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KINETICS AND MECHANISMS OF RECOMBINANT HUMAN INTERLEUKIN 1 AND TUMOR NECROSIS FACTOR-α-INDUCED CHANGES IN CIRCULATING NUMBERS OF NEUTROPHILS AND LYMPHOCYTES[1]

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Human recombinant interleukins 1α and 1β (rIL-1α and -1β) both induced monophasic peripheral neutrophilia and lymphopenia in Lewis rats 1.5 hr after i.v. injection. The kinetics of rIL-1α- and -1 β-induced neutrophilia were similar to those induced by human monocyte-derived IL-1, IL-1α, and IL-1β, and the peripheral neutrophilia was accompanied by a marked decrease in marrow neutrophils. Arachidonic acid metabolites are implicated as biochemical intermediates in the production of the neutrophilia but not lymphopenia, since indomethacin and dexamethasone both completely abrogated IL-1-induced neutrophilia but did not affect the IL-1-induced lymphopenia. Acetylsalicylic acid, a cyclooxygenase inhibitor, did not inhibit IL-1-induced neutrophilia, suggesting that products of the lipoxygenase rather than the cyclooxygenase pathway of arachidonate metabolism may contribute to the neutrophilia. Human recombinant tumor necrosis factor-α (rTNF) administered i.v. to Lewis rats induced peripheral neutropenia, two peaks of neutrophilia, and lymphopenia. A wide range of doses of rTNF resulted in an initial neutropenia at 0.5 hr after injection followed by a first peak of neutrophilia at 1.5 hr and a second peak of neutrophilia at 6 hr. The initial neutropenia and the first peak of neutrophilia were not inhibited by pretreatment of rats with dexamethasone, indomethacin, or aspirin. The second peak of neutrophilia was inhibited by both dexamethasone and indomethacin, but was not at all inhibited by aspirin, suggesting that the second peak of neutrophilia is mediated by the release of endogenous cytokines, especially by IL-1, since exogenous IL-1-induced neutrophilia is also completely inhibited by dexamethasone and indomethacin but not by aspirin. The TNF-induced peripheral neutrophilia is also accompanied by a significant depletion of bone marrow neutrophils, indicating that the source of increased circulating neutrophils is, at least in part, via recruitment of marrow neutrophils. Systemic blood pressure was not affected by IL-1 or rTNF at the dosages employed, showing that the changes in circulating leukocyte subsets were not attributable to hemodynamic changes nor to the hemodynamic change-related release of adrenal hormones. Adrenalectomy did not alter the IL-1- or rTNF-induced neutrophilia or lymphopenia, also demonstrating that neither monokine mediates its hematologic effects on peripheral blood leukocytes via the release of adrenal hormones. In conclusion, IL-1 and TNF induce neutrophilia via arachidonic acid metabolism-dependent and -independent mechanisms, respectively. The mechanisms of IL-1- and TNF-induced lymphopenia are independent of arachidonic acid metabolism and may relate to the cytokine-induced expression of endothelial leukocyte adhesion molecules as described by previous investigators in vitro.

Interleukin 1 (IL-1)[2] is a macrophage-derived endogenous mediator of numerous host responses to infection, inflammation, and neoplasia. The term interleukin 1 describes the family of monokines that includes endogenous pyrogen, leukocyte endogenous mediator (LEM), and lymphocyte-activating factor (1). Two forms of IL-1, IL-1α and IL-1β, have been cloned, and these purified recombinant forms are now available to define their activities in the various in vivo and in vitro biologic assays originally employed to investigate endogenous pyrogen, LEM, and lymphocyte-activating factor (2). IL-1 has recently been proposed as a central mediator of the "stress reaction" in a paradigm that places IL-1 in a role somewhat akin to that originally envisioned for the adrenal hormones (3). The pivotal role of IL-1 in the stress reaction is attested to by the ability of IL-1 to cause fever, neutrophilia, and the synthesis of acute phase proteins by the liver (1, 3). The hematologic features of the classical stress reaction are neutrophilia, lymphopenia, and eosinopenia (4). The striking neutrophilia that results after administration of IL-1 is the basis of the original designation as leukocyte endogenous mediator. Although Kampschmidt reported that LEM may exert a lymphopenic effect (5), IL-1 is not as widely recognized to cause lymphopenia (1, 3).

Tumor necrosis factor-α (TNF) is a macrophage-derived protein so named on the basis of its in vitro and in vivo tumoricidal activity (6). TNF has been shown to be iden-

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[1] Supported by the Cancer Research Coordinating Committee of the University of California and the University of California, Irvine School of Medicine.

[2] Abbreviations used in this paper: IL-1, interleukin 1; rIL-1, recombinant interleukin 1; LEM, leukocyte endogenous mediator; TNF, tumor necrosis factor-α; rTNF, recombinant tumor necrosis factor-α; LT, lymphotoxin.

tical with the molecule known as "cachectin" and has thereby been implicated as an endogenous mediator in the pathogenesis of endotoxic shock and in a variety of the metabolic changes that accompany shock (7). Finally, TNF has also been implicated as a pleiotropic mediator of many of the phenomena accompanying inflammation and hemopoiesis (6, 8). The study of the many biologic properties of TNF has been facilitated by the availability of the recombinant molecule. Our laboratory has been studying the regulation of leukocyte trafficking by endogenous mediators and has noted that recombinant human lymphotoxin (LT) induces peripheral neutrophilia and lymphopenia concomitantly with a depletion of bone marrow neutrophils (9). TNF is thought to bind to the same receptor as LT (10).

The purpose of the present study was to examine the hematologic effects of recombinant IL-1 (rIL-1) and TNF (rTNF) on the peripheral blood, bone marrow, and tissues of rats after i.v. administration. The effects of rIL-1 were also compared with those of monocyte-derived IL-1. In order to examine the biochemical mechanisms through which IL-1 and TNF affect leukocyte trafficking, the monokines were administered to rats pretreated with indomethacin, dexamethasone, or acetylsalicylic acid (aspirin) and to adrenalectomized rats. Indomethacin inhibits phospholipase A_2 and the cyclooxygenase pathway of arachidonic acid metabolism (11), whereas dexamethasone, a glucocorticosteroid analogue, inhibits phospholipase A_2 through a regulatory protein termed macrocortin (12). Aspirin inhibits the cyclooxygenase pathway of arachidonate metabolism. Thus, indomethacin, dexamethasone, and aspirin may be used to study the contributions of arachidonic acid metabolites to monokine-induced changes in leukocyte trafficking. Dexamethasone also may block the synthesis of endogenous cytokines such as IL-1 (13) that may follow after the administration of TNF. IL-1 and TNF were administered to adrenalectomized rats to examine their interaction with endogenous steroids, a question of interest in light of recent reports that IL-1 may possess corticotropin-releasing activity and might thereby influence the release of adrenal corticosteroids (14). TNF was administered at doses that do not cause hypotension or other signs of shock, since hypotension causes hemodynamic changes and the release of adrenal hormones and may thereby secondarily cause changes in numbers of circulating leukocytes.

