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Comparison of Peripheral Blood Leukocyte Kinetics After Live *Escherichia coli,* Endotoxin, or Interleukin-1α Administration

Studies Using a Novel Interleukin-1 Receptor Antagonist

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Objective

This study was undertaken to evaluate whether hematologic and immunologic effects observed after bacteremia and endotoxemia in the host could be replicated by administration of recombinant human interleukin-1α (IL-1α) in a primate model. Furthermore, to determine whether endogenously produced interleukin-1 (IL-1) contributes to the changes observed during endotoxemia or gram-negative septic shock, a specific IL-1 receptor antagonist (IL-1ra) was administered.

Summary Background Data

The lipopolysaccharide (LPS) component of the outer membrane of gram-negative bacteria initiates a constellation of metabolic and immunologic host responses. IL-1, a macrophage-derived cytokine, acts as a key mediator in the host response to infection and inflammation.

Methods

Baboons were randomly assigned to receive either recombinant human IL-1α, LPS, or live *Escherichia coli* both with or without concomitant administration of IL-1ra. Blood was collected hourly and analyzed using flow cytometric techniques.

Results

Both endotoxemia and live *E. coli* bacteremia induced an acute granulocytopenia; however, the granulocytopenia gradually resolved in the endotoxemic group, but was sustained in the bacteremic group. An early lymphopenia and monocytopenia was elicited by LPS or *E. coli* and persisted throughout the experiment. Recombinant human IL-1α induced the following: (1) an early, transient decline in granulocytes followed by a sustained granulocytosis; (2) a lymphopenia; and (3) a transient monocytopenia followed by a gradual return to baseline. Although IL-1ra had no effect on leukocyte kinetics with either live *E. coli* or LPS, the IL-1ra significantly abrogated the monocytopenia seen with recombinant human IL-1α administration alone.

Conclusions

These results suggest that administration of recombinant human IL-1α can replicate some of the characteristic patterns of hematologic change associated with bacteremia and endotoxemia. However, an endogenous IL-1 response is not required for these changes to occur. Rather, the data suggest that other inflammatory mediators induced by endotoxemia or gram-negative bacteremia, such as tumor necrosis factor-α (TNFα), may be involved.

The lipopolysaccharide (LPS) component of the outer membrane of gram-negative bacteria is known to initiate a wide variety of immunologic and metabolic responses.[1,2] When administered to animals and humans *in vivo,* LPS induces the endogenous synthesis of numerous cytokines, including interleukin-1 (IL-1)[3,4] and tumor necrosis factor-α (TNFα),[5,6] that are thought to act as proximal mediators in the host response to infection and inflammation.

Administration of purified LPS to normal human subjects elicits an acute granulocytopenia followed by a granulocytosis, lymphopenia, and a transient monocytopenia with a gradual return to baseline by 24 hours after administration.[7,8] In addition, *in vivo* administration of LPS to humans results in diminished *in vitro* mononuclear cell proliferative capacity that appears to be secondary to a defect in antigen presentation.[9,10] However, the contribution of the individual cytokines to these changes remains unclear.

The cytokine IL-1 is a class of macrophage-derived endogenous mediators in the hematologic and immunologic response to microbial invasion, inflammation, and tissue injury. At least two biochemically distinct gene products of the molecule exist *in vivo,* IL-1α and IL-1β. Although both forms share limited amino acid identity ($\sim$ 26%), they bind to both classes of the IL-1 receptor[11] and appear to have similar biological activity.[12] The recent availability of purified recombinant forms of IL-1 has enabled further investigation into the biological activity of this cytokine during states of infection and inflammation. Previous reports have demonstrated that a variety of hematologic and immunologic effects are mediated by IL-1, including stimulation of T-lymphocyte proliferation,[13] increased chemotaxis of granulocytes into an inflammatory site,[14] and stimulation of myelopoiesis by induction of colony-stimulating factor (GM-CSF and M-CSF) synthesis.[15,16] Further, intravenous administration of recombinant human IL-1 *in vivo* in rodents induces a rapid neutrophilia and lymphopenia.[4,17]

