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The Hematologic Effects of Chronic Administration of the Monokines Tumor Necrosis Factor, Interleukin-1, and Granulocyte-Colony Stimulating Factor on Bone Marrow and Circulation

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Monokines may contribute to the regulation of hematopoiesis and circulating numbers of leukocytes during chronic inflammation. The hematologic effects of daily intravenous injection of the recombinant monokines tumor necrosis factor (TNF), interleukin-1 (IL-1), and granulocyte-colony stimulating factor (G-CSF) were therefore studied in the bone marrow and circulation of rats over the course of a week. TNF induced daily neutrophilia and lymphopenia with no evidence of tachyphylaxis. TNF also induced a slight decrease in early myeloid forms in the marrow, but, more strikingly, induced a marked erythroid hyperplasia of late normoblasts, although no changes other than a slight reticulocytosis were noted in the peripheral red blood cell compartment. IL-1 also induced daily neutrophilia and lymphopenia with no evidence of tachyphylaxis. IL-1 differed from TNF in the induction of a significant myeloid hyperplasia and in the lack of any effect on the erythroid elements of the marrow. The lack of tachyphylaxis to the chronic administration of both TNF and IL-1 suggests that the mechanism of endotoxin-induced tachyphylaxis is not at the level of the effector cell response to these endogenous cytokines. G-CSF induced a biphasic peripheral neutrophilia first peaking on day one, reaching a nadir on day 4, and then rising progressively again until day 7. The low level of neutrophilia on day 4 is not due to marrow depletion of neutrophils secondary to the neutrophil releasing activity of G-CSF because the marrows of G-CSF-treated rats on both days 3 and 7 contained over twice the number of mature neutrophils as controls. Thus, the trough in the neutrophilia induced by G-CSF is postulated to be due to an as-yet unidentified negative feedback mechanism that inhibits its neutrophil release from the marrow. (Am J Pathol 1989, 134:149–159)

Macrophages activated by inflammatory or immune stimuli are able to secrete tumor necrosis factor alpha (TNF), interleukin-1 (IL-1), and granulocyte-colony stimulating factor (G-CSF).[1–3] TNF, IL-1, and G-CSF are present in increased concentrations in the circulation under a variety of pathologic conditions.[4,5] These monokines may therefore act in concert with other endogenous inflammatory mediators to contribute to the regulation of hematopoiesis and to the control of circulating numbers of leukocytes. Experimental administration of TNF as a single injection is known to cause lymphopenia and neutropenia followed by neutrophilia in mice,[6] rats,[7] rabbits, (Ulich TR, del Castillo J, unpublished observations) and humans.[8] IL-1α and β administered is a single injection in rats also cause lymphopenia and neutrophilia.[7] G-CSF as a single injection in several species induces a transient neutropenia followed by a relatively long-lasting neutrophilia with no changes in circulating numbers of lymphocytes.[9–11] Chronic administration of G-CSF in hamsters, monkeys, and humans induces a persistent granulocytosis.[9,10] The purpose of the present study is to further examine the hematologic effects of the chronic administration of TNF, IL-1, and G-CSF on the bone marrow and circulation.

Materials and Methods

Male Lewis rats weighing 225–275 g (Harlan-Sprague-Dawley, Indianapolis, IN) under ether anaesthesia re-

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ceived daily injections of 3×10^3 U (0.5 μg) recombinant human TNF alpha (Dr. Michael Shepard, Genentech, Inc., San Francisco, CA), 10^5 U (0.04 μg) recombinant human IL-1α (Dr. Peter Lomedico, Hoffmann-La Roche, Inc., Nutley, NJ), or 2.5×10^6 U (25 μg) recombinant human G-CSF (Dr. Larry Souza, AmGen Biologicals, Inc., Thousand Oaks, CA) intravenously via the dorsal vein of the penis. Carrier for dilution of cytokines and for negative controls consisted of 1% normal rat serum in sterile saline. The specific activity of the TNF was 6×10^6 U/mg determined by *in vitro* tumoricidal assay and less than 3×10^{-5} EU of endotoxin were present in each dose of TNF as determined by the Limulus amebocyte lysate assay. IL-1 consisted of the carboxy-terminal 154 amino acids of the 271 amino acid human IL-1α precursor with a specific activity of 2.5×10^9 U/mg and less than 1.2×10^{-6} EU of endotoxin present in each dose. G-CSF had a specific activity of 10^8 U/mg and had undetectable levels of endotoxin by the Limulus amebocyte lysate assay.[9] Hematologically significant endotoxin contamination of TNF, IL-1, and G-CSF was ruled out by abrogating their hematologic effects by boiling since endotoxin, but not the monokines, are resistant to boiling.

Peripheral blood for smears and for quantitation of the absolute number of circulating leukocytes was obtained by tail bleeding. The total circulating white blood cell count/cumm was measured with a Coulter counter (Coulter Electronics, Inc., Hialeah, FL). The percentage and absolute number of circulating white blood cell subsets was determined after performing a differential count of at least 100 cells/smear. The absolute number of red blood cells/cumm, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and the platelet count were determined in EDTA anticoagulated blood on a Coulter counter. Hematocrit was determined by centrifuging standard heparinized capillary tubes for 5 minutes in a capillary tube centrifuge. Serum bilirubin and haptoglobin were measured on the Beckman Ideal and Array (Beckman, Inc., Brea, CA), respectively, and serum iron was measured on the Dupont ACA (Dupont, Wilmington, DE).

Bone marrow was obtained from the humerus as described by Chervenick, et al[12] and the absolute number of nucleated cells/humerus was quantitated with the Coulter counter. Bone marrow differentials were performed on Wright's-Giemsa stained smears obtained from the contralateral humerus and differential counts were performed on an average of approximately 800 cells/smear according to the standard morphologic criteria for the rat as reported by Hulse.[13]

Statistical analysis of the data was performed using the paired or unpaired *t*-test on an Apple personal computer with the Statview program. A P value of ≤ 0.05 was considered to be statistically significant. Arithmetic averages are in all instances expressed as ± 1 SD. Statistical analysis was always performed on absolute numbers of cells rather than on percentages.

Results

TNF (N = 9) administered intravenously resulted in a significant neutrophilia ($P < 0.0005$) and lymphopenia ($P < 0.005$) at 1.5 hours after injection each day for 7 consecutive days (Figure 1). No differences in the magnitude of either the neutrophilia or lymphopenia were noted on any day, indicating that tachyphylaxis to the hematologic effects of TNF does not occur under the conditions of dosage and route of administration employed in the present experiment. Daily intravenous administration of carrier (N = 5) did on some days result in significant changes in circulating numbers of neutrophils or lymphocytes (Figure 2), but the magnitude of these changes were always significantly less than the corresponding neutrophilia or lymphopenia in TNF-treated rats.