MATERIALS AND METHODS

Lewis rats, male, were obtained weighing 200 to 250 g (Harlan-Sprague-Dawley, Indianapolis, IN) and received various doses (1 to 50 U as defined by the thymocyte comitogenesis assay) of human rIL-1α (Genzynme, Boston, MA) human rIL-1β (Cistron, Pine Brook, NJ) purified human monocyte IL-1α (Cytokine, Cambridge MA), ultrapure human monocyte IL-1β (Cytokine), and purified human monocyte IL-1 (Cistron). Nonglycosylated human rTNF was the gift of Genentech (South San Francisco, CA). The dosages of rTNF are expressed in units (U) of lytic activity defined as the reciprocal of the dilution required to lyse 7500 murine L929 cells as previously described in a microplate assay (15). The specific activity of the rTNF was 6×10^6 U/mg. rTNF was diluted in 1% normal Lewis rat serum in sterile phosphate-buffered saline. Indomethacin (Merck, Sharp and Dome, West Point, PA), 5 mg/kg, and dexamethasone (Organon, West Orange, NJ), 50 mg/kg, were administered i.v. 1 min before the i.v. injection of rIL-1β or rTNF. All agents were administered i.v. via the dorsal vein of the penis with the exception of aspirin (Gendex, Inc., Jersey City, NJ), 100 mg/kg, that was dissolved in ethanol and given via gavage 30 min prior to injection of IL-1 or rTNF. Peripheral blood for the purpose of performing blood smears and quantitating the absolute numbers of circulating leukocytes was obtained at various time points after injection by tail bleeding. The total circulating white blood cell count/mm^3 was determined 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 100 cells/smear on Wright-stained smears. Bone marrow was obtained from the humerus as described by Chervenick et al. (16) and the absolute number of nucleated cells per 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 500 cells/smear according to the standard morphologic criteria for the rat as reported by Hulse (17). Adrenalectomy was performed 3 to 5 days before the experiments through bilateral dorsal incisions under ether anesthesia with blunt removal by forceps of the entire suprarenal fat pad. Blood pressure measurements were obtained in representative animals by using the Harvard rat tail blood pressure system (Harvard Instruments, Cambridge, MA) Blood pressure was measured in triplicate and averaged before and at designated time points after the injection of vehicle or monokines. Histologic examination of selected organs was performed with hematoxylin and eosin-stained sections of 10% neutral buffered formalin-fixed tissue. Statistics were performed with the paired or unpaired t-test as appropriate, and a value of $p < 0.05$ was considered to be significant. Arithmetic averages are expressed as ±1SD.

RESULTS

A dose-response and kinetic study of various doses of purified human IL-1α and Il-1β and rIL-1α and rIL-1β (1, 5, 10, 25, and 50 U/rat) at various time points (0, 0.5, 1, 1.5, 2, 4, and 6 hr) after i.v. injection, in general, demonstrated a dose-dependent leukocytosis, neutrophilia, and lymphopenia (data not shown), although the magnitude and kinetics of the changes in white blood cell subsets varied somewhat among the different sources of IL-1. Purified monocyte-derived IL-1α and IL-1β induced only a mild neutrophilia at the doses tested. rIL-1β was the most potent inducer of neutrophilia, and the rIL-1β-induced monophasic neutrophilia and general leukocytosis peaked 1.5 hr after injection (Fig. 1). The rIL-1β-induced neutrophila was readily apparent at doses of 5 U/rat or more, but 1 U caused little, if any, neutrophilia.

The dose of 25 U/rat was chosen to examine the hematologic effects of rIL-1α and rIL-1β in greater detail (n

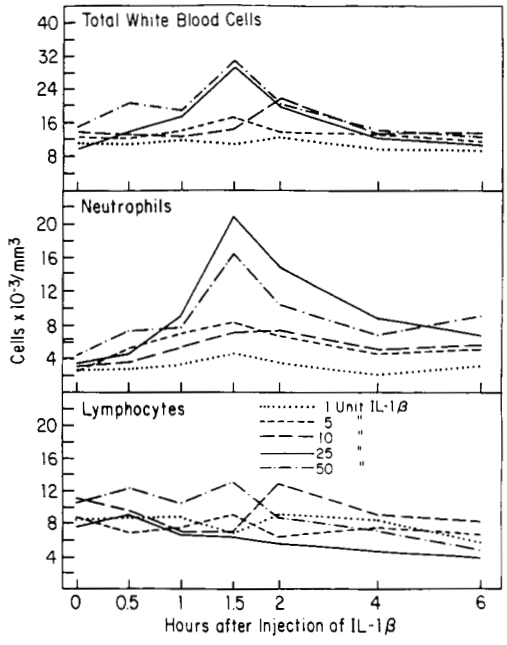

Figure 1. Human rIL-1β induces leukocytosis that is the result of a monophasic neutrophilia that peaks at 1.5 hr after i.v. injection.

= 6 for IL-1α- and IL-1β-treated rats and n = 6 for saline controls, with all groups being studied at time 0 and 1.5 hr). rIL-1α and rIL-1β induced a peripheral blood neutrophilia that was characterized by a marked relative and absolute increase in segmented neutrophils ($p < 0.01$) (Fig. 2). Occasional myelocytes and myetamyelocytes (immature neutrophils) were also present in the blood of rIL-1β-treated rats. The peripheral neutrophilia in rIL-1β-treated rats was accompanied by a marked decrease in bone marrow neutrophils from $10.9 \pm 1.2 \times 10^6$ neutrophils/humerus to $3.7 \pm 1.1 \times 10^6$ neutrophils/humerus ($p < 0.001$) (Table I). The magnitude of the rIL-1α-induced peripheral neutrophilia was not as great as that induced by rIL-1β and, in consonance with this observation, the decrease in bone marrow neutrophils was less pronounced in rIL-1α- than in rIL-1β-treated rats. The changes in the absolute numbers of total nucleated cells per humerus in the IL-1α- IL-1β- and saline-treated groups of rats objectively mirror the decrease in bone marrow neutrophils induced by the rIL-1. Thus, the total nucleated cells per humerus in control rats, IL-1α-, and IL-1β-treated rats, respectively, were $55.6 \pm 6.2 \times 10^6$, $49.5 \pm 1.1 \times 10^6$, and $44.4 \pm 2.9 \times 10^6$.

Human rIL-1α and rIL-1β both induced a significant lymphopenia ($p < 0.01$; $p < 0.001$) that included decreases in both small lymphocytes and large (granular) lymphocytes 1.5 hr after injection (Fig.2). Rats in the saline control group did not experience a lymphopenia (Fig. 2), although it should be noted that control rats in the kinetics experiment did exhibit a lymphopenia at 4 hr, but not 1.5 hr, after injection (data not shown). Careful study was also made of the morphologically "atypical" lymphocytes that comprise 3 to 4% of the circulating white blood cells in the rat (data not shown). Atypical lymphocytes that have been postulated to represent activated lymphoid cells (18) are a morphologically heterogeneous group generally composed of larger lymphoid cells with features such as lobulated and convoluted nuclei, a coarse chromatin pattern (as opposed to the finer chromatin pattern of most immature hemopoietic cells), and cytoplasmic basophilia and vacuolization. Neither rIL-1α or rIL-1β induced significant change in the numbers of circulating atypical lymphocytes.

