American Journal of Pathology, Vol. 135, No. 4, October 1989

Acute *In Vivo* Effects of IL-3 Alone and in Combination with IL-6 on the Blood Cells of the Circulation and Bone Marrow

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Recombinant human IL-3 administered intravenously to rats as a single injection induced peripheral neutrophilia and monocytosis beginning at 4 to 6 hours after injection, peaking at 8 hours, and subsiding to normal by 12 to 24 hours. IL-3 did not induce an initial neutropenia such as accompanies endotoxin-, G-CSF-, and TNF-induced neutrophilia, or lymphopenia such as accompanies endotoxin-, IL-1-, and TNF-induced neutrophilia. The IL-3-induced peripheral neutrophilia was accompanied by a decrease in mature marrow neutrophils, indicating that the mechanism of neutrophilia was through marrow release rather than by demargination, which occurs after the administration of epinephrine or IL-6. The release of mature marrow neutrophils further suggests that IL-3 either has intrinsic neutrophil-releasing activity or indirectly causes neutrophil release through the gene expression of a second cytokine. IL-3 induced a striking left-shifted myeloid hyperplasia in the bone marrow at 8 hours that morphologically was very similar to that observed after administration of endotoxin, a finding consistent with the hypothesis of previous investigators that endotoxin may in part act indirectly on hematopoietic cells by eliciting local marrow production of IL-3. Finally, IL-3 induced an increase in marrow pronormoblasts at 8 hours, consistent with the in vitro ***proliferative effect of IL-3 on erythroid stem cells. The combination of IL-3 and IL-6 induced a synergistic peripheral neutrophilia and monocytosis and a striking synergistic increase in marrow mast cells. The combination of IL-3 and IL-6 also induced an erythroid and left-shifted myeloid hyperplasia such as would be expected given the individual effects of these hematopoietic growth factors. (Am J Pathol 1989, 135:663–670)***

Interleukin-3 (IL-3) is a glycoprotein growth factor that is also known under the names multi-colony stimulating factor, burst promoting activity, mast cell growth factor, and hematopoietic cell growth factor.[1] IL-3 *in vitro* has been reported by Bot et al[2] to have a direct stimulating effect on BFU-E, CFU-GEMM, and CFU-Eo and indirect effects on CFU-G, CFU-GM, and CFU-M in the presence of monocytes. IL-3 *in vitro* was reported by Prystowsky et al[3] to support the growth of murine macrophages, neutrophils, mast cells, and megakaryocytes, and by Bruno et al[4] to increase megakaryocyte colony formation using normal human marrow cells. Lotem et al[5] observed that IL-3 induces the differentiation of myeloid leukemic cell lines. IL-3 *in vivo* was reported by Broxmeyer et al[6] to increase the number of hematopoietic progenitor cells (CFU-GM, BFU-E, and CFU-GEMM) in mice and to increase the percentage of progenitor cells in S-phase. Fabian et al[7] noted that pretreatment of bone marrow cells with IL-3 enhances their ability to reconstitute hematopoietic organs in irradiated mice.

Interleukin-6 (IL-6) is a multifunctional peptide growth factor that is also known under the names of interferon-β_2, B cell stimulating factor-2, hybridoma growth factor, hepatocyte stimulating factor, and 26 Kd protein.[8–11] IL-6 was recently found by our laboratory[12] to cause multiple hematologic effects *in vivo*, including a biphasic neutrophilia with an initial peak at 1.5 hours and a second sustained wave of neutrophilia between 4 and 12 hours; a mild lymphocytosis at 0.5 hours and a mild lymphopenia between 1.5 and 4 hours; and a reticulocytosis between 12 and 24 hours. The initial peak of IL-6-induced neutrophilia appears to be due to demargination of intravascular neutrophils rather than a result of marrow release of neutrophils. IL-6 also causes a mild left-shifted myeloid hyperplasia in the bone marrow and a tremendous erythroid hyperplasia of intermediate and late normoblasts at 12 and 24 hours after a single intravenous injection.[12]

Supported by NIH Award R01-AI26551 and by the Memorial Long Beach-UCI Cancer Program.

Accepted for publication June 7, 1989.

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The purpose of the present study was to document the acute effects (within the first 24 hours) of a single intravenous injection of IL-3 or of IL-3 and IL-6 in combination on the leukocytes of the peripheral circulation and of the bone marrow. In previous investigations with IL-3, most similar to the present one, the following observations were made. Metcalf et al[13] noted that mice injected intraperitoneally three times daily for up to 6 days experienced a twofold to threefold increase in circulating neutrophils and monocytes and tenfold rises in eosinophils. No significant changes, however, were observed in the marrow. Lord et al,[14] on the other hand, reported that IL-3 administered to mice either intravenously as a single dose or through subcutaneously implanted osmotic pumps did not affect circulating white cell counts but did cause an early increase in spleen colony units per femur despite an accompanying early drop in the cellularity of the femoral bone marrow. Kindler et al[15] reported that IL-3 administered to mice through subcutaneous osmotic pumps stimulated both myeloid and erythroid hematopoiesis in the spleen and liver but decreased total cell and progenitor numbers in the bone marrow. Kindler et al[15] did not examine the effects of IL-3 on the circulating number of leukocytes in peripheral blood.

