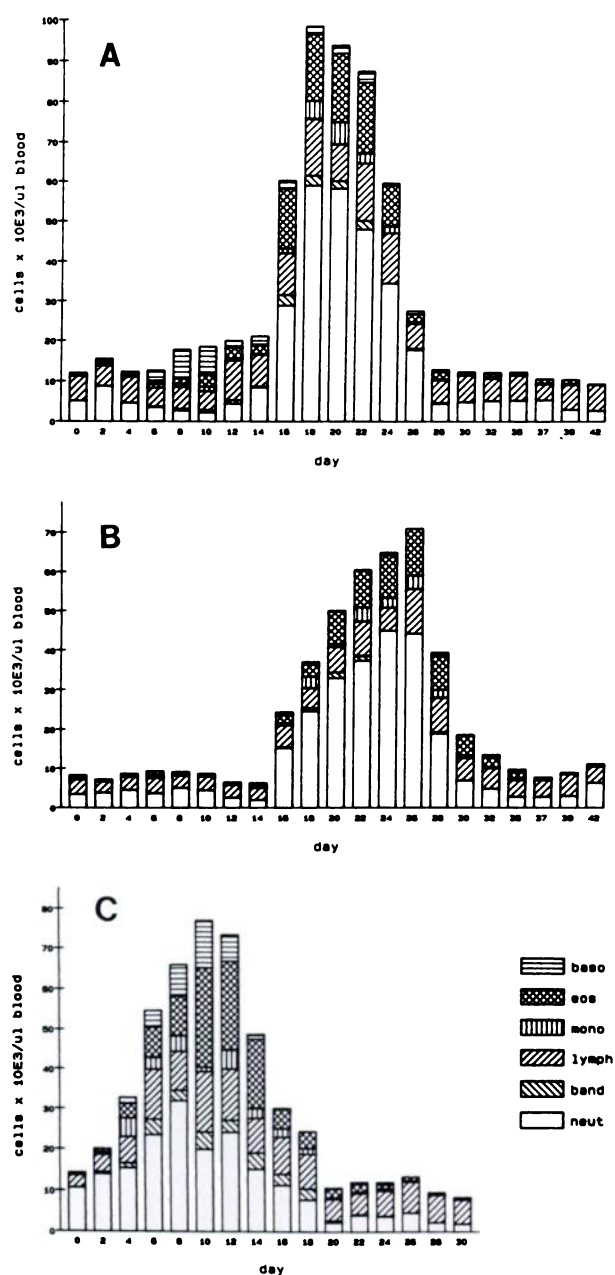In rh IL-3–pretreated animals subsequently dosed with rh GM-CSF, differential cell counts revealed a fourfold to



Fig 2. Differential cell counts in rhesus monkeys treated SC with rh IL-3 and/or rh GM-CSF (days 14 to 27). (A) 100 μg/kg/d rh IL-3 (days 0 to 13), 5.5 μg/kg/d rh GM-CSF (days 14 to 27). (B) Saline (days 0 to 13) and 5.5 μg/kg/d rh GM-CSF (days 14 to 27). (C) 100 μg/kg/d rh IL-3 simultaneously with 5.5 μg/kg/d rh GM-CSF (day 0 to 13).

14-fold elevation of maximum neutrophil counts. Basophil counts declined during rh GM-CSF treatment, reaching normal values at the end of the administration period. Eosinophil counts increased steadily, showing a maximum of 14- to 80-fold increase above control values during the second week of the rh GM-CSF treatment period. Minor changes were observed in monocyte and lymphocyte levels, with a maximum increase of fivefold to 13-fold and fivefold, respectively (Table 2, Fig 2A). Monkeys receiving only rh

GM-CSF showed an approximately threefold to ninefold maximum increase in neutrophils, a ten to 15-fold elevation in eosinophils, and a twofold to 20-fold rise in basophils (Table 2, Fig 2B). Rh IL-3–pretreated monkeys subsequently treated with rh GM-CSF showed about four times higher maximum eosinophil counts than animals treated with rh GM-CSF alone. Platelet counts increased about twofold during the rh GM-CSF administration period in all rh IL-3–pretreated monkeys. Also, however, control animals receiving saline during the rh IL-3 administrations showed a similar rise in platelet counts during their rh GM-CSF treatment period.

*Effect of simultaneous administration of rh IL-3 and rh GM-CSF on hematologic parameters.* Simultaneous administration of rh IL-3 (100 μg/kg) and rh GM-CSF (5.5 μg/kg) to monkeys for 14 days resulted in maximum WBC levels of the same magnitude as found with rh GM-CSF alone (Fig 1B) and thus failed to mimic the effect of rh IL-3 pretreatment and subsequent rh GM-CSF administration on the rise of WBCs (Table 2). Differential counts revealed stimulation of all myeloid lineages (Fig 2C).