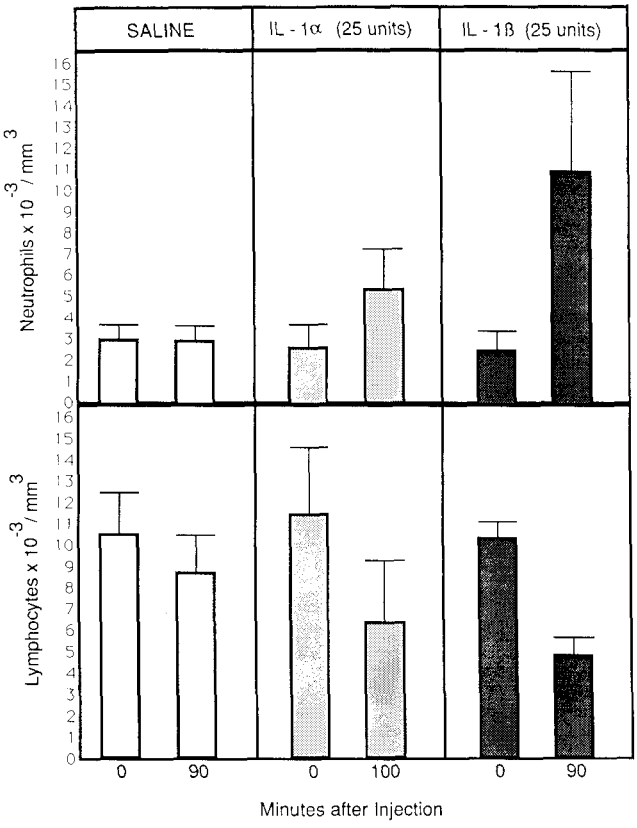

Figure 2. rIL-1α and rIL-1β (25 U each) both induce an absolute neutrophilia ($p < 0.01$; $p < 0.01$) and lymphopenia ($p < 0.01$; $p < 0.001$) 1.5 hr after i.v. injection. The magnitude of the rIL-1β-induced neutrophilia was greater than that induced by rIL-1α, although the magnitude of the lymphopenia induced by both agents was similar.

The role of arachidonic acid metabolites in IL-1-induced neutrophilia and lymphopenia was investigated by administering indomethacin (5 mg/kg) or dexamethasone (50 mg/kg) 1 min and aspirin (100 mg/kg) 0.5 hr before rIL-1β (25 U). rIL-1β was chosen for these investigations because it produced the most marked changes in circulating neutrophils and lymphocytes, as demonstrated previously. Positive controls (rIL-1β) and negative controls (indomethacin, dexamethasone, or saline) were performed concurrently on the days of the experiments (rIL-1β and indomethacin or rIL-1β and dexamethasone). Indomethacin completely abrogated the IL-1-induced neutrophilia but did not affect the IL-1-induced lymphopenia (Fig. 3). Indomethacin or saline alone did not affect the numbers of circulating neutrophils or lymphocytes. Dexamethasone also completely abrogated the neutrophilia but, again, did not diminish the lymphopenia (Fig. 3). Dexamethasone alone slightly decreased the number of circulating neutrophils and caused a mild lymphopenia at 1.5 hr and a marked lymphopenia at 6 hr. Aspirin did not inhibit the IL-1-induced neutrophilia or lymphopenia (Fig. 3).

The possible contributions of endogenous adrenal hormones to changes in circulating leukocyte subsets after IL-1 administration were investigated by injection of rIL-1β (25 U) into adrenalectomized rats. Adrenalectomy did not abrogate either the IL-1-induced neutrophilia or lymphopenia (Fig. 4), suggesting that IL-1 does not primarily exert its effects indirectly through either adrenal corticosteroids or adrenomedullary catecholamines. Adrenalectomized rats receiving saline alone (negative controls) did not experience significant changes in circulating white blood cell subsets at 1.5 hr after injection, although there was a slight increase in neutrophils at 6 hr.

Blood pressure measurements were determined in various experimental groups before and after injection of IL-1 because changes in systemic blood pressure may alter numbers of circulating white blood cells either through the release of adrenal mediators or via other mechanisms. IL-1 was not observed to significantly affect blood pressure which was found to be 103 ± 9 in uninjected rats (n = 10), 109 ± 11 in saline-treated rats (n = 8), 101 ± 7 in rIL-1α-treated rats (n = 9), and 107 ± 10 in rIL-1β-treated rats (n = 9) when blood pressure (mm Hg) was measured between 60 and 105 min after i.v. injection. Histologic examination of pulmonary parenchyma, liver, small and large intestines, spleen, and bronchus- and ileum-associated lymphoid tissue did not reveal any substantial histologic changes (other than perhaps an intravascular neutrophilia) in the tissues of IL-1α- or IL-1β-treated rats when compared with saline controls (n = 6 in each group and liver tissue was not obtained from the

TABLE I

rIL-1α and rIL-1β induce release of neutrophils from the bone marrow

White Blood Cell Differential	Cells × 10^{-6} (%)/Humerus		
	IL-1α (n = 6)	IL-1β (n = 6)	Sterile saline (n = 6)
Erythroid			
Pronormoblasts	0.76 ± 0.3 (1.5 ± 0.5)	0.59 ± 0.2 (1.3 ± 0.5)	0.86 ± 0.5 (1.5 ± 0.8)
Early normoblasts	1.43 ± 0.8 (2.8 ± 1.2)	1.84 ± 0.6 (4.1 ± 1.3)	1.65 ± 0.6 (2.9 ± 1.0)
Intermediate normoblasts	5.15 ± 1.1 (10.5 ± 1.5)	4.21 ± 0.8 (9.5 ± 1.8)	4.10 ± 0.8 (7.3 ± 1.3)
Late normoblasts	7.68 ± 1.4 (16.0 ± 3.5)	7.32 ± 1.6 (16.5 ± 3.7)	6.53 ± 1.4 (11.7 ± 2.2)
Myeloid			
Myeloblasts	1.09 ± 0.6 (2.3 ± 0.8)	1.09 ± 0.3 (2.5 ± 0.8)	0.99 ± 0.3 (1.8 ± 0.7)
Promyelocytes	1.27 ± 0.7 (2.5 ± 1.0)	1.83 ± 0.7 (4.2 ± 1.8)	1.68 ± 0.5 (3.5 ± 0.9)
Myelocytes	3.98 ± 1.0 (8.1 ± 2.0)	4.44 ± 0.7 (10.0 ± 1.7)	4.44 ± 1.1 (7.9 ± 1.8)
Metamyelocytes	1.84 ± 0.8 (3.6 ± 1.0)	3.02 ± 1.1 (6.5 ± 2.5)	2.38 ± 1.0 (4.4 ± 2.0)
Band cells	2.09 ± 0.5 (4.3 ± 1.2)	2.34 ± 1.0 (5.3 ± 2.5)	3.95 ± 2.0 (7.3 ± 3.8)
Segmented neutrophils	7.44 ± 2.6 (14.8 ± 3.0)	3.66 ± 1.1 (9.0 ± 1.8)	10.89 ± 1.2 (19.6 ± 2.0)
Eosinophils	1.86 ± 0.7 (3.6 ± 1.0)	1.98 ± 0.3 (4.4 ± 0.8)	2.86 ± 0.8 (5.1 ± 1.1)
Basophils	0.51 ± 0.4 (1.0 ± 0.8)	0.38 ± 0.4 (0.8 ± 0.9)	0.71 ± 0.6 (1.2 ± 0.9)
Monocytes	1.45 ± 0.5 (3.1 ± 1.4)	1.54 ± 0.5 (3.5 ± 1.2)	1.41 ± 0.7 (2.5 ± 1.3)
Mast cells	1.01 ± 0.5 (2.0 ± 0.6)	0.80 ± 0.5 (1.8 ± 1.3)	1.40 ± 1.1 (2.4 ± 1.7)
Histiocytes	1.72 ± 0.9 (3.3 ± 1.0)	1.45 ± 1.0 (3.2 ± 2.1)	0.99 ± 0.4 (1.7 ± 0.7)
Lymphoid			
Lymphocytes	8.76 ± 1.8 (17.8 ± 2.5)	6.05 ± 1.2 (13.5 ± 1.9)	9.20 ± 2.6 (16.4 ± 3.5)
Plasma cells	0.74 ± 0.4 (1.5 ± 0.8)	0.71 ± 0.5 (1.6 ± 1.2)	0.45 ± 0.3 (0.8 ± 0.7)
Megakaryocytes	0.71 ± 0.7 (1.3 ± 1.0)	1.05 ± 0.6 (2.3 ± 1.3)	1.14 ± 0.9 (2.0 ± 1.5)
Total nucleated cells/humerus	49.49 ± 1.1	44.37 ± 2.9	55.62 ± 6.2