Previous *in vivo* investigations with IL-3 and IL-6 in combination have not to our knowledge been reported. *In vitro,* however, IL-6 was reported by Ikebuchi et al[16] to enhance the IL-3-dependent proliferation of murine multipotential hemopoietic progenitors. More recently, Leary et al[17] reported a synergism between IL-3 and IL-6 supporting the proliferation of human hematopoietic stem cells.

In the present study, recombinant human IL-3 administered as a single intravenous injection to rats was found to cause peripheral neutrophilia and monocytosis. The increase in circulating numbers of neutrophils was due to the release of marrow neutrophils that contributed to the decrease in the total number of bone marrow cells. IL-3 also induced a striking left-shifted myeloid hyperplasia in the marrow and a hyperplasia of pronormoblasts. IL-3 and IL-6 in combination induced, in addition to those effects expected by the individual cytokines, a synergistic peripheral neutrophilia and monocytosis and a striking synergistic increase in marrow mast cells.

Materials and Methods

Recombinant human IL-3 (specific activity of 2.6×10^7 U/mg in human marrow proliferation assay) and IL-6 (specific activity of 5×10^6 U/mg in CESS cell assay of IgG induction) were the generous gift of Dr. David Urdal (Immunex Corp., Seattle, WA). Results were confirmed with recombinant human IL-3 that was the generous gift of Dr. Lawrence M. Souza (AmGen, Thousand Oaks, CA). Lewis male rats weighing approximately 350 g (Harlan-Sprague-Dawley, Indianapolis, IN) received a single intravenous injection of varying doses of IL-3 or of 10 μg Salmonella typhosa endotoxin (Sigma Chemical Co., St. Louis, MO) in a final volume of 0.5 ml 1% normal rat serum in sterile saline through the dorsal vein of the penis. Blood for the quantitation of circulating leukocytes (Coulter counter, Coulter Co., Hialeah, FL) and for blood smears was obtained by tail bleeding under ether anesthesia immediately before and at various times after the injection of IL-3 and IL-6. White blood cell differentials were performed by counting 100 white blood cells on modified Wright's-stained smears (Diff-Quik Stain Set, American Scientific Products, McGaw Park, IL). Bone marrow hematopoietic cell subsets were quantitated by the method of Chervenick et al.[18] Briefly, at the time of sacrifice one humerus was dissected free of soft tissue and the ends of both epiphyses were cut off with a scalpel. The bone marrow was eluted from the humerus by washing the marrow with 10 ml of Isoton II buffer (Coulter Co.) injected through a 21-gauge needle together with heparin and a red blood cell lysing agent (Zapoglobin, Coulter Co.), and the absolute number of nucleated cells per humerus was determined. The contralateral humerus was used to prepare bone marrow smears stained by the modified Wright's method, and differential counts were performed on at least 1000 cells per smear according to standard morphologic criteria for the rat as reported by Hulse.[19] Statistical analysis of the data was performed with either the paired or unpaired Student's *t*-test using the Statview program on an Apple computer; all averages are expressed as ± 1 standard deviation. Boiling the IL-3 completely abrogated its hematologic effects, demonstrating that endotoxin (which was heat resistant) was not responsible for the observed results.

Results

Dose-response study (data not shown) demonstrates that IL-3 administered over a wide range of doses (0.1 to 20 μg/rat) induces a peripheral neutrophilia and monocytosis beginning at 4 to 6 hours, peaking at 8 hours, and subsiding to normal by 12 to 24 hours. IL-3 at 8 hours at a dose of 10 μg/rat ($N = 12$) causes approximately a threefold increase in circulating neutrophils (Figure 1A) and a sevenfold increase in circulating monocytes (Figure 1B), but does not cause any significant changes in the numbers of circulating lymphocytes (Figure 1C). The effects of IL-3, but not those of endotoxin, are abrogated by boiling (Figure 1A–C). Endotoxin causes both an initial neutropenia and a profound lymphopenia, neither of which is observed after injection of IL-3; endotoxin does not cause