The bone marrow on day 8 at 24 hours after the last injection of TNF demonstrated a slight decrease in the average number of immature myeloid forms ($P < 0.05$ for myeloblasts and myelocytes) but a slight though statistically not significant average increase in the number of mature neutrophils (Table 1). Most strikingly, the marrow exhibited an increase in the late normoblasts of the erythroid series ($24.40 \pm 3.1 \times 10^6$ late normoblasts per humerus in TNF-treated rats *vs.* $13.08 \pm 0.6 \times 10^6$ late normoblasts per humerus in carrier-treated rats). No significant changes in the number of pronormoblasts, early normoblasts or intermediate normoblasts were noted. The nuclei of intermediate and late normoblasts exhibited a central starburst or mulberrylike pyknosis (Figure 3) that could best be appreciated in its three-dimensionality by focusing up and down on the nucleus. The pyknotic process did not involve the peripherally situated chromatin of the nucleus. A similar nuclear morphology is also present in the late normoblasts of control rats and should not be taken as a unique manifestation of TNF treatment. The peculiar pyknotic process, which differs from that observed in human marrows, did, however, appear somewhat exaggerated in the TNF-treated rats, perhaps due to the erythroid hyperplasia. The erythroid hyperplasia could not be accounted for by the daily bleedings of the rats because no similar features were noted in the daily bled carrier control rats.

The effects of chronic administration of TNF on circulating red blood cells were further investigated in two separate experiments by again injecting 3000 U TNF per day (N = 6 rats in each experiment) but without daily bleedings to avoid any possibility of iatrogenic anemia. The chronic administration of TNF under the specified condi-

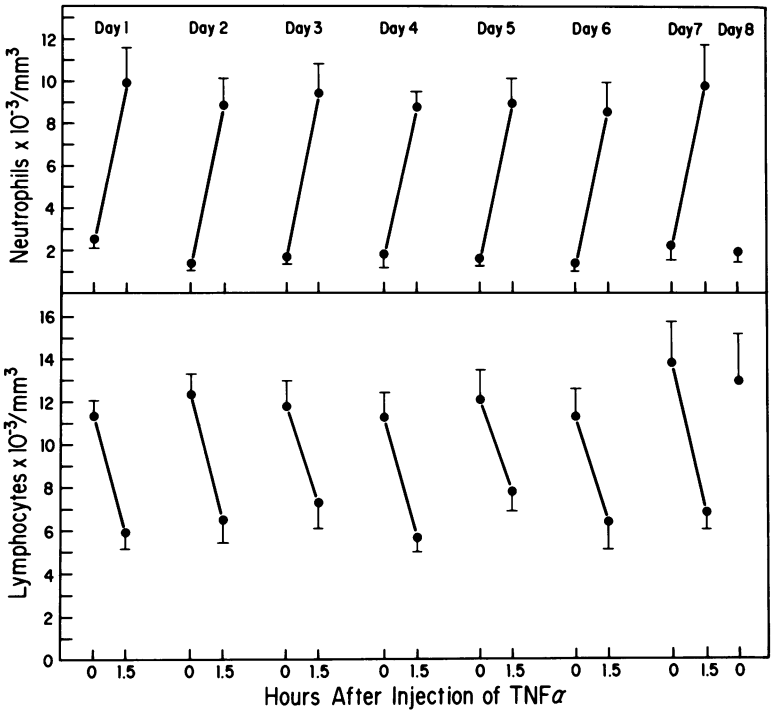


Figure 1. *TNF (0.5 μg) induces a highly significant neutrophilia ($P < 0.0005$ and lymphopenia ($P < 0.005$) every day for a week at 1.5 hours after intravenous injection ($N = 9$). There is no evidence of tachyphylaxis.*

tions of dosage and route of administration did not result in anemia as indicated by the lack of any significant decreases in total red blood cells/cumm, hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, or mean corpuscular hemoglobin concentration (Table 2). The haptoglobin and bilirubin levels were not significantly different in TNF and carrier control rats, suggesting that the normal red blood indices in TNF-treated rats were not due to a compensated hemolytic anemia. No difference in serum iron was noted in TNF-treated and control rats. The percentage of reticulocytes was slightly but significantly ($P < 0.025$) elevated in TNF-treated rats. An additional significant finding ($P < 0.05$) was a slight decrease in platelets in TNF-treated rats (757

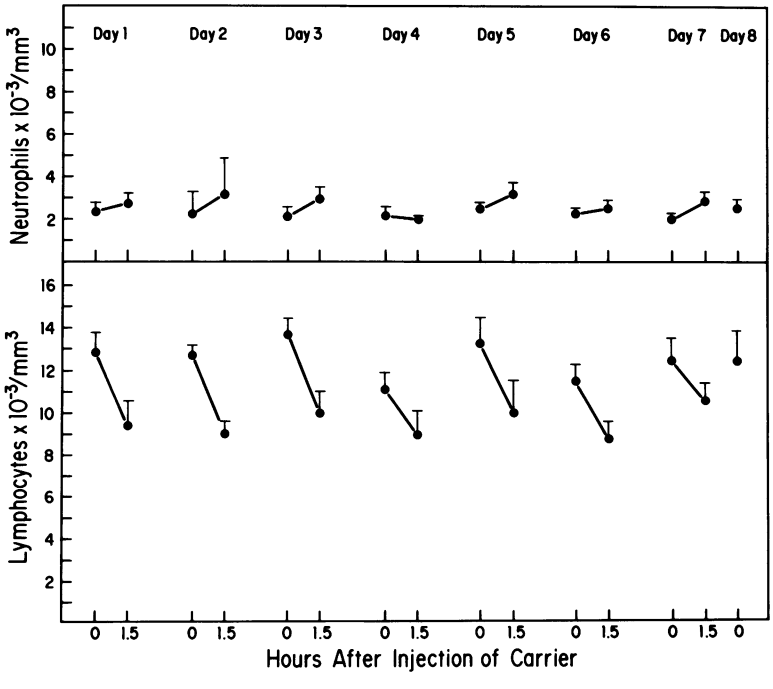


Figure 2. *Carrier ($N = 5$) generally induces little if any neutrophilia but induces a significant lymphopenia every day for a week after intravenous injection. The magnitude of the carrier-induced lymphopenia is, however, significantly less than the magnitude of TNF-induced lymphopenia.*