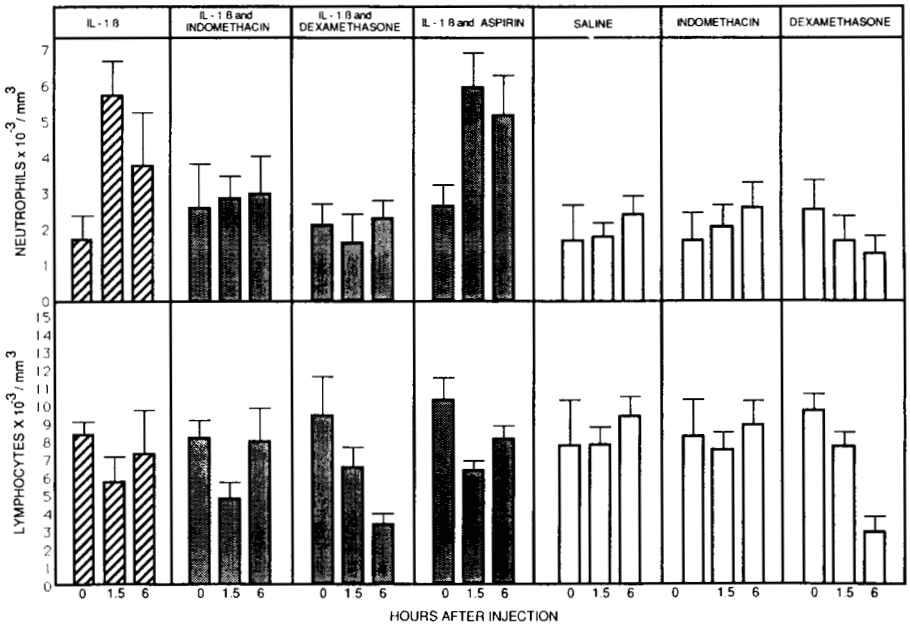

Figure 3. Indomethacin and dexamethasone, both of which inhibit phospholipase A_2, completely abrogate IL-1-induced neutrophilia, but do not affect IL-1-induced lymphopenia. Aspirin, a cyclooxygenase inhibitor, did not affect either the IL-1-induced neutrophilia or lymphopenia. IL-1 alone (positive controls performed simultaneously) induced both neutrophilia and lymphopenia. Indomethacin or saline alone or aspirin alone (data not shown) did not significantly affect numbers of circulating white blood cell subsets. The effect of dexamethasone on IL-1-induced lymphopenia is impossible to accurately interpret because dexamethasone alone caused a mild lymphopenia 1.5 hr and a marked lymphopenia at 6 hr after i.v. injection (n = 6 in each of the groups).

IL-1β-treated rats).

rTNF administered i.v. induced peripheral neutropenia, two peaks of neutrophilia, and lymphopenia (Fig. 5). A wide range of doses (500 to 50,000 U rTNF/rat) resulted in an initial neutropenia at 0.5 hr after injection followed by a first peak of neutrophilia at 1.5 hr and a second peak of neutrophilia at 6 hr. The neutropenia ($p < 0.05$ comparing time 0 with 0.5 hr), and the first peak of neutrophilia ($p < 0.0005$ comparing time 0 with 1.5) were confirmed to be statistically significant in a group of six rats receiving 3000 U rTNF/rat (Fig. 6). The initial neutropenia and neutrophilia as well as the second peak of neutrophilia were again confirmed to be statistically significant in a separate group of 12 rats receiving 3000 U rTNF as a positive control for the inhibitor studies described below (Fig. 7). Lower doses of TNF consisting of 15, 100, and 250 U rTNF/rat resulted in a monophasic neutrophilia also peaking at 1.5 to 2 hr after injection, but of lesser magnitude than noted at higher doses (data not shown). Histologic examination of the heart, lung, liver, bowel, and spleen did not demonstrate any striking differences between TNF-treated and control rats other than perhaps an intravascular neutrophilia including great numbers of neutrophils present in the red pulp of the spleen of TNF-treated rats.

The initial TNF-induced neutropenia was not inhibited by indomethacin, dexamethasone, or aspirin (Fig. 7). The initial peak of neutrophilia that occurred at 1.5 hr was also not inhibited by indomethacin, dexamethasone, or aspirin (Fig. 7). The initial peripheral neutrophilia was accompanied by a decrease in bone marrow neutrophils ($p < 0.005$) (Table II). The number of segmented neutrophils per humerus decreased from 10.6 ± 2.5 × 10^6 in vehicle control-treated rats to 3.6 ± 1.8 × 10^6 in rTNF-treated rats 1.5 hr after injection.

The second peak of TNF-induced neutrophilia was inhibited by both dexamethasone and indomethacin. Dexamethasone is known to inhibit the synthesis of IL-1 (13),

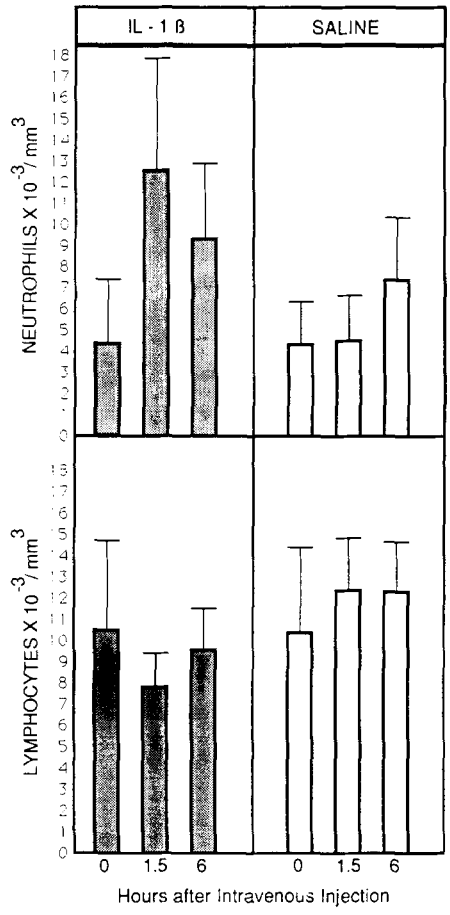

Figure 4. Adrenalectomized rats responded to IL-1 by demonstrating a neutrophilia ($p < 0.001$) and lymphopenia ($p < 0.05$) similar to non-adrenalectomized rats. Saline did not induce either neutrophilia or lymphopenia in adrenalectomized rats at 1.5 hr after injection, although there was a slight increase in neutrophils at 6 hr. The results indicate that IL-1 does not exert its effects on circulating white blood cell subsets through the adrenal gland.