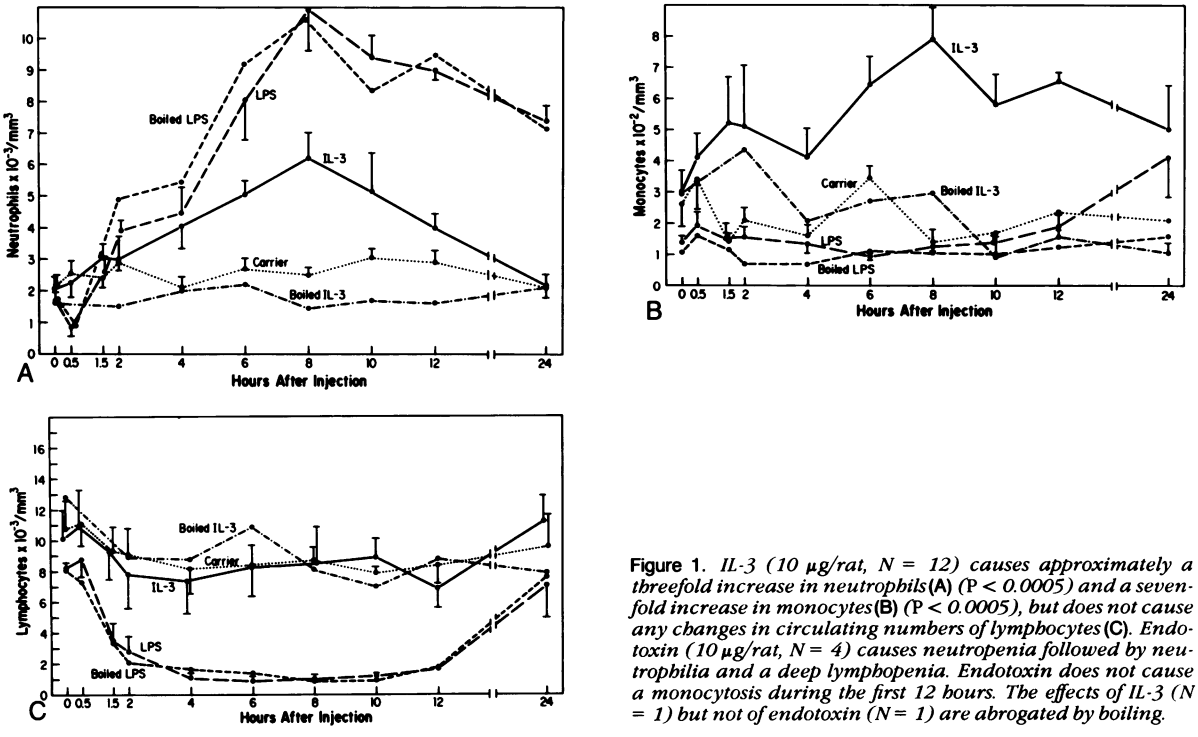

Figure 1. *IL-3 (10 μg/rat, N = 12) causes approximately a threefold increase in neutrophils* (A) ($P < 0.0005$) *and a sevenfold increase in monocytes* (B) ($P < 0.0005$), *but does not cause any changes in circulating numbers of lymphocytes* (C). *Endotoxin (10 μg/rat, N = 4) causes neutropenia followed by neutrophilia and a deep lymphopenia. Endotoxin does not cause a monocytosis during the first 12 hours. The effects of IL-3 (N = 1) but not of endotoxin (N = 1) are abrogated by boiling.*

a monocytosis at 8 hours such as occurs in IL-3-treated rats. IL-3 also does not cause a very early neutropenia at 1, 5, 10, or 15 minutes after injection of 10 μg IL-3/rat (N = 2 for IL-3-treated rats and N = 2 for carrier-treated rats, data not shown) such as occurs in G-CSF-treated rats and humans.[20]

The bone marrow (Table 1) of IL-3-treated rats at 8 hours demonstrated a marked left-shifted myeloid hyperplasia (Figure 2) with a twofold to threefold increase in myeloblasts ($P < 0.0005$) and a decrease in mature neutrophils ($P < 0.0005$). The marrow of IL-3-treated rats at 24 hours was similar to that of carrier-treated control rats. The bone marrow of endotoxin-treated rats at 24 hours was morphologically similar to that of the IL-3 group at 8 hours, although endotoxin caused a greater left-shifted myeloid hyperplasia and a greater decrease in mature