Table 1. *Daily Intravenous Injection of TNF for 1 Week Induces Hyperplasia of Late Erythroid Normoblasts and a Slight Decrease in Early Myeloid Cells*

	Bone marrow: Cells × 10^{-6}/humerus (%)	
	TNFα (N = 9)	Carrier (N = 5)
Erythroid		
Pronormoblasts	0.69 ± 0.3 (1.0 ± 0.4)	0.49 ± 0.1 (0.8 ± 0.1)
Early normoblasts	0.72 ± 0.4 (1.1 ± 0.6)	0.62 ± 0.1 (1.0 ± 0.2)
Intermediate normoblasts	5.84 ± 1.8 (9.0 ± 2.0)	5.48 ± 0.6 (9.1 ± 1.4)
Late normoblasts	24.40 ± 3.1 (38.5 ± 2.5)	13.08 ± 0.6 (21.7 ± 1.5)
Myeloid		
Myeloblasts	0.86 ± 0.4 (1.3 ± 0.6)	1.28 ± 0.2 (2.1 ± 0.5)
Promyelocytes	0.46 ± 0.1 (0.7 ± 0.3)	0.77 ± 0.1 (1.2 ± 0.3)
Myelocytes	2.72 ± 0.8 (4.2 ± 1.1)	4.11 ± 1.1 (6.7 ± 1.7)
Metamyelocytes	1.00 ± 0.3 (2.3 ± 0.4)	1.78 ± 0.3 (3.0 ± 0.7)
Band cells	1.80 ± 0.8 (2.7 ± 1.0)	2.14 ± 0.9 (3.4 ± 1.2)
Segmented neutrophils	13.03 ± 2.6 (20.6 ± 3.7)	12.92 ± 2.6 (21.1 ± 2.5)
Eosinophils	1.15 ± 0.6 (1.8 ± 0.9)	1.44 ± 0.8 (3.0 ± 1.3)
Basophils	0.22 ± 0.1 (0.3 ± 0.1)	0.28 ± 0.1 (0.4 ± 0.2)
Monocytes	0.29 ± 0.2 (0.4 ± 0.3)	0.99 ± 0.5 (1.7 ± 1.1)
Mast cells	0.42 ± 0.2 (0.6 ± 0.3)	0.75 ± 0.2 (1.2 ± 0.3)
Histiocytes	0.59 ± 0.5 (0.8 ± 0.6)	0.66 ± 0.3 (1.0 ± 0.4)
Lymphoid		
Lymphocytes	8.79 ± 1.2 (14.0 ± 2.5)	13.26 ± 1.8 (21.8 ± 1.1)
Plasma cells	0.34 ± 0.2 (0.5 ± 0.3)	0.33 ± 0.1 (0.5 ± 0.2)
Megakaryocytes	0.13 ± 0.1 (0.2 ± 0.1)	0.21 ± 0.1 (0.3 ± 0.2)
Total nucleated cells	63.45 ± 8.5	60.59 ± 6.4

± 59 × 10^3 platelets/cumm, N = 5) as compared with carrier controls (854 ± 102 × 10^3 platelets/cumm, N = 6). The bone marrows of the 12 TNF-treated and 12 carrier control rats studied in these additional two experiments were also examined and confirmed the results presented in Table 1.

IL-1 (N = 6) administered intravenously resulted in a significant neutrophilia ($P < 0.0005$) and lymphopenia ($P < 0.005$) at 1.5 hours after injection each day for 7 consecutive days (Figure 4). No evidence of tolerance was noted to the peripheral hematologic effects of IL-1. Daily intravenous injection of carrier (N = 2) again resulted in little or no increase in neutrophils and a much lesser lymphopenia than noted in cytokine-treated rats (Figure 5).

The bone marrow on day 8 at 24 hours after the last injection of IL-1 demonstrated a generalized myeloid hyperplasia (Figure 6) with statistically significant increases in the number of myeloblasts ($P < 0.025$), promyelocytes ($P < 0.01$), and especially mature segmented neutrophils (18.4 ± 3.9 × 10^6/humerus in IL-1-treated rats *vs.* 11.6 ± 2.1 × 10^6/humerus in carrier control-treated rats, $P < 0.05$) (Table 3). No significant quantitative changes were noted in the erythroid or lymphoid series of the marrow or in the number of megakaryocytes.

G-CSF (N = 3) administered intravenously on a daily basis resulted in a first peak of neutrophilia 1 day after injection ($P < 0.005$) followed by progressively lesser neutrophilia ($P < 0.025$) until a nadir was reached on day 4 that was then followed by a progressively increasing neutrophilia ($P < 0.01$) until day 7 (Figure 7). G-CSF-treated rats were not bled at 1.5 hours (as were TNF- and IL-1-treated rats) because the peak of G-CSF-induced neutrophilia is between 12 and 24 hours. The bone marrow at 24 hours after the last injection demonstrated a dramatic

Table 2. *Daily Intravenous Injection of TNF for 1 Week Causes a Slight Reticulocytosis but Does Not Affect Other Red Blood Cell Indices*

Red blood cell indices	*N*	TNF	*N*	Carrier
Red blood cells × 10^6/mm^3	5	7.88 ± 0.2	6	7.92 ± 0.2
Hematocrit (%)	12	48.0 ± 0.7	6	48.1 ± 0.4
Hemoglobin (g/dl)	5	15.4 ± 0.3	6	16.5 ± 1.4
Mean corpuscular volume (μm^3)	5	53.8 ± 0.4	6	51.8 ± 4.7
Mean corpuscular hemoglobin (pg)	5	19.6 ± 0.3	6	19.1 ± 2.1
Mean corpuscular hemoglobin concentration (g/dl)	5	36.4 ± 0.8	6	37.2 ± 2.3
Reticulocytes (%)	6	6.0 ± 1.0	6	4.1 ± 1.6
Haptoglobin (mg/dl)	9	8.0 ± 4.0	6	7.0 ± 2.0
Bilirubin, direct (mg/dl)	12	0.0 ± 0.0	6	0.0 ± 0.0
Bilirubin, total (mg/dl)	12	0.2 ± 0.0	6	0.3 ± 0.1
Serum iron (mg/dl)	10	262 ± 42	5	247 ± 57