*Total histamine (TH) levels in blood of rh IL-3 and rh GM-CSF–treated monkeys.* Mean levels of TH in blood of healthy monkeys determined during the pretreatment period was 12.5 ± 10.2 ng/mL. Figure 3A shows the changes in TH levels in the blood of monkeys receiving rh IL-3 (100 μg/kg) and subsequently rh GM-CSF (5.5 μg/kg). In monkeys treated with rh IL-3, TH levels increased steadily during the rh IL-3 treatment period, reaching approximately 400- to 700-fold higher maximum levels (8,310 and 4,720 ng/mL blood, respectively) than normal values by days 8 to 10. Thereafter TH levels declined and returned to normal values within 2 weeks. On day 14, when blood TH levels were determined from all rh IL-3–treated monkeys, a dose-dependent increase could be evaluated showing an approximately 140-fold increase (mean of two animals 1,695 ng/mL blood) at a daily dose of 11 μg to a 350-fold (4,528 ng/mL) elevation at 33 μg and a maximum of 80-fold (6,877 ng/mL) rise at a dose of 100 μg/kg body weight. Rh GM-CSF–treated monkeys exhibited a moderate increase of TH blood levels, with about sixfold to 52-fold increased maximum values compared with control levels (80 and 650 ng/mL
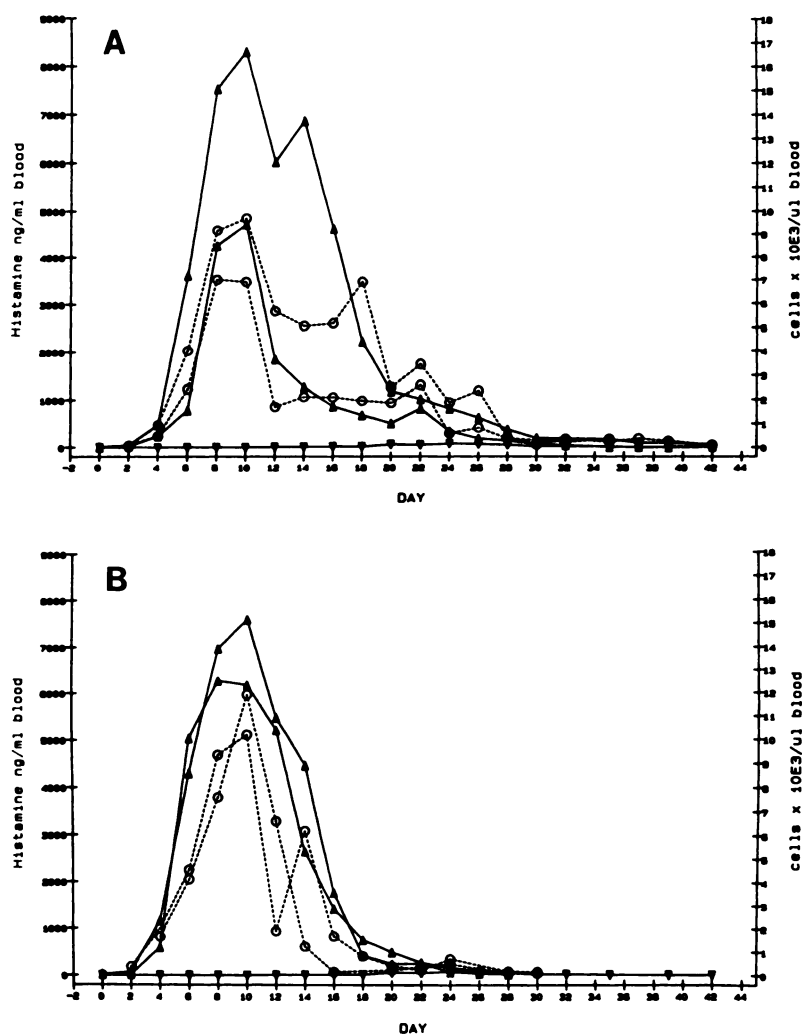




Fig 3. TH levels in blood and basophil counts in rhesus monkeys injected SC with rh IL-3 and rh GM-CSF. (A) Treatment with 100 μg/kg/d rh IL-3 (days 0 to 13); subsequent administrations of rh GM-CSF (5.5 μg/kg/d, days 14 to 27). (B) Simultaneous administration of 100 μg/kg/d rh IL-3 and 5.5 μg/kg/d rh GM-CSF (days 0 to 13). The daily doses were divided into three administrations. (▲) Histamine ng/mL blood (mean of duplicates). (○) Basophil counts × 10³ per μL blood. (▼) Histamine levels of monkeys treated with rh GM-CSF alone.

blood, respectively). The increases in TH values during the rh IL-3 treatments were found to be related to the rise in circulating basophils (regression coefficient = 0.89). The mean histamine content per basophil, calculated from the regression line, was about 1 pg.