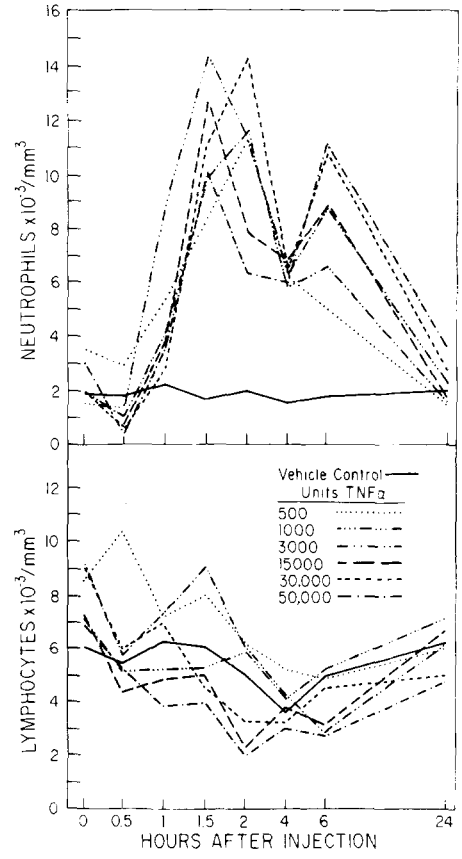

Figure 5. TNF over a wide range of doses (500 to 50,000 U/rat) causes an initial neutropenia 0.5 hr after injection followed by a major first peak of neutrophilia at 1.5 to 2 hr and a lesser second peak of neutrophilia at 6 hr after injection. Vehicle control does not cause any change in circulating numbers of neutrophils. TNF also causes a lymphopenia at 1.5 to 2 hr that is more apparent at the higher doses in this figure. Vehicle control causes a lymphopenia at 4 hr after injection.

consistent with the hypothesis that the second peak of neutrophilia reflects the synthesis and release of endogenous IL-1 by the rat in response to the injection of rTNF. Dexamethasone and indomethacin abrogate exogenous IL-1-induced neutrophilia, but not the initial TNF- or LT-induced neutrophilia, also suggesting that the second peak of TNF-induced neutrophilia may be to a large extent due to the release of endogenous IL-1. Aspirin did not inhibit the second peak of neutrophilia as is consistent with the fact that aspirin has no inhibitory effect on either IL-1-, TNF-, or LT-induced neutrophilia (9).

TNF caused a progressive lymphopenia (Fig. 5). The lymphopenia was statistically significant at 0.5 hr ($p < 0.01$) and 1.5 hr ($p < 0.0005$) after injection (Fig. 6). Rats receiving vehicle control injection did not exhibit significant lymphopenia at 0.5 hr but did have a slight lymphopenia at 1.5 hr, although the lymphopenia was much less pronounced than in TNF-treated rats. The foregoing results were confirmed in a second group of 12 TNF and 10 vehicle control-treated rats with the exception that the vehicle control-treated rats did not exhibit any lymphopenia at all at 1.5 hr in this later experiment (Fig. 7). Indomethacin, dexamethasone, or aspirin did not inhibit the TNF-induced lymphopenia (Fig. 7).

Vehicle control (carrier)-injected rats experienced a lymphopenia 4 hr after injection that negates the ability to interpret the effects of TNF on circulating numbers of lymphocytes at this time point. The phenomenon of carrier-induced lymphopenia at 4 hr has been repeatedly observed in our laboratory and we therefore investigated the possibility that the lymphopenia might be due to the release of endogenous adrenal hormones. In another set of six saline-injected intact rats and in six adrenalectomized saline-injected rats we found that the lymphopenia that occurs at 4 hr in intact rats is abrogated by adrenalectomy (Fig. 8), strongly suggesting that the release of adrenal glucocorticosteroids is responsible for the observed lymphopenia.

Blood pressure was measured in uninjected control rats, vehicle control rats, and 3000 U rTNF-injected rats to exclude the possibility that hypotensive hemodynamic changes or related secondary pathophysiologic homeostatic mechanisms might contribute to the observed changes in numbers of leukocyte subsets. The blood pressure was 101 ± 9 ($n = 6$) in vehicle control rats and 108 ± 10 ($n = 6$) in TNF-injected rats, confirming that hypotension (which has been reported after comparatively massive doses of TNF) did not contribute to the present results. TNF-injected rats did have a slightly lower heart rate (382 ± 39 beats/min; $n = 5$) than vehicle control-injected rats (434 ± 27 beats/min; $n = 5$).

Adrenalectomized rats were given 3000 U TNF or vehicle control to further probe the possibility that endogenous adrenal hormones might contribute to either the

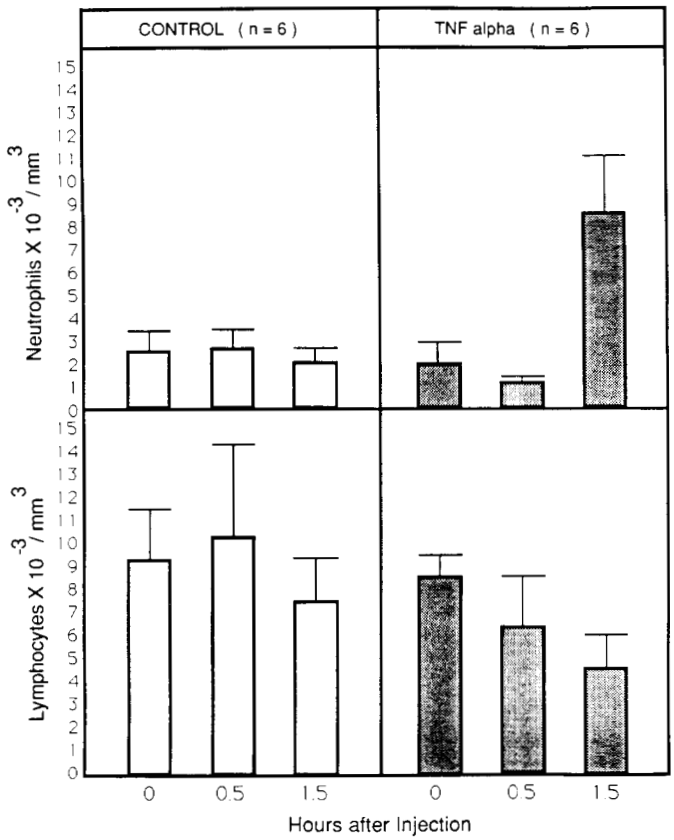

Figure 6. TNF at the dose of 3000 U/rat causes a statistically significant neutropenia at 0.5 hr ($p < 0.05$), neutrophilia at 1.5 hr ($p < 0.0005$), and lymphopenia ($p < 0.0005$).

neutrophilia (e.g., via the release of epinephrine) or lymphopenia (e.g., via the release of glucocorticosteroids). Adrenalectomy, however, did not alter the TNF-induced neutropenia ($p < 0.05$), neutrophilia ($p < 0.01$), or lymphopenia ($p < 0.05$ at 0.5 hr and $p < 0.0005$ at 1.5 hr) (Fig. 9). Vehicle control-treated adrenalectomized rats did not demonstrate any significant changes in circulating numbers of neutrophils or lymphocytes at any of the time points examined.

Rats (n = 2) receiving 3000 U boiled TNF did not experience any significant changes in circulating leukocyte subsets, thus confirming that endotoxin was not responsible for the experimental observations (endotoxin but not TNF is resistant to boiling).