Table 1. *Acute* In Vivo *Effects of IL-3 on the Bone Marrow*

	Cells × 10^{-6}/humerus (%)			
Differential	Carrier ($N = 6$)	IL-3 ($N = 6$) at 8 hours	IL-3 ($N = 4$) at 24 hours	LPS ($N = 4$) at 24 hours
Erythroid				
Pronormoblasts	0.39 ± 0.1 (0.6 ± 0.2)	0.77 ± 0.1 (1.5 ± 0.3)	0.52 ± 0.3 (0.8 ± 0.4)	0.31 ± 0.1 (0.6 ± 0.2)
Early normoblasts	0.65 ± 0.1 (1.0 ± 0.1)	0.83 ± 0.2 (1.6 ± 0.4)	1.11 ± 0.5 (1.8 ± 0.7)	0.58 ± 0.2 (1.2 ± 0.4)
Intermediate normoblasts	6.59 ± 0.9 (10.3 ± 1.0)	4.89 ± 1.3 (9.3 ± 1.8)	6.32 ± 0.8 (10.5 ± 0.8)	4.36 ± 0.7 (8.9 ± 1.4)
Late normoblasts	12.74 ± 1.7 (20.5 ± 1.8)	8.83 ± 1.1 (17.6 ± 2.2)	12.89 ± 0.1 (21.5 ± 1.3)	7.92 ± 0.3 (16.2 ± 0.9)
Myeloid				
Myeloblasts	1.84 ± 0.3 (2.8 ± 0.3)	5.10 ± 0.7 (9.9 ± 1.7)	1.93 ± 0.1 (3.3 ± 0.3)	7.05 ± 0.3 (14.3 ± 0.4)
Promyelocytes	0.95 ± 0.2 (1.5 ± 0.3)	1.53 ± 0.3 (2.9 ± 0.7)	1.07 ± 0.2 (1.7 ± 0.4)	3.36 ± 0.5 (6.8 ± 1.2)
Myelocytes	5.31 ± 0.5 (8.5 ± 0.5)	3.55 ± 0.6 (6.8 ± 0.9)	5.16 ± 0.4 (8.5 ± 0.8)	4.85 ± 0.6 (9.8 ± 1.2)
Metamyelocytes	1.99 ± 0.2 (3.1 ± 0.4)	1.46 ± 0.2 (2.6 ± 0.3)	1.74 ± 0.6 (2.9 ± 0.9)	1.47 ± 0.2 (3.1 ± 0.4)
Band cells	2.02 ± 0.2 (3.2 ± 0.5)	1.43 ± 0.2 (2.7 ± 0.4)	1.63 ± 0.7 (2.7 ± 1.1)	0.85 ± 0.3 (1.7 ± 0.5)
Segmented neutrophils	11.52 ± 1.0 (18.2 ± 0.9)	8.31 ± 0.5 (16.6 ± 1.1)	10.71 ± 0.6 (17.8 ± 0.8)	3.51 ± 0.9 (7.1 ± 1.6)
Eosinophils	2.03 ± 0.2 (3.2 ± 0.3)	1.33 ± 0.2 (2.5 ± 0.2)	2.06 ± 0.7 (3.5 ± 1.2)	1.56 ± 0.3 (3.3 ± 0.9)
Monocytes	1.45 ± 0.3 (2.2 ± 0.4)	1.15 ± 0.2 (2.3 ± 0.4)	1.13 ± 0.2 (1.9 ± 0.4)	1.02 ± 0.2 (2.1 ± 0.4)
Mast cells	0.81 ± 0.2 (1.2 ± 0.3)	1.13 ± 0.2 (2.2 ± 0.5)	0.79 ± 0.4 (1.3 ± 0.8)	0.79 ± 0.3 (1.7 ± 0.6)
Histiocytes	1.95 ± 0.3 (3.5 ± 0.4)	1.12 ± 0.3 (2.1 ± 0.4)	1.58 ± 0.4 (2.6 ± 0.5)	1.11 ± 0.5 (2.2 ± 1.0)
Lymphoid				
Lymphocytes	11.87 ± 1.4 (18.6 ± 0.8)	9.28 ± 1.6 (17.9 ± 2.6)	10.17 ± 1.2 (16.9 ± 1.1)	9.45 ± 0.8 (19.2 ± 1.4)
Plasma cells	0.81 ± 0.2 (1.2 ± 0.3)	0.60 ± 0.1 (1.1 ± 0.3)	0.96 ± 0.7 (1.6 ± 1.2)	0.62 ± 0.3 (1.3 ± 0.7)
Megakaryocytes	0.38 ± 0.1 (0.6 ± 0.2)	0.19 ± 0.1 (0.3 ± 0.1)	0.44 ± 0.4 (0.7 ± 0.7)	0.29 ± 0.1 (0.5 ± 0.2)
Total nucleated cells	63.38 ± 6.0	51.57 ± 4.1	60.21 ± 3.83	49.10 ± 1.8