IL-1 has occasionally been detected in the circulation after sepsis and endotoxemia in humans[18,19] and animals.[20] Circulating levels of IL-1 in septic human patients[19] were comparable to the levels measured in our septic baboon model.[23] The current study was undertaken to further delineate the contributory role of IL-1 as a mediator in the alterations in peripheral blood leukocyte kinetics associated with endotoxemia and sepsis in a primate model. The recent discovery of a novel IL-1 receptor antagonist (IL-1ra)[21,22] aided in this effort by enabling effective inhibition of IL-1 binding to its type I receptor, thereby allowing further definition of the role of IL-1 in mediating endotoxin-induced peripheral blood leukocyte changes. Finally, a comparison between the relative effects of lethal *Escherichia coli* bacteremia and sublethal endotoxemia on peripheral blood leukocyte kinetics was made using the same *in vivo* primate model. The metabolic and physiologic responses to recombinant human IL-1α, LPS, and *E. coli* administration in this model have been reported previously[23,24]

MATERIALS AND METHODS

Reagents

Recombinant human IL-1α (lot no. 117–271) was provided by Hoffmann-La Roche Inc. (Nutley, NJ). *Salmonella typhosa* LPS (phenol-extracted; lot no. 126F-4020) was obtained from Sigma Chemical Co. (St. Louis, MO). Recombinant human IL-1ra was provided by Synergen Inc. (Boulder, CO). The endotoxin content of the IL-1α and the IL-1ra was less than 3 pg/mg protein. Before intravenous administration, the IL-1α, IL-1ra, and LPS were diluted with physiologic saline (0.9% sodium chloride) containing 0.5 mg/mL of human serum albumin. Due to the high degree of structural homology between human and *Papio* sp. cytokines, a primate model allowed for utilization of human recombinant forms of cytokines (*i.e.,* IL-1α and IL-1ra) in this experiment to more closely mimic the human response. Lyophilized *E. coli* (strain 086:B7), which was provided by Dr. G. Tom Shires, was used to grow cultures on tryptic soy broth agar slants; viability counts of the inoculum were determined by standard dilution techniques. *E. coli* bacteria

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were diluted with physiologic saline to obtain a final concentration of 10^{11} colony-forming units (CFU)/mL.

Treatment of the Animals

Male and female *Papio* sp. baboons weighing 14 to 21 kg were obtained from the National Primate Pool through Buckshire Laboratories (Chelmsford, PA). Animals were quarantined at the Research Animal Resource Center, Cornell University Medical College, for a minimum of 3 weeks before the study to confirm the absence of disease. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Cornell University Medical College, and the animal facilities are approved by both the U.S. Department of Agriculture and the American Association of Laboratory Animal Care (AALAC). Baboons were fasted overnight and the next morning they were anesthetized with intramuscular ketamine hydrochloride (15 mg/kg). While the baboons received sodium pentobarbital anesthesia (5 mg/kg/hr intravenously), catheters for hemodynamic monitoring were inserted. The animals were then covered with a blanket and allowed to equilibrate for a minimum of 1 hour, after which baseline blood collections were obtained. After this baseline period, the animals were randomly assigned, three to a group, to receive one of the following treatments: (1) recombinant human IL-1α (10 μg/kg), (2) recombinant human IL-1α (0.1 μg/kg), (3) *S. typhosa* LPS (500 μg/kg), or (4) vehicle alone (an equivalent volume of 0.5 mg/mL of human serum albumin in physiologic saline). Three additional groups of baboons, two per group, received a simultaneous infusion of the following: (1) recombinant human IL-1α (10 μg/kg) plus IL-1ra (10 mg/kg), (2) LPS (500 μg/kg) plus IL-1ra (10 mg/kg), or (3) IL-1ra (10 mg/kg) alone. Another group of baboons (n = 12) received 10^{11} CFU/kg of live *E. coli* intravenously while a final group (n = 6) simultaneously received *E. coli* (10^{11} CFU/kg) plus IL-1ra (10 mg/kg) bolus followed by a continuous intravenous infusion of 25 μg/kg/min. The recombinant human IL-1α, recombinant human IL-1ra, and LPS were administered into a deep central vein as a single bolus over 30 seconds (unless otherwise stated). Live *E. coli* was administered into a central vein over 5 minutes as a bolus infusion.