DISCUSSION

The regulation of leukocyte trafficking, that is, the regulation of the release and removal of leukocyte subsets into and from the circulation by endogenous mediators is a complex and multifactorial phenomenon. Among the originally and best studied regulators of leukocyte trafficking are the adrenal medullary catecholamines and the adrenal glucocorticosteroids (19). The release of adrenal hormones affects the levels of circulating neutrophils, lymphocytes, and eosinophils (19, 20). Another endogenous regulator of leukocyte trafficking was originally described as "leukocyte endogenous mediator" (21). Leukocyte endogenous mediator induces neutrophilia and lymphopenia and has been shown to be identical to IL-1, a cytokine with physiologic and immunologic properties that had been originally designated under such names as "endogenous pyrogen" and "lymphocyte activating factor."

The ability of recombinant human IL-1α and IL-1β to induce neutrophilia via the release of bone marrow neutrophils, that is, in a fashion analogous to the neutrophilia induced by LEM (1, 21) has been confirmed by our results. The kinetics of rIL-1α- and rIL-1β-induced neutrophilia are similar to those of monocyte-derived IL-1 species and to those reported for LEM. The rIL-1α and rIL-1β used in the present study were nonglycosylated, suggesting that glycosylation is not required for the hematologic effects expressed by IL-1. Additionally, rIL-1α and rIL-1β have been documented to induce lymphopenia in the face of a generalized leukocytosis. Kampschmidt et al. (5) have previously reported that LEM induces lymphopenia in rats. Although lymphopenia was clearly evident at 1.5 hr after injection of 25 U IL-1α and IL-1β, a time point when no suggestion of lymphopenia was present in saline controls, note should be taken that a lymphopenia was observed at 4 hr in saline controls during kinetic experiments. The kinetics of changes in circulating white blood cell subsets are an indispensable part of the study of the effects of both endogenous and exogenous substances on leukocyte trafficking, as a single substance (for example, endotoxin) may produce both neutropenia and neutrophilia at various times after administration. Moreover, the time at which a substance induces changes in circulating leukocyte numbers may suggest mechanisms of action, as, for example, in the case of the delayed neutrophilia produced by endotoxin when compared with IL-1 (21). The kinetics of the rIL-1α- and rIL-1β-induced lymphopenia, as well as the fact that adrenalectomy did not abrogate the lymphopenia, suggest that the IL-1-induced lymphopenia is independent of the release of adrenal hormones. The possibility that IL-1 might have affected circulating lymphocytes via adrenal corticosteroids would have been consistent with the reported corticotropin-releasing activity of IL-1 (14).

Recently, our laboratory reported that several stable analogues of prostaglandins induce neutrophilia in a dose-response dependent fashion (22) and in adrenalectomized as well as intact rats (23). Therefore, we chose to test the hypothesis that IL-1 induces neutrophilia via the formation of arachidonic acid metabolites. IL-1 is known to cause the release of cyclooxygenase products in several target cells (3). Indomethacin and dexamethasone, both inhibitors of arachidonic acid metabolism, were, therefore, administered to rats before the injection of IL-1. Indomethacin and dexamethasone both completely abrogated the induction of neutrophilia by IL-1. These results are consistent with the hypothesis that IL-1-induced neutrophilia is caused by the formation of arachdonic acid metabolites in the bone marrow (most likely within neutrophils themselves, but possibly in another marrow cell that could release the arachidonate metabolites into the marrow milieu). Aspirin, a cyclooxygenase inhibitor, did not block IL-1-induced neutrophilia as is consistent with previously reported results by Merriman et al. (24). The observation that two inhibitors of phospholipase A_2 abrogated neutrophilia, whereas an inhibitor of cyclooxygenase metabolism did not, suggests that the lipoxygenase pathway and possibly leukotrienes may be involved in the etiogenesis of IL-1-induced neutrophilia.