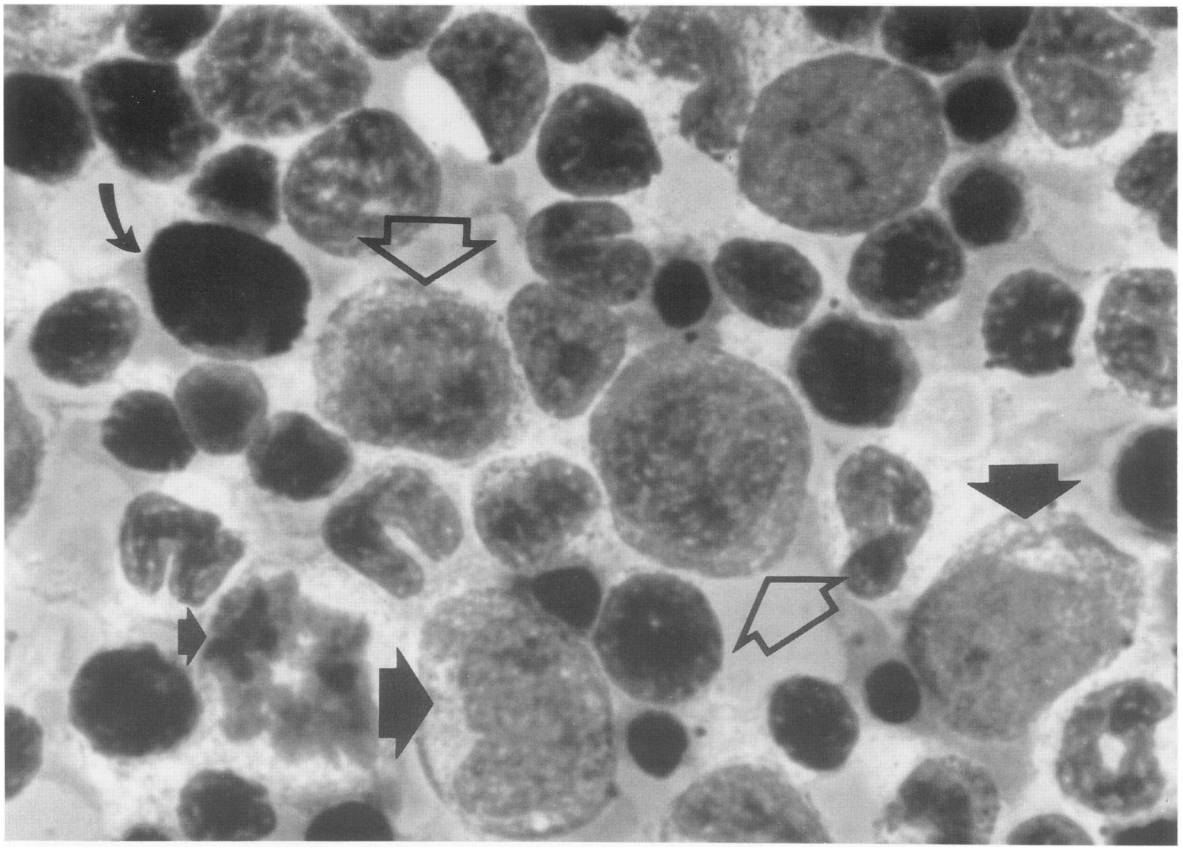

Figure 2. *The bone marrow smear of an IL-3-treated rat at 8 hours shows a left-shifted myeloid hyperplasia with an increase in myeloblasts (open arrows), promyelocytes (large solid arrows), and mitoses (small solid arrow). A mast cell (curved arrow) is also present, but there was no increase in the number of marrow mast cells. The cells with the hyperchromatic nuclei are erythroid precursors at varying stages of maturation. (Wrights-Giemsa, ×1000.)*

marrow neutrophils. The more marked effects of endotoxin on the bone marrow are consistent with the greater peripheral neutrophilia induced by endotoxin as opposed to IL-3.

IL-3 also induced a twofold increase in pronormoblasts ($P < 0.005$), a change consistent with the *in vitro* effects of IL-3 on erythroid stem cells. No increase in pronormoblasts was noted in endotoxin-treated rats, but interestingly both IL-3- and endotoxin-treated rats demonstrated a mild decrease in the late erythroid cells of the marrow (intermediate normoblasts and late normoblast, $P < 0.005$), suggesting the possibility that an increase in erythroid differentiation had occurred.

IL-3 and IL-6 in combination (Figure 3A–B) induced a greater peripheral neutrophilia ($P < 0.05$ at 4, 6, 10, and 12 hours) and monocytosis than either IL-3 or IL-6 alone. The increases in circulating neutrophils and monocytes were either less than additive, additive, or synergistic (ie, greater than additive), depending on the time after injection. IL-3-plus IL-6-treated rats developed a mild lymphopenia at 1.5 to 4 hours as would be expected in rats treated with IL-6 alone (data not shown). The marrows of IL-3-plus IL-6-treated rats at 24 hours (Table 2) demonstrated an erythroid hyperplasia and slight left-shifted myeloid hyperplasia as would be expected due to the individual effects of IL-3 and IL-6.[12] Additionally, IL-3 and IL-6 in combination induced an increase in mast cells that was very striking and easily appreciated in marrow smears (Figure 4). IL-3-plus IL-6-treated rats demonstrated $3.2 \pm 0.8 \times 10^6$ mast cells/humerus compared with approximately $0.8 \pm 0.2 \times 10^6$ mast cells/humerus in carrier-, IL-3-, or IL-6-treated rats ($P < 0.005$).

Discussion

IL-3 *in vivo* was documented as inducing peripheral neutrophilia and monocytosis accompanied by a decrease in mature marrow neutrophils and a left-shifted myeloid hyperplasia in the bone marrow. IL-3 does not induce an initial neutropenia such as accompanies endotoxin-, G-CSF-, and TNF-induced neutrophilia,[20–23] and IL-3 does not induce lymphopenia such as accompanies endotoxin-, IL-1, and TNF-induced neutrophilia.[21–23] The decrease in mature marrow neutrophils that accompanies IL-3-induced neutrophilia suggests that the mechanism of neu-