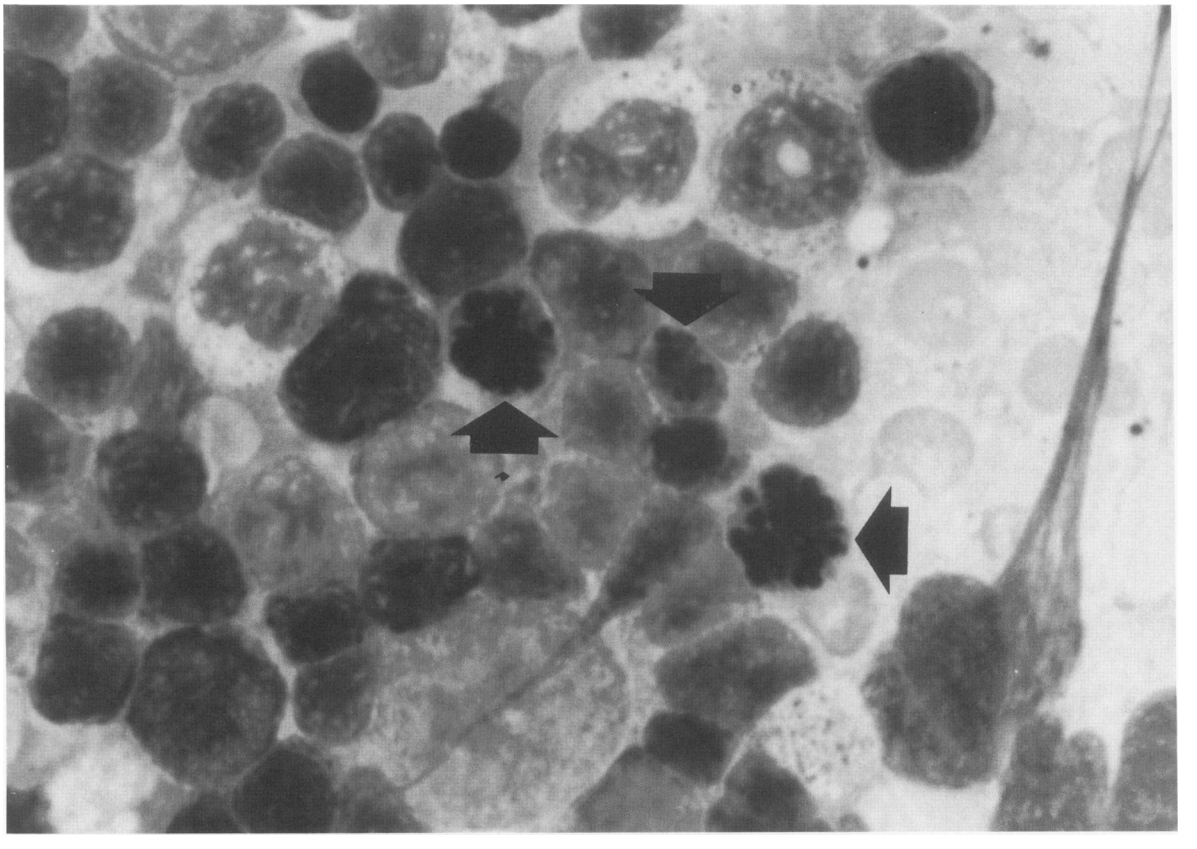
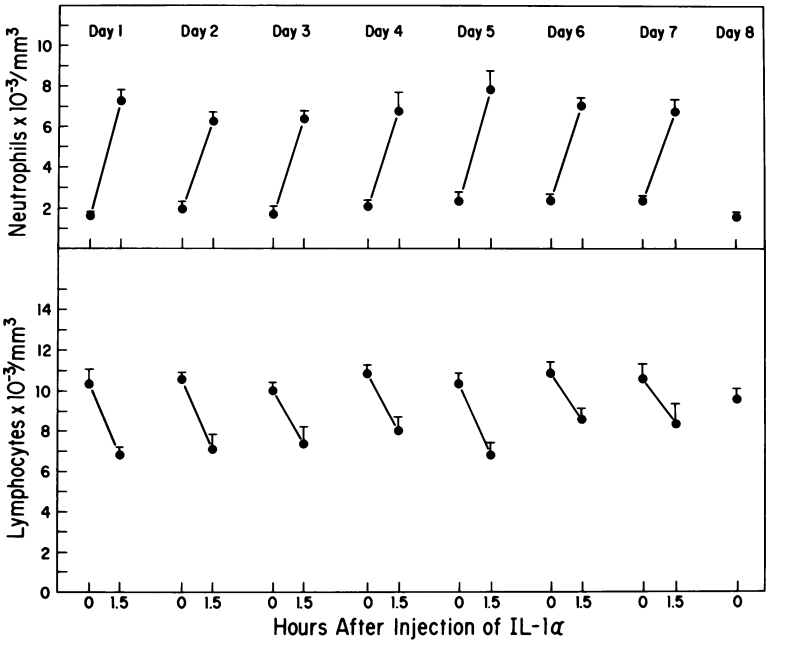


Figure 3. *The bone marrow after daily injection of TNF (0.5 μg) for one week demonstrates a dramatic hyperplasia of late erythroid normoblasts with an exaggerated starburstlike nuclear fragmentation. (×1000)*

Figure 4. *IL-1 (0.4 μg) induces a highly significant neutrophilia (P < 0.0005) and lymphopenia (P < 0.005) every day for a week at 1.5 hours after intravenous injection (N = 6). There is no evidence of tachyphylaxis.*



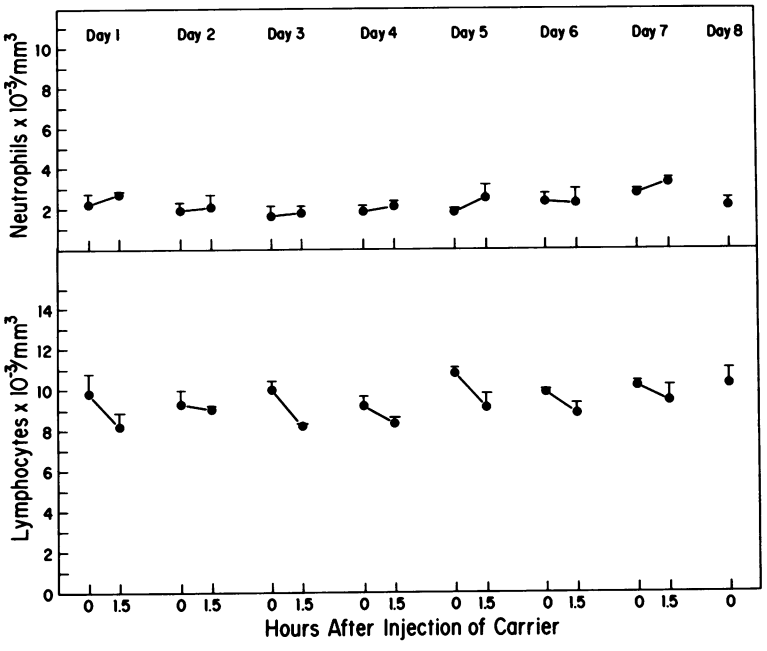


Figure 5. *Carrier control-treated rats (N = 2) demonstrate little or no increases in circulating numbers of neutrophils and lesser decreases in the number of circulating lymphocytes as compared to IL-1-treated rats.*