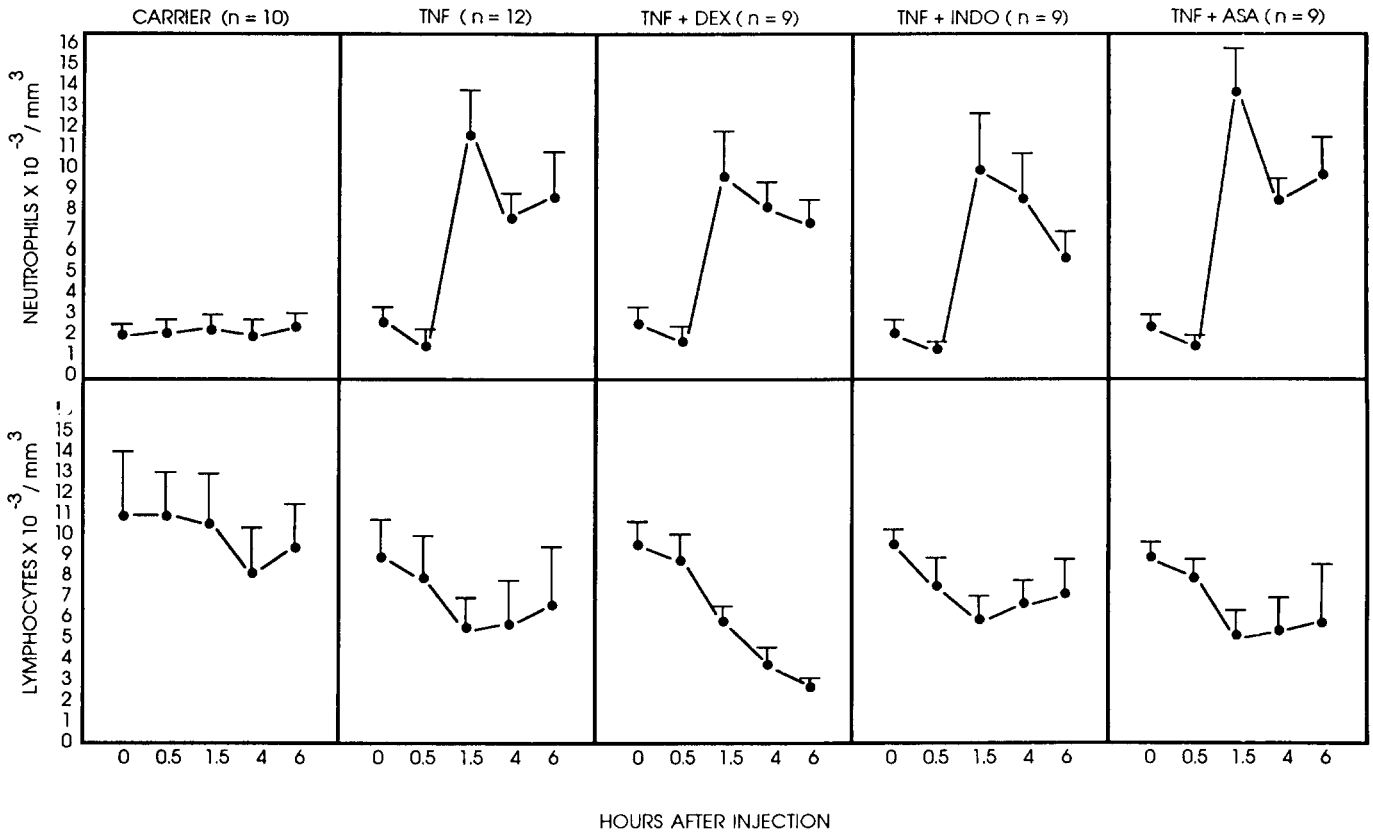

Figure 7. Dexamethasone (n = 9), indomethacin (n = 9), and aspirin (n = 9), do not inhibit the initial neutropenia or first peak of neutrophilia that is caused by TNF alone (n = 12). Dexamethasone and indomethacin, but not aspirin, inhibit the second peak of neutrophilia that is observed in rats receiving TNF alone. $p < 0.05$ in all cases for the neutropenia occurring at 0.5 hr; $p < 0.05$ in all cases for the first peak of neutrophilia; $p < 0.05$ for the second peak of neutrophilia occurring between 4 and 6 hr in TNF alone and TNF plus aspirin-treated groups; $p < 0.05$ for the progressive decrease in neutrophils occurring between 4 and 6 hr in dexamethasone- and indomethacin-pretreated rats. No significant changes in numbers of neutrophils are seen in carrier controls (n = 10) or in rats treated with dexamethasone, indomethacin, or aspirin alone (data not shown). Since dexamethasone and indomethacin, but not aspirin, block IL-1-induced neutrophilia, the data support the hypothesis that the second peak of neutrophilia is at least partially attributable to the release of endogenous IL-1. Neither indomethacin nor aspirin inhibit the lymphopenia noted at 1.5 hr in TNF-treated rats. The effect of dexamethasone on TNF-induced lymphopenia is difficult to assess because of the progressive lymphopenia produced by dexamethasone itself. Carrier-treated rats experience a lymphopenia at 4 hr, but not at 0.5 or 1.5 hr, after injection.

TABLE II

Human rTNF-α induces release of neutrophils from the bone marrow

White Blood Cell Differential	Cells × 10^{-6} (%)/Humerus	
	TNF-α (n = 6)	Vehicle (n = 6)
Erythroid		
Pronormoblasts	0.69 ± 0.3 (1.3 ± 0.7)	0.50 ± 0.3 (0.8 ± 0.5)
Early normoblasts	1.48 ± 0.6 (2.7 ± 1.0)	1.17 ± 0.5 (1.8 ± 0.6)
Intermediate normoblasts	6.38 ± 1.5 (12.0 ± 1.8)	6.44 ± 1.0 (10.5 ± 0.6)
Late normoblasts	10.07 ± 2.3 (18.9 ± 2.6)	10.22 ± 2.4 (16.5 ± 1.4)
Myeloid		
Myeloblasts	1.54 ± 0.2 (2.9 ± 0.6)	1.52 ± 0.7 (2.8 ± 0.6)
Promyelocytes	1.11 ± 0.3 (2.0 ± 0.5)	1.15 ± 0.2 (1.9 ± 0.4)
Myelocytes	4.93 ± 1.2 (9.3 ± 1.5)	5.45 ± 0.6 (9.0 ± 1.3)
Metamyelocytes	1.78 ± 0.5 (3.3 ± 0.6)	1.86 ± 0.1 (3.0 ± 0.2)
Band cells	1.23 ± 0.3 (2.3 ± 0.5)	2.03 ± 0.5 (3.2 ± 0.6)
Segmented neutrophils	3.60 ± 1.8 (6.8 ± 3.3)	10.57 ± 2.5 (17.1 ± 2.2)
Eosinophils	2.27 ± 0.8 (4.9 ± 1.3)	2.66 ± 0.7 (4.3 ± 1.1)
Basophils	0.29 ± 0.2 (0.5 ± 0.4)	0.44 ± 0.5 (0.7 ± 0.8)
Monocytes	1.26 ± 0.6 (2.2 ± 1.0)	1.40 ± 0.4 (2.3 ± 0.8)
Mast cells	0.73 ± 0.3 (1.4 ± 1.0)	0.94 ± 0.6 (1.5 ± 1.0)
Histiocytes	2.12 ± 1.0 (3.8 ± 1.4)	1.79 ± 0.9 (2.9 ± 1.5)
Lymphoid		
Lymphocytes	11.30 ± 2.2 (21.5 ± 3.4)	11.92 ± 1.7 (19.8 ± 1.8)
Plasma cells	1.70 ± 0.8 (3.3 ± 1.8)	0.78 ± 0.2 (1.2 ± 0.3)
Megakaryocytes	0.46 ± 0.3 (0.9 ± 0.6)	0.45 ± 0.2 (0.7 ± 0.4)
Total nucleated cells/humerus	52.94 ± 8.7	61.29 ± 9.1

The IL-1-induced lymphopenia was not blocked by indomethacin, suggesting that arachidonate metabolites are not involved in the mechanism responsible for lymphopenia. The experiments with adrenalectomized rats indicate that endogenous steroids also do not appear to be implicated in the IL-1-induced lymphopenia. IL-1 is well known to interact with lymphocytes in the role of "lymphocyte-activating factor" and possibly circulating

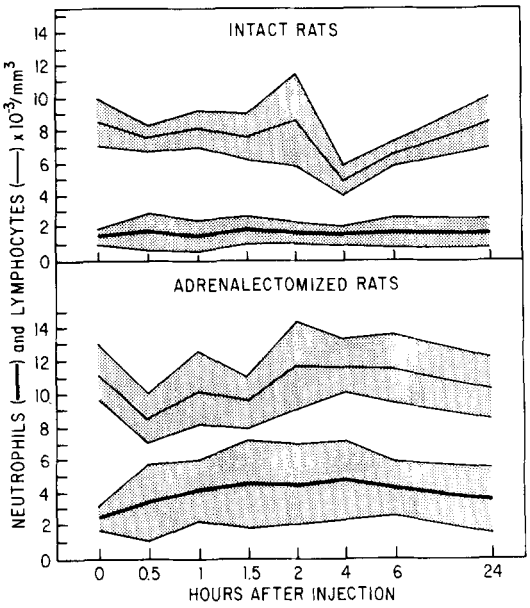

Figure 8. Adrenalectomy prevents the lymphopenia that occurs at 4 hr in intact saline-injected rats, suggesting that the lymphopenia that is consistently observed in intact control rats at 4 hr is most likely due to the release of endogenous adrenocorticosteroids (*shaded area* represents 1 SD).

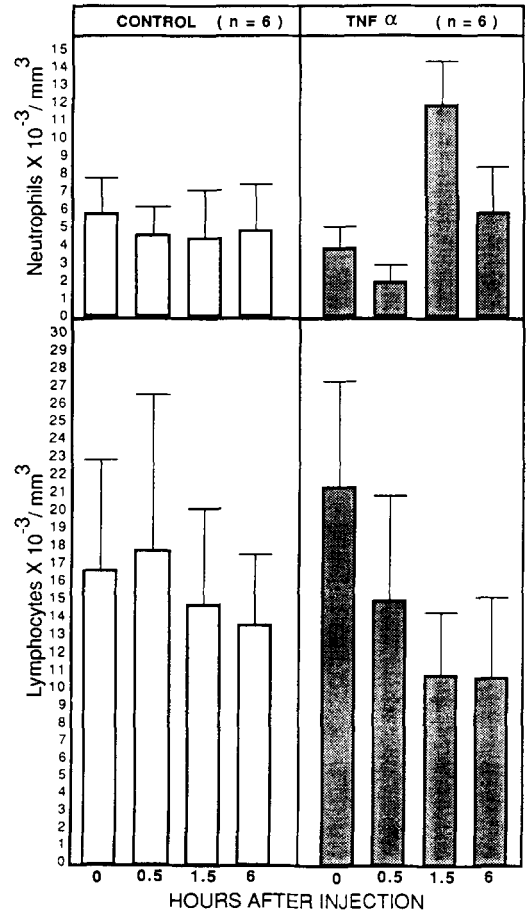

Figure 9. Adrenalectomy does not prevent either the TNF-induced neutropenia ($p < 0.05$), neutrophilia ($p < 0.01$), or lymphopenia ($p < 0.005$ at 1.5 hr), indicating that TNF does not mediate its effects on circulating leukocytes via the adrenal gland.

lymphocytes interacting with IL-1 may be induced to leave the peripheral circulation by increased adherence to endothelium or by emigration into lymphoid tissues. IL-1 has been reported to increase the binding of human lymphocytes to endothelial cell monolayers (25).