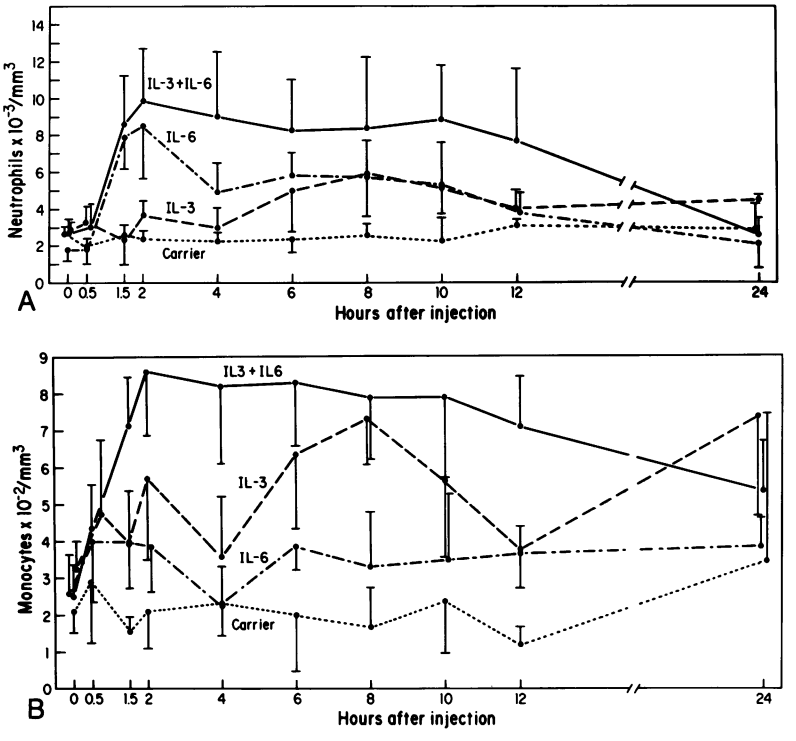

Figure 3. *IL-3 (10 μg) and IL-6 (0.4 μg) in combination induces a peripheral neutrophilia (A) greater than that induced by either IL-3 (10 μg) or IL-6 (0.4 μg) alone (P < 0.05 at 4, 6, 10, and 12 hours). The increase in circulating number of neutrophils induced by the combination of IL-3 and IL-6 was either less than additive, additive (at 2 hours and 6 to 10 hours), or synergistic (ie, greater than additive at 4 and 12 hours), depending on the time after injection. The combination of IL-3 and IL-6 also induced a greater monocytosis (B) than that noted with IL-3 alone.*

trophilia is not primarily through demargination such as occurs after administration of IL-6[12] or epinephrine.[24,25]

The results of the present study complement those of previous investigators who examined the *in vivo* effects of IL-3 on numbers of leukocytes in the circulation and bone marrow. Lord et al[14] did not observe any changes in peripheral white cell counts in IL-3-treated mice, but the times examined and the data are not reported. Lord et al[14] did observe a decrease in the cellularity of the bone marrow at 6 hours along with an increase in the cellularity of the spleen, and postulated that a migration of CFU-S occurs from the marrow to the spleen. Our data suggest

Table 2. *Acute* In Vivo *Effects of IL-3 and IL-6 on the Bone Marrow*

	Cells × 10^{-6}/humerus (%)			
Differential	Carrier (*N* = 6)	IL-3 (24 hours) *N* = 4	IL-6 (24 hours)[12] *N* = 6	IL-3 + IL-6 (24 hours) *N* = 8
Erythroid				
Pronormoblasts	0.39 ± 0.1 (0.6 ± 0.2)	0.52 ± 0.3 (0.8 ± 0.4)	0.73 ± 0.3 (0.9 ± 0.4)	0.80 ± 0.2 (0.9 ± 0.3)
Early normoblasts	0.65 ± 0.1 (1.0 ± 0.1)	1.11 ± 0.5 (1.8 ± 0.7)	1.00 ± 0.1 (1.3 ± 0.3)	1.10 ± 0.3 (1.3 ± 0.4)
Intermediate normoblasts	6.59 ± 0.9 (10.3 ± 1.0)	6.32 ± 0.8 (10.5 ± 0.8)	8.97 ± 1.1 (11.6 ± 2.0)	9.01 ± 2.0 (11.9 ± 3.0)
Late normoblasts	12.74 ± 1.7 (20.5 ± 1.8)	12.89 ± 0.1 (21.5 ± 1.3)	20.89 ± 3.4 (26.6 ± 3.1)	20.89 ± 3.2 (26.3 ± 3.6)
Myeloid				
Myeloblasts	1.84 ± 0.3 (2.8 ± 0.3)	1.93 ± 0.1 (3.3 ± 0.3)	2.73 ± 1.5 (3.3 ± 1.5)	3.42 ± 0.8 (4.2 ± 0.8)
Promyelocytes	0.95 ± 0.2 (1.5 ± 0.3)	1.07 ± 0.2 (1.7 ± 0.4)	1.59 ± 0.6 (2.0 ± 0.5)	1.58 ± 0.5 (1.9 ± 0.6)
Myelocytes	5.31 ± 0.6 (8.5 ± 0.5)	5.16 ± 0.4 (8.5 ± 0.8)	5.10 ± 0.7 (6.5 ± 0.6)	6.03 ± 1.1 (7.5 ± 1.2)
Metamyelocytes	1.99 ± 0.2 (3.1 ± 0.4)	1.74 ± 0.6 (2.9 ± 0.9)	2.71 ± 0.4 (3.4 ± 0.4)	2.14 ± 0.4 (2.6 ± 0.4)
Band cells	2.02 ± 0.2 (3.2 ± 0.5)	1.63 ± 0.7 (2.7 ± 1.1)	3.22 ± 1.3 (4.0 ± 1.1)	1.80 ± 0.3 (2.2 ± 0.3)
Segmented neutrophils	11.52 ± 1.0 (18.2 ± 0.9)	10.71 ± 0.6 (17.8 ± 0.8)	13.12 ± 2.0 (16.7 ± 0.9)	10.81 ± 1.4 (13.6 ± 1.9)
Eosinophils	2.03 ± 0.2 (3.2 ± 0.3)	2.06 ± 0.7 (3.5 ± 1.2)	1.38 ± 0.8 (1.7 ± 0.9)	1.54 ± 0.5 (1.9 ± 0.6)
Monocytes	1.45 ± 0.3 (2.2 ± 0.4)	1.13 ± 0.2 (1.9 ± 0.4)	0.85 ± 0.3 (1.1 ± 0.4)	1.43 ± 0.4 (1.8 ± 0.6)
Mast cells	0.81 ± 0.2 (1.2 ± 0.3)	0.79 ± 0.4 (1.3 ± 0.8)	0.78 ± 0.3 (0.9 ± 0.2)	3.24 ± 0.8 (4.0 ± 1.1)
Histiocytes	1.95 ± 0.3 (3.5 ± 0.4)	1.58 ± 0.4 (2.6 ± 0.5)	0.95 ± 0.2 (1.2 ± 0.4)	0.96 ± 0.1 (1.2 ± 0.1)
Lymphoid				
Lymphocytes	11.87 ± 1.4 (18.6 ± 0.8)	10.17 ± 1.2 (16.9 ± 1.1)	13.03 ± 3.5 (16.5 ± 2.9)	13.02 ± 2.2 (16.8 ± 2.4)
Plasma cells	0.81 ± 0.2 (1.2 ± 0.3)	0.96 ± 0.7 (1.6 ± 1.2)	0.84 ± 0.2 (1.6 ± 0.2)	1.09 ± 0.6 (1.3 ± 0.7)
Megakaryocytes	0.38 ± 0.1 (0.6 ± 0.2)	0.44 ± 0.4 (0.7 ± 0.7)	0.46 ± 0.1 (0.5 ± 0.1)	0.49 ± 0.2 (0.6 ± 0.3)
Total nucleated cells	63.38 ± 6.0	60.21 ± 3.83	78.55 ± 11.3	79.35 ± 55

