In Vivo Hematologic Effects of Recombinant Interleukin-6 on Hematopoiesis and Circulating Numbers of RBCs and WBCs

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Interleukin-6 (IL-6) administered as a single intravenous (IV) injection caused the following changes in the peripheral circulation of rats: (a) a biphasic neutrophilia with an initial peak at 1.5 hours and a second sustained wave of neutrophilia between four and 12 hours, (b) a mild lymphocytosis at 0.5 hours and a mild lymphopenia between 1.5 and four hours, and (c) a reticulocytosis between 12 and 24 hours. The bone marrow showed no significant changes at 1.5 hours, suggesting that the peripheral neutrophilia at that time is caused by demargination of intravascular neutrophils and not by release of marrow neutrophils. The bone marrow at 12 hours showed a mild left-shifted myeloid hyperplasia of myeloblasts and promyelocytes and a tremendous erythroid hyperplasia of intermediate and late normoblasts. The bone marrow at 24 hours showed a continued mild myeloid hyperplasia and striking erythroid hyperplasia. In conclusion, IL-6 in vivo acts as a stimulus for myelopoiesis and erythropoiesis and causes accompanying peripheral changes in the number of neutrophils, lymphocytes, and RBCs.

NUMEROUS recombinant cytokines and colony-stimulating factors (CSFs) have recently been documented to contribute to hematopoiesis and to regulation of circulating numbers of neutrophils and mononuclear cells.[1-8] The regulation of hematopoiesis, of the rate of release of blood cells into the circulation, and of the egress of leukocytes from the circulation into tissues is clearly a complex process controlled by multiple cell types and by multiple members of the so-called "cytokine cascade." Interleukin-6 (IL-6) is a multifunctional peptide growth factor[9] that enhances the multi-CSF (IL-3)-dependent proliferation of multipotential hematopoietic progenitors in vitro.[10] IL-6 is the name currently most often used to describe a molecule also known as interferon (IFN)-β_2, B-cell stimulating factor-2, hybridoma growth factor, hepatocyte-stimulating factor, and 26-Kd protein.[11-13] This article reports the in vivo effects of a single intravenous (IV) injection of recombinant human IL-6 on hematopoiesis and on circulating numbers of RBCs and WBCs in the Lewis rat.

MATERIALS AND METHODS

Recombinant human IL-6 (Lot 917-9-42) of the sequence published by Hirano et al[14] and secreted by transfected yeast cells was obtained from Immunex (Seattle, WA) with a protein concentration of 66 μg/mL and containing 3×10^5 U/mL biologic activity. One unit of IL-6 is defined as the amount required to augment the production of immunoglobulin by the CESS cell line to the half-maximal level.[15] Lewis male rats weighing ~250 g (Harlan-Sprague-Dawley, Indianapolis) received a single IV injection of varying doses of IL-6 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, Hialeah, FL) and for blood smears was obtained by tail bleeding under ether anesthesia immediately before and at various time points after injection of IL-6. WBC differentials were performed by counting 100 WBCs on modified Wright's-stained smears (Diff-Quik Stain Set; American Science Products, McGaw Park, IL). Reticulocyte preparations were made by vital staining with methylene blue. Bone marrow hematopoietic cell subsets were quantitated by the method of Chervenick et al.[16] When the rats were killed, 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 Isoton II buffer (Coulter) injected through a 21-gauge needle together with heparin and a RBC lysing agent (Zapoglobin, Coulter), 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 1,000 cells/smear according to standard morphologic criteria for the rat as reported by Hulse.[17] Statistical analysis of the data was performed with either the paired or unpaired *t* test using the Statview program on an Apple computer, and all averages are expressed at ±1 SD. Heating of the IL-6 at 80°C for one hour completely abrogated its hematologic effects, demonstrat-