At hourly intervals for the subsequent 8 hours, whole blood was collected in edetic acid (EDTA)-coated tubes. At the end of the 8-hour period, the baboons were killed with an intravenous overdose of sodium pentobarbital (100 mg/kg).

Differential Leukocyte Counts

One hundred microliters of whole blood was aliquoted and the erythrocytes lysed by the addition of 1.9 mL of bicarbonate-buffered ammonium chloride solution (Ortho Diagnostic Systems, Raritan, NJ). This suspension was then directly aspirated into a Spectrum III flow cytometer (Becton-Dickinson Immunocytometry Systems, Braintree, MA) for a quantitative, three-part (lymphocyte, monocyte, and granulocyte), differential cell count by light scatter analysis as previously described.[7]

Lymphocyte Subset Analysis

Aliquots of whole blood (150 μL) were diluted 1:2 with phosphate-buffered saline. Saturating amounts of the following fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies were then added: anti-CD2 and anti-CD8 (Ortho Diagnostic Systems), anti-CD4 and anti-CD20 (Coulter Cytometry, Hialeah, FL), and anti-HLA–DR (Becton-Dickinson, San Jose, CA). These antibodies directed against human lymphocyte surface markers have been previously shown to cross react with primate lymphocyte markers.[25] After incubating for 30 minutes on ice, erythrocytes were lysed as described above. Leukocytes were recovered by centrifugation and resuspended in 1 mL of phosphate-buffered saline containing 1% bovine serum albumin and 0.1% sodium azide for flow microfluorimetry analysis. Control samples consisted of unstained cells or cells stained with monoclonal mouse immunoglobulin G-FITC.

The argon ion laser of the flow cytometer was operated at 75 mW, and all data analyses were performed on a 2140 computer system (Becton-Dickinson Immunocytometry Systems). Lymphocytes were gated by simultaneous forward *versus* 90-degree light scatter with an elliptical region set around the lymphocyte cluster. Green fluorescence histograms were generated with mutually exclusive counting regions set to quantify fluorescence-negative (control-stained) and fluorescence-positive events. Data were obtained as the percentage of fluorescence-positive lymphocytes relative to total lymphocytes counted.

Proliferation Assays

Mononuclear cells were isolated from whole blood by Ficoll-Hypaque separation[26] and suspended in RPMI-1640 containing 110 μg/mL of sodium pyruvate, 100 units of penicillin/streptomycin, 5% horse serum, and 292 mcg/mL of glutamine. Cells were plated in triplicate in round-bottom, 96-well microtiter plates at a concentration of 10^5 cells/well and stimulated with phytohemagglutinin (PHA) (Burroughs-Wellcome, Dartford, England) at final concentrations of 2 μg/mL and 5 μg/mL. Cultures were incubated at 37 C in a 5% carbon dioxide atmosphere for 72 hours. After a 6-hour pulse with tritiated (1 μCi/well) thymidine (New England Nuclear,

Boston, MA), cells were harvested onto glass fiber filter discs using a cell harvester (Flow Laboratories, Rockville, MD). Tritiated thymidine incorporation was assessed by scintillation counting.

Cortisol/TNF Assay/Data Analysis

Plasma cortisol concentrations were measured by a direct radioimmunoassay as reported previously.[27,28] Cross reactivity of the antibody was 5% with 11-deoxy-17 OH-corticosterone (substance S) and less than 0.5% with other relevant glucocorticoid and sex steroids. Quality control samples were run in each group. Plasma TNFα was measured by an enzyme-linked immunosorbent assay (ELISA) as previously described.[5] The sensitivity of the assay was 25 pg/mL. Changes in leukocyte counts, cortisol/cytokine levels, and proliferation values are expressed as the arithmetic mean ± standard error of the mean. Statistical analyses were performed by a repeated measures analysis of variance (ANOVA). Differences between groups were assessed by Newman-Keuls' multiple range test. Statistical significance was indicated at a 95% confidence level.