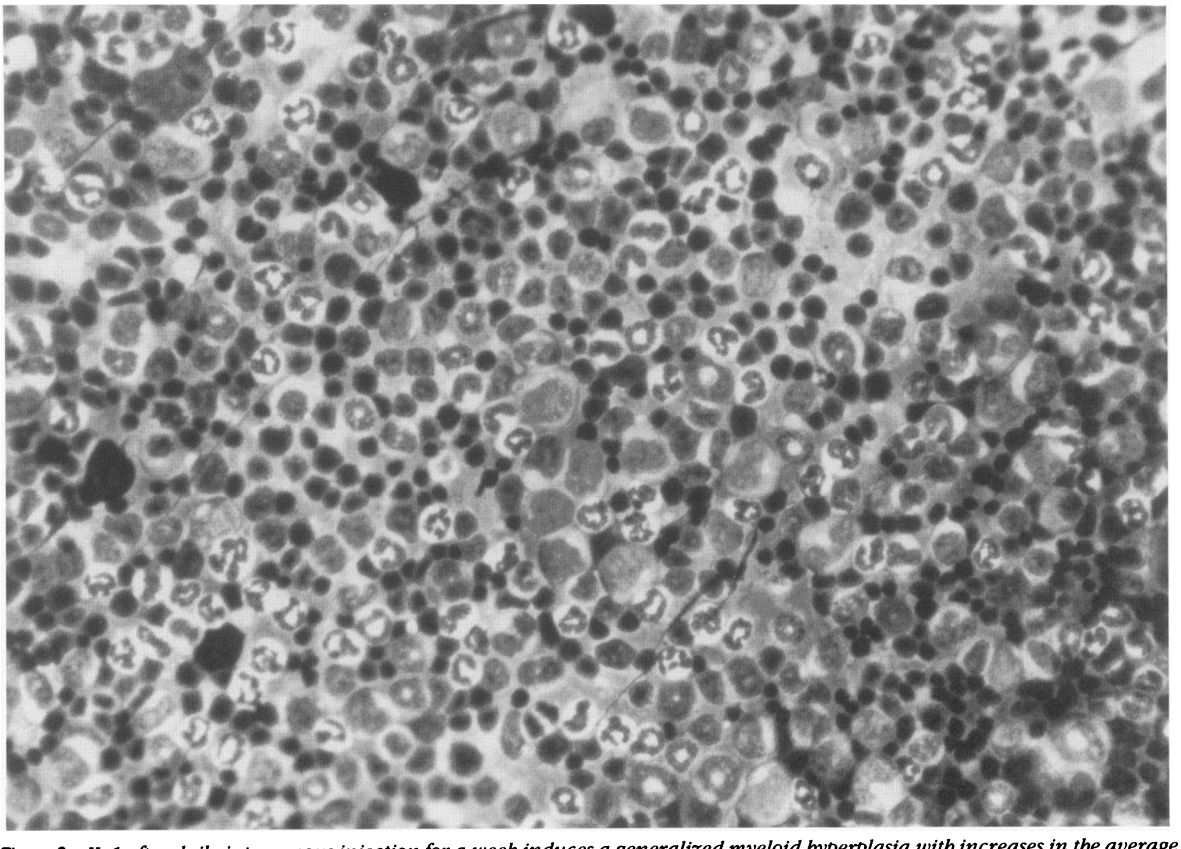


Figure 6. *IL-1 after daily intravenous injection for a week induces a generalized myeloid hyperplasia with increases in the average number of all stages of myeloid maturation and with statistically significant increases in the number of myeloblasts, promyelocytes, and especially mature segmented neutrophils. (×400)*

Table 3. *Daily Intravenous Injection of IL-1 for 1 Week Induces Myeloid Hyperplasia*

	Bone marrow: Cells × 10^{-6}/humerus (%)	
	Carrier (N = 2)	IL-1α (N = 6)
Erythroid		
Pronormoblasts	0.68 ± 0.6 (1.0 ± 1.0)	0.59 ± 0.3 (0.7 ± 0.4)
Early normoblasts	1.08 ± 0.8 (1.6 ± 1.2)	0.72 ± 0.3 (0.9 ± 0.4)
Intermediate normoblasts	6.95 ± 0.9 (10.1 ± 0.7)	6.24 ± 1.6 (7.8 ± 2.0)
Late normoblasts	13.03 ± 3.1 (18.7 ± 3.5)	15.78 ± 3.3 (19.9 ± 4.5)
Myeloid		
Myeloblasts	1.63 ± 0.4 (2.3 ± 0.4)	3.24 ± 0.7 (4.0 ± 0.7)
Promyelocytes	0.86 ± 0.1 (1.2 ± 1.2)	2.36 ± 0.6 (2.9 ± 0.7)
Myelocytes	5.67 ± 0.4 (8.2 ± 1.0)	6.26 ± 1.5 (7.7 ± 1.4)
Metamyelocytes	2.71 ± 0.8 (3.9 ± 1.4)	3.43 ± 1.4 (4.2 ± 1.5)
Band cells	2.54 ± 0.3 (3.7 ± 0.7)	4.36 ± 1.3 (5.3 ± 1.3)
Segmented neutrophils	11.62 ± 2.1 (16.7 ± 2.1)	18.38 ± 3.9 (23.1 ± 5.0)
Eosinophils	2.43 ± 0.6 (3.8 ± 0.7)	1.26 ± 0.2 (1.5 ± 0.2)
Basophils	0.55 ± 0.3 (0.7 ± 0.4)	0.36 ± 0.1 (0.4 ± 0.1)
Monocytes	1.57 ± 0.5 (2.3 ± 0.9)	0.89 ± 0.4 (1.4 ± 0.5)
Mast cells	1.10 ± 0.3 (1.6 ± 0.5)	1.01 ± 0.6 (1.2 ± 0.7)
Histiocytes	2.87 ± 0.1 (4.4 ± 0.4)	1.72 ± 0.9 (2.6 ± 1.0)
Lymphoid		
Lymphocytes	12.06 ± 1.0 (17.4 ± 0.6)	11.79 ± 2.6 (14.6 ± 2.1)
Plasma cells	1.12 ± 0.6 (1.6 ± 1.0)	0.93 ± 0.4 (1.1 ± 0.6)
Megakaryocytes	0.06 ± 0.1 (0.8 ± 0.1)	0.59 ± 0.1 (0.7 ± 0.1)
Total nucleated cells	69.07 ± 3.6	79.91 ± 8.7