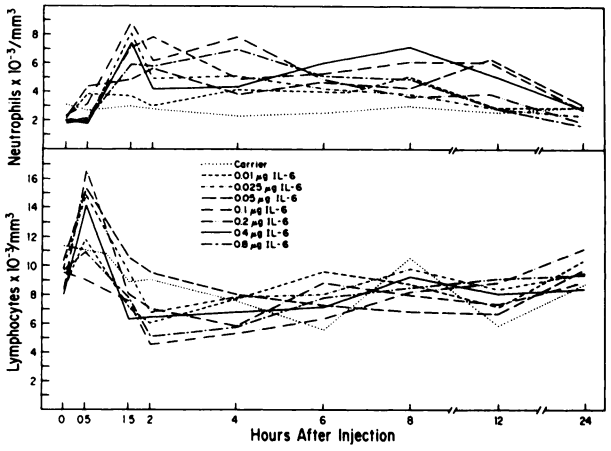

Fig 1. A dose–response study of the effects of IL-6 (n = 1 rat at each dose) showed an initial peak of neutrophilia at 1.5 hours followed by a more sustained second wave of neutrophilia variably reaching a maximum between four and eight hours. An early lympocytosis occurred at 0.5 hours followed by lymphopenia between two and four hours. The magnitude of these peripheral hematologic changes did not show any clear dose–response dependence at doses >0.1 μg/rat.

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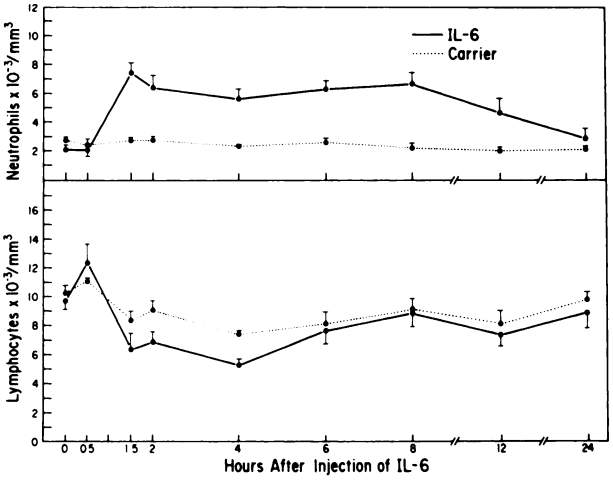

Fig 2. IL-6–induced neutrophilia ($P < .0005$ comparing time 0 to 1.5 hours), lymphocytosis ($P < .025$ comparing 0 to 0.5 hours) and lymphopenia ($P < 0.005$ at two and four hours compared either with time 0 or with carrier controls) were all significant at a dose of 0.4 μg IL-6/rat.

ing that endotoxin (which is heat resistant) was not responsible for the observed results.

RESULTS

A dose-response study of the kinetics of IL-6–induced neutrophilia and lymphopenia (Fig 1) shows that IL-6 over a wide range of doses induces an initial peak of neutrophilia at 1.5 hours, followed by a more sustained second wave of neutrophilia. Of interest is the lack of any significant increase in the magnitude of the first peak of neutrophilia over a relatively wide range of doses of IL-6 and the observation that no increase in circulating band cells occurred at this time (data not shown), both observations suggesting that the first peak of neutrophilia might be due to peripheral demargination rather than bone marrow release of neutrophils. The second broader wave of neutrophilia variably reached a maximum between four and eight hours. IL-6 induced a very transient lymphocytosis at 0.5 hours, followed by a mild lymphopenia between 1.5 and four hours. The lymphocytosis and lymphopenia did not show any clear evidence of dose–response dependence at doses >0.1 μg/rat.

The kinetics of peripheral neutrophilia and lymphopenia were studied in a larger number of rats (n = 18) at the dose of 0.4 μg IL-6/rat (Fig 2). The significance of the neutrophilia ($P < .0005$ comparing time 0 to 1.5 hours), of the early lymphocytosis ($P < .025$ comparing time 0 to 0.5 hours), and of the lymphopenia ($P < .005$ at two and four hours compared either with carrier controls or time 0) were statistically confirmed. Carrier-control–treated rats did not experience any significant changes in circulating numbers of neutrophils and experienced a slight lymphopenia between two and four hours as previously reported by our laboratory.[1,2]