In Fig 3B the TH values and basophil counts of monkeys receiving simultaneous treatment with rh IL-3 (100 µg/kg) and rh GM-CSF (5.5 µg/kg) are shown. It can be seen that TH levels increased rapidly in response to the combination of both cytokines, reaching 500- to 600-fold increased maximum TH values (6,250 and 7,570 ng/mL blood, respectively) by days 8 and 10. This is a similar response to that observed in animals treated with rh IL-3 alone.

*Plasma histamine levels in monkeys treated with rh IL-3 and rh GM-CSF.* Plasma histamine (PH) levels in response to rh IL-3 and subsequent rh GM-CSF treatment are shown in Table 3. Monkeys treated with a daily dose of 100 µg/kg rh IL-3 responded with an increase of PH from about 0.024 ± 0.006 ng/mL determined in control plasma samples to maximum values between 0.15 and 1.24 ng/mL plasma by days 14 and 17, respectively. Rh GM-CSF–treated monkeys showed maximum PH levels in the range of 0.13 to 0.36 ng/mL plasma. PH values expressed as percentage of TH levels were in the range of 0.01% to 0.1%, showing no significant difference between control animals and monkeys receiving the various treatments. The simultaneous administration of rh IL-3 and rh GM-CSF resulted in higher maximum PH levels (in the range of 2.0 to 9.4 ng/mL at day 10) compared with the administration of a single cytokine. Increased PH levels returned to normal values within 1 to 2 weeks after the end of rh IL-3 treatment. Around day 10 of rh IL-3 treatment four out of eight monkeys developed skin lesions, located mainly on the inner areas of the hind limbs, which disappeared within 1 week. The lesions were characterized as urticaria by histologic examination of skin punctures.

*Serum Abs against rh IL-3 and rh GM-CSF in monkeys.* All monkeys treated with rh IL-3 developed IgG Abs directed against the human protein. Serum Abs became detectable by day 10 of rh IL-3 treatment, reached maximum levels about 2 weeks later, and declined thereafter. No

dose–response relationship was found between the dose of rh IL-3 administered and the Ab titers. An interesting observation was that monkeys treated simultaneously with rh IL-3 and rh GM-CSF developed about five to ten times higher Ab titers than animals receiving the same dose of rh IL-3 alone (results not shown). No Abs directed against rh GM-CSF could be detected in sera of monkeys receiving rh GM-CSF.

Sera of two rh IL-3–treated monkeys were tested on reducing the IL-3–induced thymidine incorporation in the CML myeloblast assay.[22] Whereas sera collected before the beginning of treatment showed no inhibitory effect, serum samples collected on day 14 of rh IL-3 administration were found to reduce thymidine incorporation by 90% of control values at a serum dilution of 1:1,500.

## DISCUSSION

A number of human hematopoietic growth factors have been identified by their varying biological properties in vitro.[28-30] Recently most of the cytokines have also been cloned for large-scale production and subsequently tested in vivo and used for clinical trials.[31] The use of some of these growth factors such as GM-CSF, G-CSF, or erythropoietin have already been shown to represent an excellent therapeutic strategy, most impressively in cytopenic patients.[32-34]

The in vitro effects of rh IL-3 on human BM cultures have previously been described, showing that rh IL-3 is a potent growth factor for both unipotent and multipotent progenitor cells[35,36] and, in addition, supports the differentiation of basophils and eosinophils.[16-18] In the present study, almost identical results were obtained using simian BM cells and rh IL-3 in a suspension culture system. In particular, rh IL-3 induced (1) an approximately twofold elevation of total cell number compared with control cultures, (2) a dose- and time-dependent formation of basophils and rise of intracellular histamine, and (3) an enhanced formation of blasts and eosinophils compared with control. Furthermore, rh IL-3 was found to exhibit colony-stimulating activity on enriched BM progenitors in semisolid media (data not shown).