RESULTS

Baboons infused with human serum albumin exhibited only minimal changes in leukocyte kinetics during the 8-hour experiment (Table 1). Similarly, baboons receiving infusions of only IL-1ra had no significant changes in leukocyte counts from baseline values (Table 1).

Granulocytes

A lethal dose of *E. coli* resulted in an acute nadir of circulating granulocytes to below 10% of baseline values, and the counts remained significantly decreased throughout the study (Fig. 1A). Similarly, administration of a sublethal dose of LPS resulted in a nadir (19% of baseline value) of circulating granulocytes by 2 hours after infusion (Fig. 1B). However, unlike the animals treated with *E. coli* that demonstrated a sustained granulocytopenia, granulocyte counts gradually recovered to baseline values by 4 hours in the animals treated with LPS (Fig. 1B). IL-1ra treatment did not attenuate the observed granulocytopenia and had no apparent effect on granulocyte kinetics during *E. coli* bacteremia (Fig. 1A). In contrast, with concomitant treatment with LPS and IL-1ra, peak granulocyte counts were greater than those seen with LPS alone by the end of 8 hours (Fig. 1B).

After administration of recombinant human IL-1α (10 μg/kg), a biphasic increase in the granulocyte count was observed. Within 1 hour, the number of circulating granulocytes had risen to three times the baseline value and returned to baseline at 2 hours. By 8 hours, the count had gradually increased to greater than five times the baseline value (Fig. 1C). A similar trend was evident with the lower dose of recombinant human IL-1α (0.1 μg/kg); however, the magnitude of the peak rise in the granulocyte count was less than that observed with the higher (10 μg/kg) dose of recombinant human IL-1α, suggestive of a dose-response effect (Table 1). The simultaneous administration of recombinant human IL-1α and IL-1ra completely blocked the transient decline in circulating granulocytes at 2 hours induced by recombinant human IL-1α.

Monocytes

A precipitous monocytopenia (< 10% of baseline values) occurred within 1 hour after either LPS or *E. coli* administration (Figs. 2A and 2B). This monocytopenia

Table 1. PERIPHERAL BLOOD LEUKOCYTE KINETICS AFTER LIVE *E. COLI*, LPS, OR RECOMBINANT HUMAN IL-1α ADMINISTRATION

	HSA only*	IL-1ra only	IL-1 (10 μg/kg)	IL-1 (0.1 μg/kg)	IL-1 + IL-1ra	LPS only	LPS + IL-1ra	*E. coli* only	*E. coli* + IL-1ra
Granulocyte (hr)									
0	9771 ± 3144†	5541 ± 1139	6267 ± 3311	5910 ± 1294	2692 ± 50	5645 ± 1774	4432 ± 1716	9773 ± 1305	10486 ± 2004
2	14522 ± 1906	7924 ± 1877	4707 ± 1552	9855 ± 1343	13391 ± 1899	963 ± 208	713 ± 437	1013 ± 296	1860 ± 542
8	11314 ± 1695	4622 ± 1586	19375 ± 1208	13139 ± 1500	11766 ± 0.5	4430 ± 567	6905 ± 793	3285 ± 978	5158 ± 1232
Lymphocyte (hr)									
0	2044 ± 395	1298 ± 120	2469 ± 44	2056 ± 447	1090 ± 136	2258 ± 675	1100 ± 56	1755 ± 168	1979 ± 229
2	1649 ± 595	1717 ± 48	1432 ± 122	961 ± 185	715 ± 143	601 ± 214	247 ± 13	642 ± 94	677 ± 131
8	1561 ± 236	754 ± 303	670 ± 152	821 ± 84	474 ± 124	337 ± 24	162 ± 19	373 ± 40	392 ± 79
Monocytes (hr)									
0	683 ± 311	376 ± 57	223 ± 16	231 ± 31	260 ± 82	324 ± 75	279 ± 6	321 ± 28	284 ± 39
2	473 ± 182	578 ± 90	32 ± 6	61 ± 14	280 ± 16	22 ± 4	25 ± 1	24 ± 4	22 ± 2
8	438 ± 180	281 ± 8	392 ± 14	548 ± 77	581 ± 35	40 ± 17	14 ± 0.5	33 ± 5	38 ± 7

* HSA; human serum albumin (vehicle control).
† Mean ± SEM leukocytes/mm^3.