Bone marrow differentials were performed at 1.5, 12, and 24 hours in IL-6– and carrier-treated rats (Table 1). The bone marrow at 1.5 hours in IL-6–treated rats was not significantly different from controls, confirming that the peripheral neutrophilia at 1.5 hours is not due to marrow release of neutrophils. The bone marrow at 12 hours in IL-6–treated rats demonstrated a mild left-shifted myeloid hyperplasia with a doubling in the number of myeloblasts ($P < .0005$) and promyelocytes ($P < .0005$) but no significant change in the number of mature neutrophils. A striking increase in late normoblasts was noted at 12 hours ($P < .005$) and was accompanied by an increase in peripheral blood reticulocytes to 9.14% ± 0.9% as compared with 2% to 4% in control rats. The bone marrow at 24 hours in IL-6–treated rats continued to demonstrate a mild but more generalized myeloid hyperplasia. The marked erythroid hyperplasia of the marrow and a peripheral reticulocytosis

Table 1. Interleukin-6 Induces a Mild Myeloid Hyperplasia and a Striking Erythroid Hyperplasia at 12 and 24 Hours

Differential	Cells × 10^{-6} (%)/Humerus			
	Carrier (n = 6)	IL-6 (n = 6) 1.5 h	IL-6 (n = 6) 12 h	IL-6 (n = 6) 24 h
Erythroid				
Pronormoblasts	0.56 ± 0.3 (0.9 ± 0.4)	1.30 ± 0.4 (2.0 ± 0.6)	1.05 ± 0.1 (1.3 ± 0.2)	0.73 ± 0.3 (0.9 ± 0.4)
Early normoblasts	0.85 ± 0.3 (1.4 ± 0.5)	0.87 ± 0.1 (1.3 ± 0.2)	1.24 ± 0.4 (1.6 ± 0.5)	1.00 ± 0.1 (1.3 ± 0.3)
Intermediate normoblasts	6.09 ± 0.7 (10.2 ± 0.8)	7.00 ± 0.6 (10.9 ± 0.6)	8.79 ± 1.1 (11.4 ± 1.3)	8.97 ± 1.1 (11.6 ± 2.0)
Late normoblasts	11.79 ± 1.0 (19.9 ± 1.4)	12.99 ± 0.8 (20.3 ± 0.6)	25.39 ± 1.6 (33.8 ± 2.1)	20.89 ± 3.4 (26.6 ± 3.1)
Myeloid				
Myeloblasts	1.48 ± 0.2 (2.5 ± 0.3)	1.66 ± 0.4 (2.5 ± 0.6)	2.84 ± 0.3 (3.6 ± 0.3)	2.73 ± 1.5 (3.3 ± 1.5)
Promyelocytes	0.88 ± 0.1 (1.4 ± 0.2)	0.87 ± 0.1 (1.3 ± 0.2)	1.93 ± 0.2 (2.5 ± 0.2)	1.59 ± 0.6 (2.0 ± 0.5)
Myelocytes	5.09 ± 0.6 (8.6 ± 0.7)	4.70 ± 0.6 (7.8 ± 0.5)	6.64 ± 0.4 (8.6 ± 0.5)	5.10 ± 0.7 (6.5 ± 0.6)
Metamyelocytes	2.02 ± 0.3 (3.4 ± 0.5)	2.05 ± 0.4 (3.2 ± 0.5)	1.80 ± 0.2 (2.3 ± 0.3)	2.71 ± 0.4 (3.4 ± 0.4)
Band cells	1.98 ± 0.2 (3.3 ± 0.3)	2.56 ± 0.5 (4.0 ± 0.7)	1.74 ± 0.2 (2.2 ± 0.3)	3.22 ± 1.3 (4.0 ± 1.1)
Segmented neutrophils	10.83 ± 1.2 (18.3 ± 1.4)	11.98 ± 0.7 (18.6 ± 0.5)	10.47 ± 1.8 (13.5 ± 2.4)	13.12 ± 2.0 (16.7 ± 0.9)
Eosinophils	2.11 ± 0.2 (3.4 ± 0.5)	1.93 ± 0.5 (3.3 ± 1.0)	1.04 ± 0.3 (1.3 ± 0.3)	1.38 ± 0.8 (1.7 ± 0.9)
Basophils	0.21 ± 0.1 (0.3 ± 0.1)	0.11 ± 0.1 (0.1 ± 0.1)	0.10 ± 0.1 (0.1 ± 0.1)	0.20 ± 0.2 (0.2 ± 0.2)
Monocytes	1.39 ± 0.2 (2.7 ± 0.3)	1.28 ± 0.2 (2.0 ± 0.4)	0.94 ± 0.2 (1.2 ± 0.2)	0.85 ± 0.3 (1.1 ± 0.4)
Mast cells	0.80 ± 0.2 (1.7 ± 0.3)	0.84 ± 0.1 (1.3 ± 0.2)	0.56 ± 0.1 (0.7 ± 0.1)	0.78 ± 0.3 (0.9 ± 0.2)
Histiocytes	1.64 ± 0.4 (2.8 ± 0.9)	1.40 ± 0.4 (2.3 ± 0.5)	1.63 ± 0.6 (2.1 ± 0.8)	0.95 ± 0.2 (1.2 ± 0.4)
Lymphoid				
Lymphocytes	10.51 ± 1.6 (17.7 ± 1.7)	11.36 ± 1.0 (17.8 ± 0.9)	9.43 ± 0.8 (12.2 ± 0.7)	13.03 ± 3.5 (16.5 ± 2.9)
Plasma cells	0.57 ± 0.1 (0.9 ± 0.3)	0.53 ± 0.1 (0.8 ± 0.2)	0.83 ± 0.2 (1.0 ± 0.2)	0.84 ± 0.2 (1.6 ± 0.2)
Megakaryocytes	0.37 ± 0.1 (0.6 ± 0.2)	0.35 ± 0.1 (0.5 ± 0.2)	0.46 ± 0.1 (0.6 ± 0.1)	0.46 ± 0.1 (0.5 ± 0.1)
Total nucleated cells/humerus	59.17 ± 4.9	63.78 ± 4.6	76.88 ± 2.8	78.55 ± 11.3