hyperplasia of segmented neutrophils (29.6 ± 4.9 × 10^6/humerus in G-CSF-treated rats *vs.* 12.2 ± 1.0 × 10^6/humerus in carrier-treated control rats, Table 4). The neutrophils of G-CSF-treated rats often were hypersegmented (Figure 8). To explore the mechanisms of the trough in G-CSF induced neutrophilia, 1 rat each was injected with G-CSF every day or every 12 hours and killed at 3 days. Although the magnitude of neutrophilia was greater in the rat receiving G-CSF every 12 hours, both rats exhibited the same pattern of increasing tolerance to G-CSF on the second and third days as noted previously (Figure 9). The bone marrows of both these rats after 3 days exhibited a marked myeloid hyperplasia (Table 5) similar in magnitude to that seen in G-CSF-treated rats killed after 7 days. The trough in G-CSF-induced neutrophilia is thus not due to marrow depletion of neutrophils, nor is the progressive

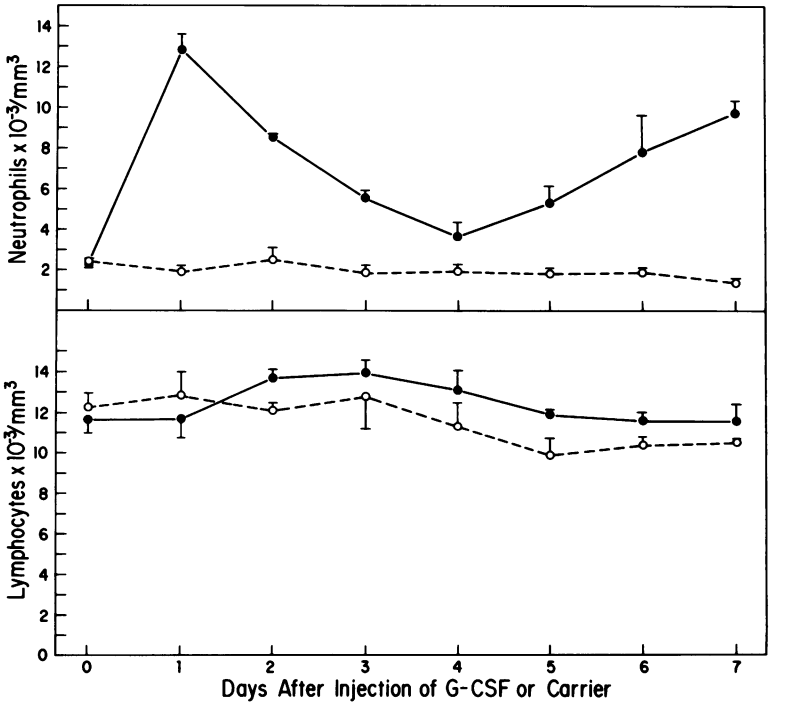


Figure 7. *G-CSF (N = 3) injected intravenously on a daily basis induces a peak of neutrophilia after the first day followed by a progressive tolerance to the neutrophilic effect of G-CSF between the second and fourth days. A progressive increase in the neutrophilic effect of G-CSF is again apparent beginning on the fifth day. The trough in peripheral neutrophilia occurs despite a myeloid hyperplasia in the bone marrow, suggesting that inhibition of neutrophil release rather than myeloid depletion of the marrow is responsible for the transient refractory state to the neutrophilic effect of G-CSF.*

Table 4. *Daily Intravenous Injection of G-CSF for 1 Week Induces a Striking Hyperplasia of Segmented and Hypersegmented Neutrophils*

	Bone marrow: Cells × 10^{-6}/humerus (%)	
	Carrier (N = 2)	G-CSF (N = 3)
Erythroid		
Pronormoblasts	0.20 ± 0.1 (0.3 ± 0.1)	0.26 ± 0.1 (0.3 ± 0.1)
Early normoblasts	0.54 ± 0.3 (0.8 ± 0.4)	0.77 ± 0.4 (1.0 ± 0.5)
Intermediate normoblasts	7.94 ± 0.1 (12.5 ± 0.2)	3.91 ± 1.4 (5.4 ± 2.3)
Late normoblasts	12.88 ± 0.2 (20.4 ± 0.9)	12.12 ± 3.2 (16.6 ± 3.5)
Myeloid		
Myeloblasts	1.60 ± 0.2 (2.5 ± 0.3)	2.93 ± 0.7 (4.1 ± 1.2)
Promyelocytes	1.07 ± 0.1 (1.7 ± 0.1)	1.16 ± 0.1 (1.8 ± 0.4)
Myelocytes	4.01 ± 0.1 (6.3 ± 0.1)	4.50 ± 0.6 (6.5 ± 0.1)
Metamyelocytes	1.28 ± 0.1 (2.0 ± 0.2)	1.58 ± 0.4 (2.2 ± 0.9)
Band cells	1.78 ± 0.6 (2.8 ± 0.9)	1.78 ± 0.1 (2.4 ± 0.3)
Segmented neutrophils	12.24 ± 1.0 (19.4 ± 2.2)	29.62 ± 4.9 (40.7 ± 0.8)
Eosinophils	2.28 ± 0.7 (3.6 ± 1.1)	0.86 ± 0.6 (1.1 ± 0.6)
Basophils	0.44 ± 0.1 (0.7 ± 0.1)	0.23 ± 0.1 (0.3 ± 0.1)
Monocytes	1.39 ± 0.2 (2.2 ± 0.2)	0.78 ± 0.6 (1.0 ± 0.7)
Mast cells	0.82 ± 0.1 (1.6 ± 0.2)	0.38 ± 0.1 (0.5 ± 0.1)
Histiocytes	1.67 ± 0.1 (2.6 ± 0.2)	0.69 ± 0.3 (0.9 ± 0.5)
Lymphoid		
Lymphocytes	12.48 ± 0.4 (19.7 ± 0.1)	10.49 ± 3.2 (1.43 ± 3.2)
Plasma cells	0.28 ± 0.2 (0.4 ± 0.4)	0.50 ± 0.1 (0.7 ± 0.1)
Megakaryocytes	0.31 ± 0.7 (0.5 ± 0.1)	0.16 ± 0.1 (0.1 ± 0.1)
Total nucleated cells	63.21 ± 2.0	72.72 ± 11.5

increase in neutrophils after day 4 simply due to regeneration of a depleted reserve of marrow neutrophils.