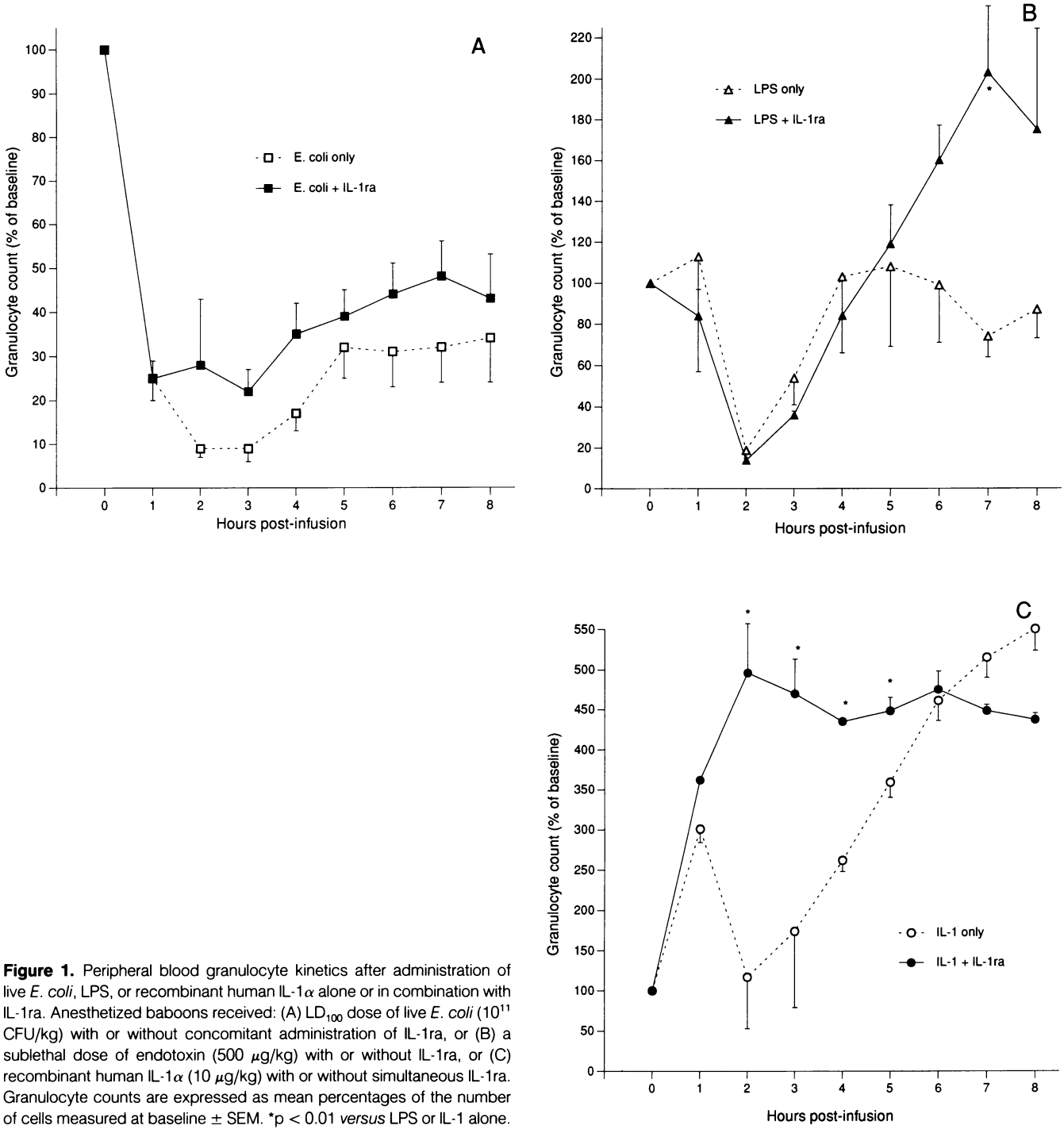

Figure 1. Peripheral blood granulocyte kinetics after administration of live *E. coli*, LPS, or recombinant human IL-1α alone or in combination with IL-1ra. Anesthetized baboons received: (A) LD_{100} dose of live *E. coli* (10^{11} CFU/kg) with or without concomitant administration of IL-1ra, or (B) a sublethal dose of endotoxin (500 μg/kg) with or without IL-1ra, or (C) recombinant human IL-1α (10 μg/kg) with or without simultaneous IL-1ra. Granulocyte counts are expressed as mean percentages of the number of cells measured at baseline ± SEM. *$p < 0.01$ *versus* LPS or IL-1 alone.

was of similar magnitude in both groups, persisted throughout the 8-hour experiment, and was unaffected by IL-1ra (Figs. 2A and 2B).

After administration of recombinant human IL-1α (either 10 or 0.1 μg/kg), a sharp decline (88% of baseline) in circulating monocytes was observed by 1 hour with a gradual recovery of counts back to baseline values by 8 hours (Fig. 2C). Concomitant administration of recombinant human IL-1α with IL-1ra significantly attenuated the monocytopenia observed with recombinant human IL-1α alone (Fig. 2C).

Lymphocytes

Both LPS and *E. coli* infusion induced an acute decline (to 44% and 55% of baseline values, respectively) in circulating lymphocytes within 1 hour of administration (Figs. 3A and 3B). The lymphopenia persisted through-