also persisted. No significant increases in lymphocytes, plasma cells, or megakaryocytes were noted. The myeloid and erythroid hyperplasia of IL-6–treated rats is reflected in the hypercellularity of their bone marrows (77 and 79 $\times$ 10^6 nucleated cells/humerus at 12 and 24 hours as compared with 59 $\times$ 10^6 nucleated cells/humerus in controls).

DISCUSSION

IL-6 is a multifunctional peptide growth factor[9-13] that was recently reported to enhance IL-3–dependent proliferation of multipotential hematopoietic progenitors in vitro.[10] The present study provides in vivo confirmation that IL-6 is a stimulus for myelopoiesis and especially erythropoiesis. A single IV injection of 0.4 μg IL-6/rat causes a striking myeloid and erythroid hyperplasia at 12 and 24 hours.

IL-6–induced neutrophilia is not accompanied by a decrease in marrow neutrophils at 1.5 hours such as is dramatically caused by a single injection of IL-1 or TNF[1,2] or accompanying G-CSF–induced neutrophilia at four to 12 hours.[7] Thus, IL-6 is not a neutrophil-releasing factor as are IL-1, TNF, and G-CSF. The first peak of IL-6–induced neutrophilia is therefore most likely due to demargination as is also consistent with the observation that the magnitude of this neutrophilia is very similar to that induced by epinephrine (a known demarginating agent) in rats.[18] The second wave of IL-6–induced neutrophilia might be due to a combination of "overflow" because of increased myelopoiesis, IL-6–induced expression of endogenous releasing factors, and perhaps continued demargination of peripheral neutrophils. IL-6 causes a transient lymphocytosis at 0.5 hours that our laboratory has not observed in either control, IL-1–, TNF-, or G-CSF–treated rats.[1,2,7] IL-6 causes a mild lymphopenia as compared with controls between two and four hours, a phenomenon that is observed to a greater degree with IL-1– and TNF-treated rats, but not in G-CSF–treated rats. Lymphopenia is also a feature of endotoxemia,[19] and endotoxin induces expression of IL-6 in vitro.[12]

The in vivo hematopoietic effects of IL-6 in the present experimental model suggest that the action of IL-6 takes place at the level of an early multipotential stem cell and provide a basis for future investigations of the possible clinical applications of IL-6 in hematologic disorders in which hypoplasia of myeloid and erythroid elements is of pathogenetic significance.

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