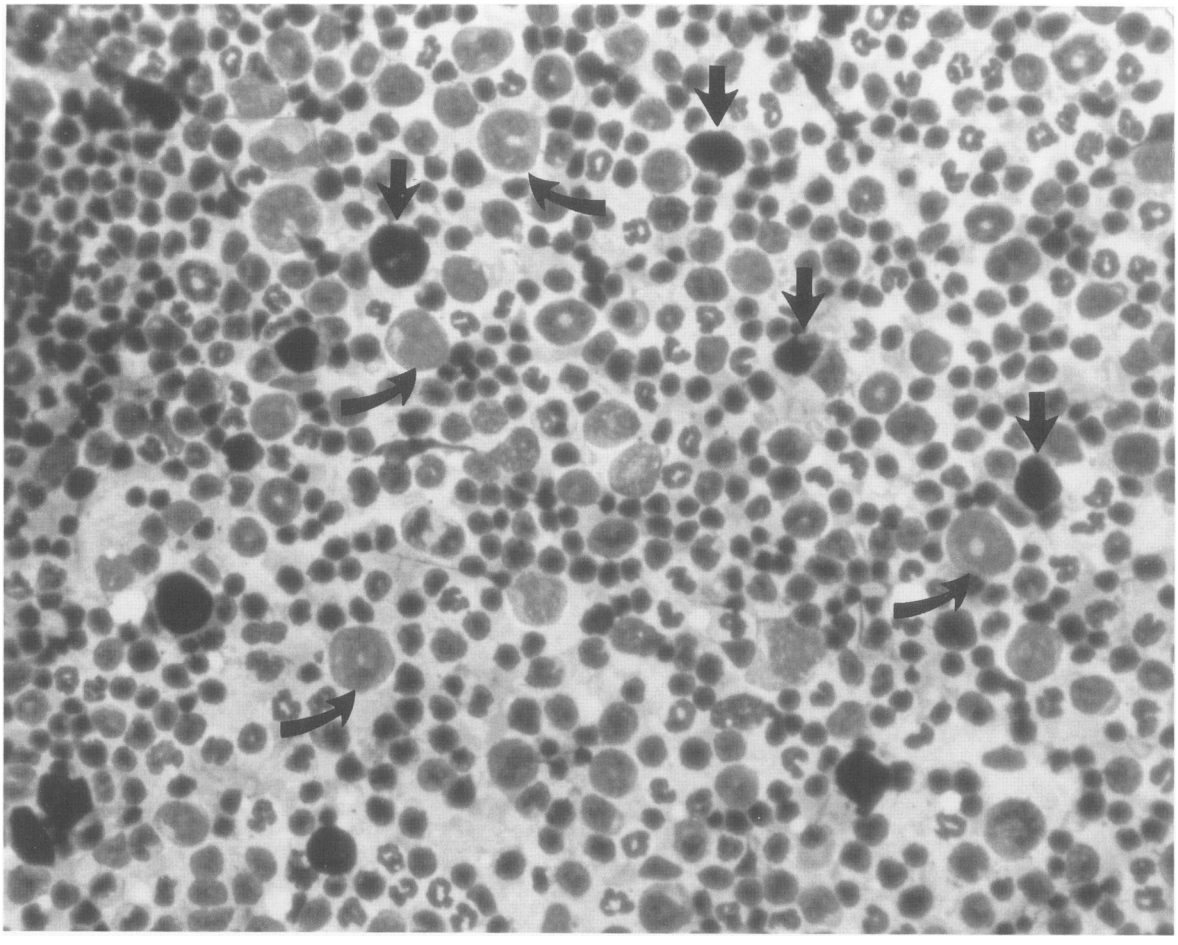

Figure 4. *IL-3 and IL-6 in combination induced a striking increase ($P < 0.005$) in the number of marrow mast cells (straight arrows). Neither IL-3 nor IL-6 alone caused an increase in marrow mast cells. Also demonstrated at this relatively low power (original magnification ×250) is the prominent right-shifted erythroid hyperplasia (small cells with hyperchromatic nuclei) characteristic of IL-6-treated rats and the left-shifted myeloid hyperplasia (some promyelocytes are indicated with curved arrows) that is induced independently by both IL-6 and more especially IL-3 at an earlier timepoint. (Wrights-Giemsa, ×400.)*

that a substantial portion of the decrease in the cellularity in the humerus and of the increase in splenic cellularity may be due to migration of mature neutrophils from the marrow to the circulation with sequestration of these neutrophils in the spleen. Lord et al[14] also noted that the effects of IL-3 on the marrow in their model were similar to those of endotoxin (LPS) and suggested that LPS may act indirectly on hematopoietic precursor cells by eliciting local production of IL-3 from adjacent T cells or stromal cells. The bone marrow differentials performed in the present study show that IL-3 and LPS both cause a left-shifted myeloid hyperplasia and a decrease in mature marrow neutrophils. IL-3 and LPS also are similar in that both cause a peripheral neutrophilia. IL-3, however, does not cause an initial peripheral neutropenia or a dramatic lymphopenia such as occurs after administration of LPS. Furthermore, the peripheral monocytosis noted after injection of IL-3 is not seen after injection of LPS. LPS most likely exerts its hematologic effects through the release of a wide spectrum of adrenal hormones and of cytokines that may include IL-3, TNF, IL-1, G-CSF, GM-CSF, and IL-6.[12,20-26]

Metcalf et al[13] noted increases in circulating numbers of neutrophils, monocytes, and eosinophils in the peripheral blood in their model, but did not observe any significant changes in the bone marrow. The study of Metcalf et al, however, was based on chronic daily injections of IL-3 and is, therefore, not strictly comparable to the present study. That Metcalf et al[13] did not observe any significant changes in the marrow is consistent with the present finding that the marrows of IL-3-treated rats at 24 hours were indistinguishable from the marrows of carrier-treated control rats.

Kindler et al[15] also studied the effects of chronic administration of IL-3 in a murine model and noted enhancement of the myeloid and erythroid lineages as well as a decrease in marrow cellularity. Continuous infusion of IL-3 was considered necessary by these authors because