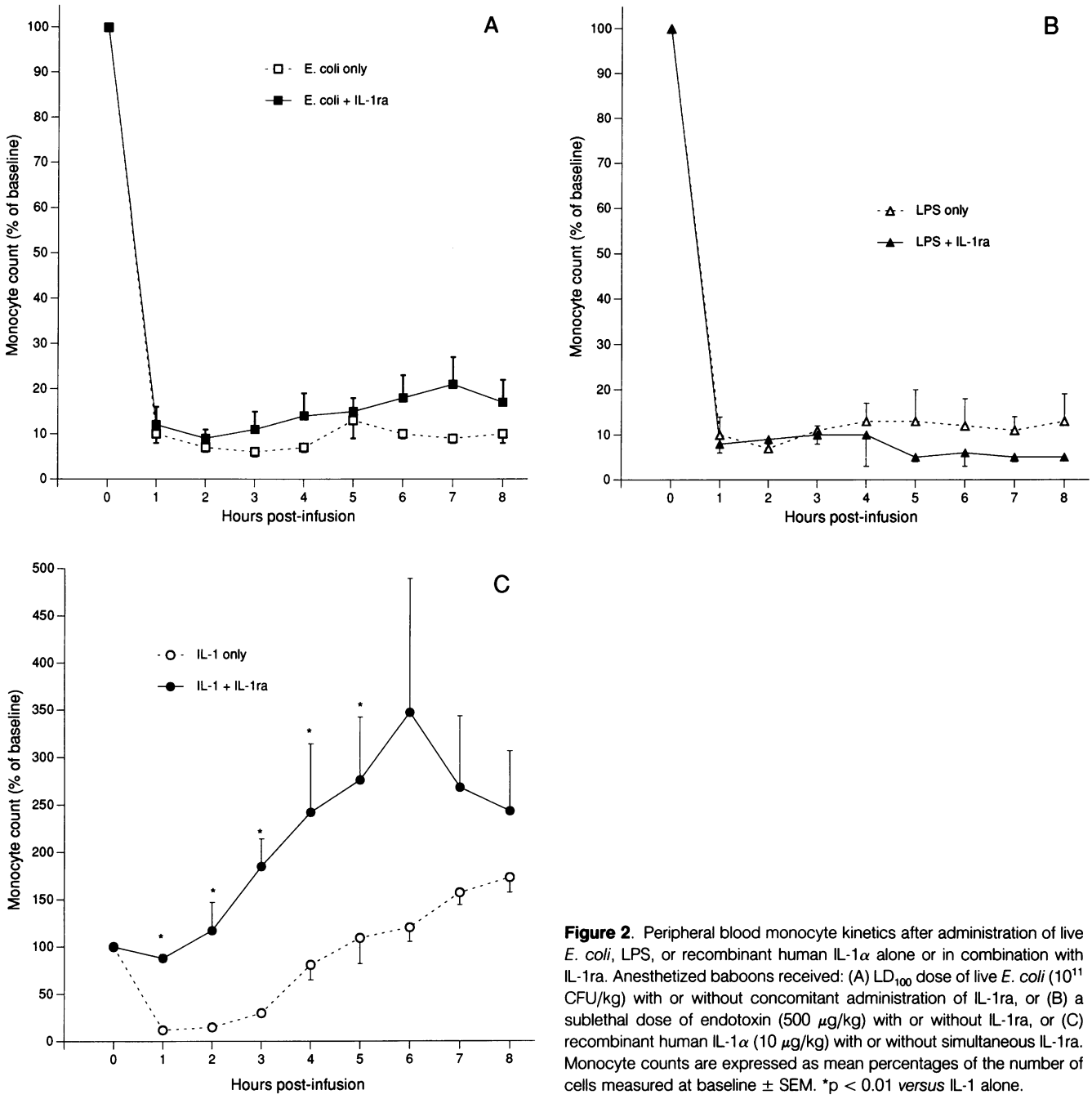

Figure 2. Peripheral blood monocyte kinetics after administration of live *E. coli*, LPS, or recombinant human IL-1α alone or in combination with IL-1ra. Anesthetized baboons received: (A) LD_{100} dose of live *E. coli* (10^{11} CFU/kg) with or without concomitant administration of IL-1ra, or (B) a sublethal dose of endotoxin (500 μg/kg) with or without IL-1ra, or (C) recombinant human IL-1α (10 μg/kg) with or without simultaneous IL-1ra. Monocyte counts are expressed as mean percentages of the number of cells measured at baseline ± SEM. *$p < 0.01$ *versus* IL-1 alone.

out the course of the experiment, reaching a nadir at 8 hours in both treatment groups. IL-1ra had no apparent effect on lymphocyte kinetics in endotoxemia or bacteremia.

A significant lymphopenia was observed within 1 hour of recombinant human IL-1α infusion (either 10 or 0.1 μg/kg) that persisted throughout the experiment, reaching a nadir (25% of the baseline value) at 8 hours after infusion (Fig. 3C). Co-administration of recombinant human IL-1α with IL-1ra only partially attenuated the persistent gradual lymphopenia observed during the 8-hour experiment (Fig. 3C).

After administration of either live *E. coli*, LPS, or recombinant human IL-1α, alterations in lymphocyte subpopulations were characterized by a slight increase (~ 10% to 15%) in the percentage of lymphocytes expressing the pan T-cell or CD2 cell surface marker at 2 to 3 hours after infusion accompanied by a concomitant decrease in pan B-cell (CD20+ or HLA–DR) lymphocyte percentages compared to baseline (Figs. 4A–4C). In all three treatment groups, the increase in the percentage of CD2+ lymphocytes could be accounted for by a transient increase in the helper/inducer (CD4+) subpopulation of T lymphocytes, whereas the percentage of sup-