TNF and LT are cytokines that, similarly to IL-1α and IL-1-β, appear to serve as central mediators in the regulation of many of the inflammatory, immunologic, and physiologic responses of the body to various forms of tissue injury or stress (6). Although TNF is a monokine and LT is a lymphokine and although their amino acid homology is less than 30% (6), both molecules are thought to bind to the same receptor (10). Our laboratory recently demonstrated that recombinant human LT induces a dose-response-dependent neutrophilia and lymphopenia in the rat after i.v. injection (9). The LT-induced neutrophilia and lymphopenia are not inhibited by pretreatment of rats with either indomethacin, dexamethasone, or aspirin (9).

TNF was found to be similar to IL-1 and LT in that all induce an initial peak of neutrophilia 1.5 to 2.0 hr after i.v. injection and all also produce a progressive lymphopenia that is significant by 1.5 hr. TNF differs from IL-1 and LT in that TNF over a dose range of 500 to 50,000 U/rat induced a clear neutropenia 0.5 hr after injection, whereas neutropenia was not observed at the same time point after injection of doses of IL-1 ranging from 1 to 50 U/rat or doses of LT ranging from 100 to 75,000 U/rat. The possibility cannot be excluded that IL-1 or LT may induce neutropenia at higher doses or earlier time points. Endotoxin is well documented to cause neutropenia preceding neutrophilia and the observation that TNF induces an initial neutropenia supports the concept that many of the biologic effects of endotoxin are mediated via TNF. Passive immunization against TNF protects mice from the lethal effects of endotoxin (26). TNF also differed from IL-1 in the appearance of a distinct second peak of neutrophilia in the majority of rats at 6 hr after injection of TNF. A second peak of neutrophilia has been observed in our laboratory in the few rats studied at 4 and 6 hr after injection of 30,000, 50,000, or 75,000 U of LT/rat suggesting that LT may be similar although less potent than TNF in the ability to cause a second wave of neutrophilia.

TNF induced a neutropenia at 0.5 hr that was not inhibited by pretreatment of rats with indomethacin, dexamethasone, or aspirin. The biochemical second messages of TNF-induced neutropenia thus would not appear to be arachidonic acid metabolites, a possibility that had been considered in our laboratory because of the known ability of leukotriene B_4 to cause neutropenia (27). TNF induced an initial wave of neutrophilia at 1.5 hours that was not inhibited by indomethacin, dexamethasone, or aspirin. TNF is analogous in this regard to LT, but differs dramatically from IL-1 since the neutrophilia induced by injection of IL-1 is completely abrogated by pretreatment of rats with either indomethacin or dexamethasone. This foregoing observation is consistent with the belief that TNF and LT, but not IL-1, bind to the same receptor (5) and is also consistent with the observation that many of the biologic properties of TNF and LT are shared.

TNF induced a second wave of neutrophilia at 6 hr that was markedly inhibited by pretreatment of rats with both indomethacin and dexamethasone but was not inhibited

by aspirin. The hypothesis that the second wave of neutrophilia is due to the release of endogenous IL-1 is supported by the following pieces of evidence: 1) exogenous IL-1-induced neutrophilia is abrogated by indomethacin and dexamethasone, but not by aspirin; 2) dexamethasone inhibits the synthesis of endogenous IL-1 (13); 3) TNF has been demonstrated to induce the release of IL-1 from mononuclear cells and endothelial cells in vitro (28, 29); and 4) perhaps most important, TNF has been shown to induce two waves of fever in the rabbit with the second wave being accompanied by an increase in serum IL-1-like activity (30). The observation that TNF at very low doses causes a monophasic neutrophilia is similar to the observation that LT causes a monophasic neutrophilia at lower doses and a biphasic neutrophilia at higher doses, although in terms of lytic units a much higher dose of LT than of TNF is required to induce biphasic neutrophilia.

Adrenalectomy did not alter the TNF-induced neutrophilia or lymphopenia, suggesting that TNF does not mediate its hematologic effects on peripheral blood leukocytes via the release of adrenal hormones. As mentioned previously, both adrenal medullary and cortical hormones can affect the numbers of circulating neutrophils and lymphocytes (19), and experiments with adrenalectomized rats were therefore considered necessary to rule out any contribution of adrenal products to the observed changes in white blood cells. IL-1, another monokine, has been reported to possess corticotropin-releasing activity (14), raising the possibility of an immune-adrenal axis. Hypotension and shock are effects of TNF that have been described after administration of much higher doses of TNF than those used in the present study. Hypotension can cause the release of adrenal hormones and the hemodynamic changes that accompany hypotension and shock might in themselves affect the numbers of circulating leukocytes (31). Blood pressure measurements of the rats in our study indicated that 3000 U TNF/rat does not cause hypotension. Furthermore, no mortality or clinically apparent morbidity was observed after administration of 3000 U TNF/rat.

TNF induced a significant lymphopenia at 1.5 hr after injection and is similar in this regard to both LT and IL-1. TNF differed somewhat from LT and IL-1 at the doses studied in our laboratory, in that the TNF-induced lymphopenia persisted at 6 hr, whereas the numbers of circulating lymphocytes in IL-1- or LT-treated rats tended to return to normal by 6 hr. Possibly, the lymphopenia observed at 6 hr in TNF-treated rats was in part, due to the release of the same endogenous cytokines that induces the second wave of neutrophilia, a possibility that could not be answered with the inhibitor experiments since indomethacin and aspirin do not inhibit IL-1-, TNF-, or LT-induced lymphopenia and dexamethasone itself causes a progressive lymphopenia. The study of the kinetics of cytokine-induced lymphopenia is further complicated by the lymphopenia that consistently occurs at 4 hr in vehicle control-treated rats. Experiments with adrenalectomized rats demonstrated that the lymphopenia occurring at 4 hr in control rats is abrogated by adrenalectomy, an observation that is consistent with the previously reported release of glucocorticosteroids by ether anesthesia (32). TNF-induced lymphopenia may be postulated to be mechanistically related to the TNF-induced expression of endothelial leukocyte adhesion molecules that has been described by other investigators in vitro (33, 34).

In summary, the kinetics and some of the mechanisms contributing to IL-1- and TNF-induced changes in leukocyte trafficking have been elucidated. IL-1 and TNF represent two monokines that share the common property of inducing neutrophilia, but do so via independent biochemical pathways. IL-1 and TNF both induce a lymphopenia that does not appear to be mediated by either arachidonic acid metabolites or endogenous adrenal hormones. Neither the IL-1- or TNF-induced changes in circulating leukocyte subsets could be attributed to an effect on systemic blood pressure. Knowledge of the effects of TNF on circulating white blood cells will be especially important in the use of TNF as a biologic response modifier in the therapy of human neoplasms. TNF has recently been reported to induce both neutrophilia and lymphopenia in patients (35–37), thus suggesting that the knowledge obtained in the rat model may be applicable to the human situation. The demonstration that IL-1α and IL-1β, TNF, and LT induce neutrophilia indicates that both macrophage and lymphocyte products may play a role in the mediation of the acute inflammatory reaction, as is also teleologically consistent with the observations that one or more of the IL-1 and TNF family may cause myeloid differentiation (38), functional activation of neutrophils (6, 8), and acute dermal inflammation (39).

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