Discussion

The monokines TNF, IL-1, and G-CSF may be important endogenous mediators of the hematologic and inflammatory effects of endotoxin and gram-negative bacteremia.[14] The chronic administration of endotoxin, depending on the dose, the rate of administration, and the biologic response being measured, is known to result in varying degrees of tolerance or so-called tachyphylaxis.[15-18] The development of tolerance to the inflammatory effects of endotoxin could theoretically be due to: 1) a decreased elaboration of endogenous mediators of inflammation in response to endotoxin; 2) desensitization of responding leukocytes to endogenous mediators of inflammation, for example by down-regulation of receptors, or 3) a change in the clearance of endotoxin. Hesse et al[19] have demonstrated that decreased elaboration of TNF and IL-1 by Kupffer cells may contribute to the phenomenon of LPS-induced tachyphylaxis. In the present study, by quantitating the changes in circulating numbers of leukocytes, we report on the lack of tolerance to TNF and IL-1 during a week of daily intravenous injections. The lack of tolerance to these monokines is teleologically consistent with the host's constant need to be able to mount an inflammatory response and suggests that the mechanism of tachyphylaxis is not due to densensitization of responder neutrophils or lymphocytes. Because the half-life of the circulating neutrophil is only approximately 8 hours, however, the possibility cannot be ruled out that desensitization of neutrophils might occur after a more sustained exposure to TNF or IL-1.

The bone marrow after a week of daily injections of TNF demonstrated a slight but significant hypoplasia of immature myeloid forms and no significant change in the number of mature neutrophils. The *in vivo* effects of TNF could be considered quite consistent with the somewhat paradoxical *in vitro* observations of previous investigators that TNF suppresses neutrophil colony growth[20-22] but promotes myeloid maturation.[23] Clearly, however, the increased demand for marrow production of neutrophils that is created by TNF's daily release of over 70% of marrow neutrophils into the circulation[1] could not be accommodated without a substantial increase in myelopoiesis. The net *in vivo* effect of TNF must, therefore, be an increase in neutrophil colony growth, a mechanism for which is available in the known ability of TNF to induce the release of G-CSF.[24] The present observation that chronic administration of TNF causes hypoplasia of immature myeloid forms without any decrease in mature marrow neutrophils may best be explained by the combined effects of G-CSF or another colony-stimulating factor to increase the production of immature myeloid cells to keep pace with the increased demand for myelopoiesis[25] and the direct effect of TNF to cause a more rapid than usual maturation of immature myeloid forms into mature neutrophils.[23]

TNF was serendipitously noted to induce a marked hyperplasia of erythroid precursors within the bone marrow.

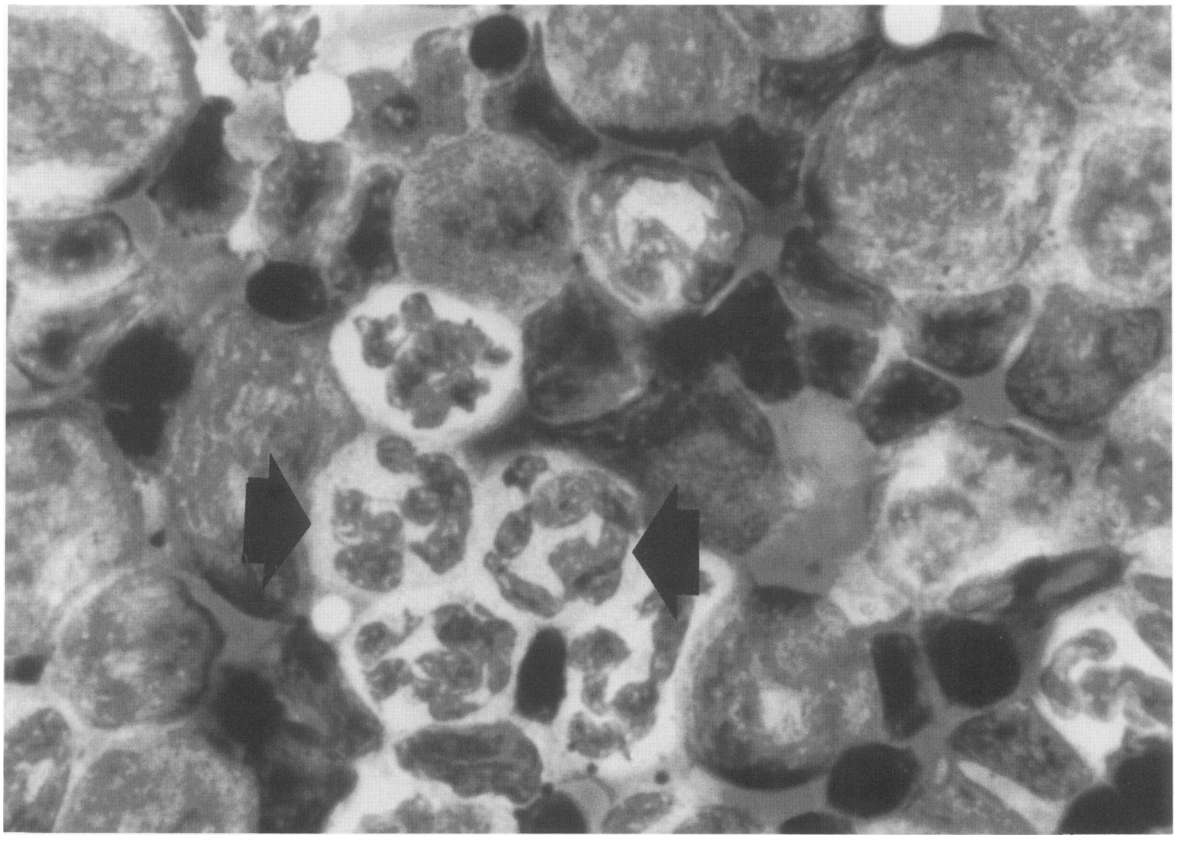


Figure 8. *G-CSF (25 μg) induces a marked hyperplasia of the mature neutrophils of the marrow. The majority of the mature neutrophils are readily appreciated to be hypersegmented (arrows) in comparison with the neutrophils of control rats. (×1000)*

No significant changes other than a slight reticulocytosis were noted within the peripheral red blood cell compartment. *In vitro* study of the effect of TNF on erythroid colony forming units may be needed to determine whether the effects of TNF on erythroid precursors *in vivo* are direct or indirectly mediated by other endogenous cytokines in the cytokine cascade. TNF might be postulated to induce the production not only of G-CSF but also of erythropoietin or other erythroid growth factors.