of the rapid blood clearance of IL-3. Kindler et al[15] suggested that IL-3 could double hematopoietic activity in less than 3 days as indicated by the extrapolated total number of hematopoietic cells. In the present study, IL-3 was shown to be effective as a single intravenous injection and to more than double the number of myeloblasts in the marrow within 8 hours. An increase in myelopoiesis as judged by an increase in early myeloid cells in bone marrow smears could, in fact, be seen as early as 0.5 hours after injection of IL-3 (data from two rats not shown). The present study also documents the erythropoietic effects of IL-3 *in vivo*.

The combination of IL-3 and IL-6 was shown in the present study to induce a synergistic neutrophilia and monocytosis and a synergistic increase in marrow mast cells. Ogawa and colleagues[16,17] previously reported on the synergistic *in vitro* effects of IL-3 and IL-6 in supporting and hastening the growth of murine and human blast cell colonies. In those *in vitro* experiments, the effects of the growth factors were measured over a time course of many days during which time the progenitor cells were in prolonged contact with IL-3 and IL-6. In contrast, in the present *in vivo* experiments, the hematopoietic effects of the growth factors were apparent well within the first 24 hours although the precursor cells of the marrow were most likely only briefly exposed to IL-3 and IL-6 because of the short half-lives of these factors *in vivo*. *In vivo* experiments allow the observation of marrow releasing activity and peripheral neutrophilia, phenomena that are not observable *in vitro*. On the other hand, *in vivo* experiments do not allow a distinction between the direct and indirect effects of exogenously administered cytokines and future study of the effects of exogenous IL-3 on endogenous cytokine gene expression will be necessary to determine whether the neutrophil releasing activity observed in the present model is due directly to IL-3 or indirectly to another cytokine such as, for example, G-CSF. In any case, IL-3 and IL-6, like G-CSF and GM-CSF, may be considered as potentially useful in clinical settings in which restoration of hematopoietic activity and release of marrow neutrophils into the circulation is desirable.

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Acknowledgment

We thank Dr. David Urdal and Dr. Lawrence Souza for their generous gifts of recombinant human IL-3 and IL-6.