These in vitro observations provided a rationale for initiating a study to assess the role of recombinant human IL-3 in

Table 3. Maximum Values of Plasma Histamine in Rhesus Monkeys Treated With rh IL-3 and rh GM-CSF

| Treatments | Cytokine Doses (µg) Protein/kg/d | | Maximum Plasma Histamine ng/mL§ | | | | Day of Maximum Plasma Histamine Level Animal | |
| | | | Before Treatment Animal | | During Treatment Animal | | | |
| | rh IL-3 | rh GM-CSF | 1 | 2 | 1 | 2 | 1 | 2 |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| rh GM-CSF* day 14 to 27 | — | 5.5 | 0.025 | 0.031 | 0.362 | 0.133 | 15 | 17 |
| rh IL-3† day 0 to 13; subsequently rh GM-CSF day 14 to 27 | 100 | 5.5 | 0.026 | 0.022 | 1.224 | 0.156 | 17 | 13 |
| rh IL-3;‡ simultaneously rh GM-CSF day 0 to 13 | 100 | 5.5 | 0.023 | 0.019 | 9.457 | 2.077 | 9 | 9 |

*Monkeys received a daily dose of 5.5 µg/kg rh GM-CSF SC over a period of 14 days.

†Monkeys were treated daily SC with 100 µg/kg rh IL-3 over a period of 14 days, thereafter receiving rh GM-CSF 5.5 µg/kg/d SC for additional 2 weeks.

‡Monkeys were treated daily with 100 µg/kg rh IL-3 and 5.5 µg/kg rh GM-CSF simultaneously for 14 days.

§Mean of duplicates.

regulating hematopoiesis in vivo in nonhuman primates. The SC administration of rh IL-3 to healthy rhesus monkeys induced a moderate elevation (about twofold to threefold above controls) of WBC caused mainly by a dose-dependent increase in basophils and eosinophils during the second week of a 14-day administration period. Thus rh IL-3 by itself was found to be a growth factor for basophils and eosinophils in vivo, reflecting the situation in vitro in BM cultures. Furthermore, our results clearly demonstrate that rh IL-3 treatments induced a dose-dependent formation of intracellular histamine in rhesus monkey blood.

Interestingly, in monkeys basophil counts and histamine levels began to decline after day 10 of the rh IL-3 administration, which may be explained by the neutralization of the biological effect of rh IL-3 by anti-rh IL-3 antibodies detected in the sera of rh IL-3–treated monkeys by day 10.

Recombinant human GM-CSF has previously been administered to rhesus monkeys and was found to be a growth factor for neutrophils and eosinophils.[37,38] In this study rh GM-CSF was given in vivo (1) in combination with rh IL-3, (2) after a 14-day IL-3 pretreatment period, and (3) after pretreatment with saline. Rh IL-3–pretreated monkeys were found to respond to rh GM-CSF by a twofold enhanced increase of WBCs compared with the WBC response in monkeys receiving rh GM-CSF subsequent to a saline treatment period. Differential blood cell counts revealed that this enhanced increase in WBC was due to an increased formation of neutrophils and eosinophils. These results provide evidence that rh IL-3 primes the hematopoietic system for subsequent GM-CSF effects, probably due to a rh IL-3–expanded stem cell pool, which, in response to the sequential stimulation with rh GM-CSF, differentiates into neutrophils and eosinophils. However, in rh IL-3–primed monkeys, WBCs declined during rh GM-CSF administration in contrast to saline-pretreated animals. This phenomenon may indicate that the self-renewal capacity of the stem-cell pool generated during rh IL-3 administration is limited.

Simultaneous administration of both cytokines failed to induce an enhanced WBC increase (as observed in rh IL-3–pretreated monkeys) when compared with rh GM-CSF treatment alone. These results might be explained by a competitive recruitment of myeloid cells from a stem-cell pool expressing receptors for both IL-3 and GM-CSF. Probably IL-3 and GM-CSF–responsive stem cells with self-renewal capacity were driven into a myeloid differentiation pathway by rh GM-CSF before a substantial self-renewal could occur.

In this study we could demonstrate that rh IL-3 is a potent growth factor for blood basophils in vivo. Blood basophils, on the other hand, are the effector cells of allergic events. Recent data indicate that peripheral human blood basophils express receptors for IL-3.[39] In addition, it was shown that rh IL-3 could represent a signal for histamine release from basophils in vitro.[40] In this study rh IL-3 as well as rh GM-CSF application was followed by an elevation of plasma histamine. However, it must be pointed out that plasma histamine levels represented less then 0.1% of total histamine values. These results support the suggestion that rh IL-3 as well as rh GM-CSF has no substantial histamine-releasing activity in vivo. This is also in agreement with the observation that no monkey treated with these cytokines suffered from life-threatening allergic reactions. Most of the monkeys developed transient, urticarialike skin lesions during the second week of rh IL-3 treatment. Nevertheless, at present it remains unclear whether this side effect is due to basophil/mast-cell activation or to anti-rh IL-3 antibodies.

We conclude that IL-3 is an in vivo basophil differentiation factor and is capable of priming hematopoiesis for subsequent GM-CSF actions. It must be deduced from this study, however, that the clinical use of rh IL-3 has to be monitored by careful attention to allergic events and by continuous control of plasma histamine levels.

## ACKNOWLEDGMENT

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