The bone marrow of IL-1-treated rats demonstrated an increase in the average number of all myeloid forms and a statistically significant increase in the numbers of myeloblasts, promyelocytes, and especially mature segmented neutrophils. IL-1 thus differed from TNF that had caused a decrease in early myeloid forms and no significant increase in mature neutrophils. IL-1 did not affect the erythroid compartment of the marrow. Several other groups of investigators in a variety of species and animal models have recently also presented evidence that IL-1 by itself or in conjunction with other cytokines such as G-CSF may promote myelopoiesis.[28-28] Because IL-1 is known to induce the release of G-CSF,[29] some of the *in vivo* effects of IL-1 may be indirectly due to the release of other myelopoietic factors. Of note, though, are the differences in the hematologic effects of IL-1 and TNF, supporting the conclusion of Burchett et al[30] that IL-1 and TNF may, in addition to their common effects, be partially independently regulated and play partially independent roles in the host response.

G-CSF induced a biphasic peripheral neutrophilia. The mechanism of the early and transient trough in the circulating numbers of neutrophils after chronic injection of G-CSF might reasonably be postulated to be due to the granulocyte releasing effect of G-CSF and to represent the result of a depleted marrow reserve of neutrophils. The bone marrow after 3 days, however, was shown in the present study to contain over twice as many mature neutrophils as control or time zero marrows. The trough in G-CSF-induced peripheral neutrophilia might therefore be postulated to be due to an as-yet unidentified inhibitor of neutrophil release, although other mechanisms of tolerance are also possible.

In conclusion, the effects of the chronic administration of exogenous TNF, IL-1, and G-CSF on hematopoiesis and on circulating numbers of leukocytes support the many lines of evidence that these monokines may contribute to the pathogenesis of acute and chronic inflammation. The present results also underscore the fact that

the *in vivo* effects of these cytokines (such as the erythroid hyperplasia induced by TNF, the myeloid hyperplasia induced by IL-1, and the trough in G-CSF-induced neutrophilia) are not predictable by their *in vitro* actions. Many of the *in vivo* effects of cytokines may be exerted indirectly by inducing the release of a host of both known and unknown members and secondary mediators of the cytokine cascade.

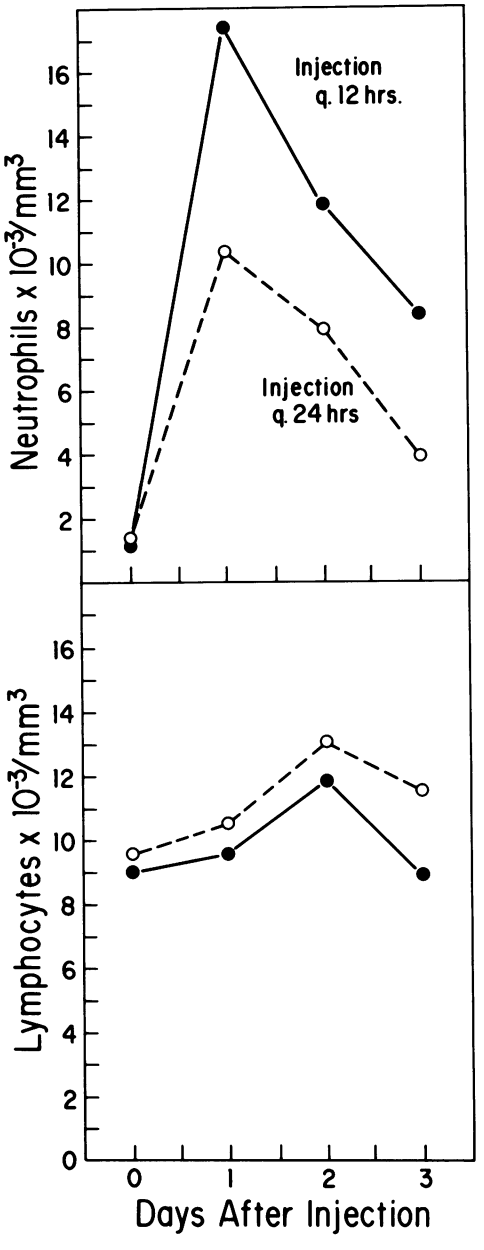


Figure 9. *The phenomenon of progressive tolerance of the neutrophilic effects of G-CSF after the second and third days was confirmed in 1 rat receiving G-CSF (25 μg) every 24 hours as described previously and 1 rat receiving G-CSF (25 μg) every 12 hours. The kinetics of neutrophilia are qualitatively the same in both rats although the magnitude of the neutrophilia is greater in the rat receiving an injection every 12 hours.*

Table 5. *Although the Number of Circulating Neutrophils Has Temporarily Decreased After 3 Days of Administration of G-CSF, the Marrow Reserve of Segmented Neutrophils Has Doubled*

	Bone marrow: Cells × 10^{-6}/humerus (%)	
	G-CSF q. 24 hrs	G-CSF q. 12 hrs
Erythroid		
Pronormoblasts	1.30 (1.6)	0.40 (0.5)
Early normoblasts	1.46 (1.8)	0.89 (1.1)
Intermediate normoblasts	6.77 (8.3)	4.23 (5.2)
Late normoblasts	15.74 (19.3)	13.51 (16.6)
Myeloid		
Myeloblasts	4.48 (5.5)	7.00 (8.6)
Promyelocytes	2.44 (3.0)	2.68 (3.3)
Myelocytes	5.30 (6.5)	4.47 (5.5)
Metamyelocytes	3.26 (4.0)	2.19 (2.7)
Band cells	3.85 (4.6)	2.68 (3.3)
Segmented neutrophils	22.19 (27.2)	25.96 (31.9)
Eosinophils	1.30 (1.6)	1.05 (1.3)
Basophils	0.32 (0.4)	0.40 (0.5)
Monocytes	2.52 (3.1)	2.03 (2.5)
Mast cells	0.65 (0.8)	2.11 (2.6)
Histiocytes	1.14 (1.4)	1.30 (1.6)
Lymphoid		
Lymphocytes	8.24 (10.1)	9.11 (11.2)
Plasma cells	0.40 (0.5)	0.89 (1.1)
Megakarocytes	0.24 (0.3)	0.40 (0.5)
Total nucleated cells	81.60	81.40

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