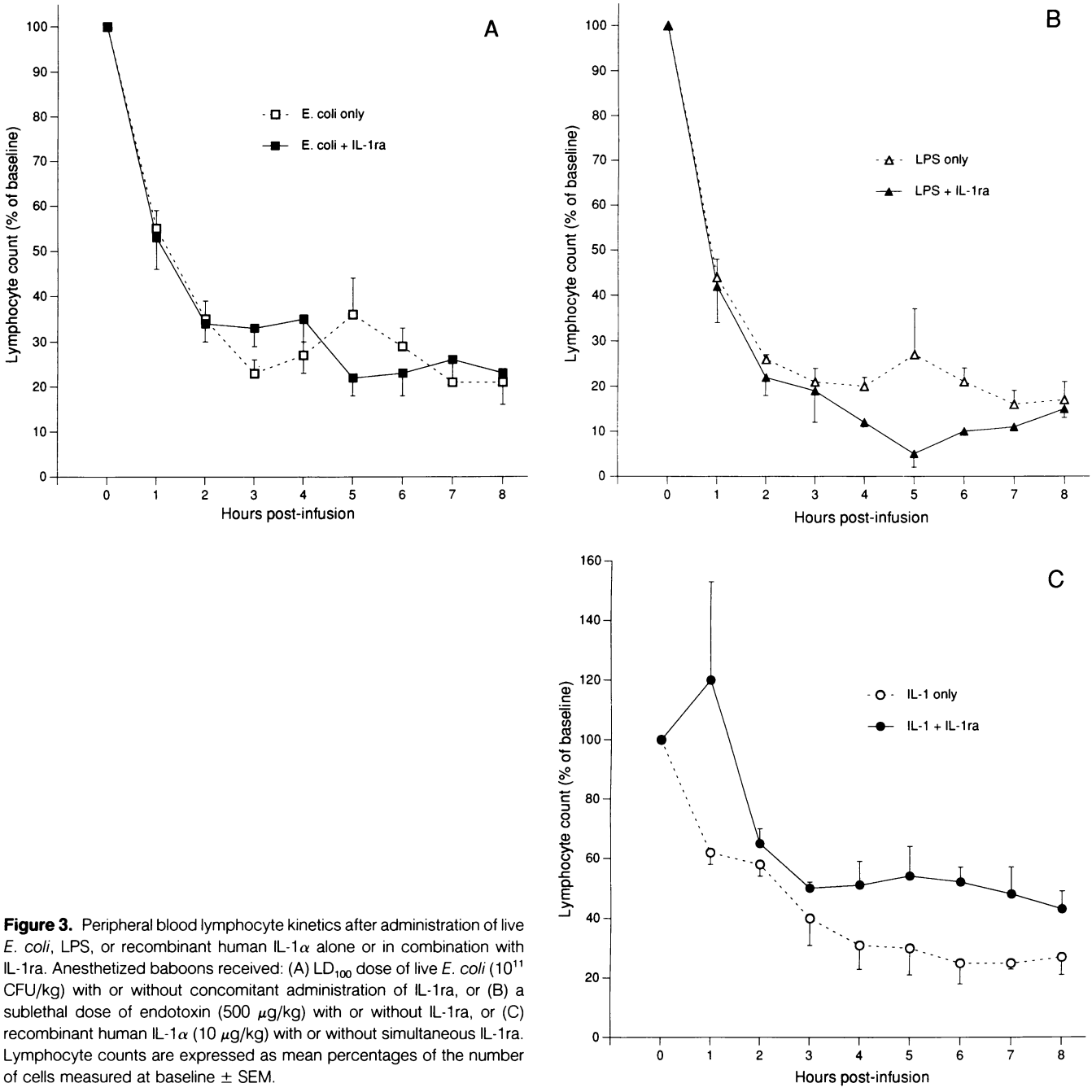

Figure 3. Peripheral blood lymphocyte kinetics after administration of live *E. coli*, LPS, or recombinant human IL-1α alone or in combination with IL-1ra. Anesthetized baboons received: (A) LD_{100} dose of live *E. coli* (10^{11} CFU/kg) with or without concomitant administration of IL-1ra, or (B) a sublethal dose of endotoxin (500 μg/kg) with or without IL-1ra, or (C) recombinant human IL-1α (10 μg/kg) with or without simultaneous IL-1ra. Lymphocyte counts are expressed as mean percentages of the number of cells measured at baseline ± SEM.

pressor/cytotoxic (CD8+) T lymphocytes remained relatively constant. These changes in lymphocyte subpopulations gradually returned to baseline values by 8 hours after infusion. Concomitant treatment with IL-1ra had no apparent effect on lymphocyte subpopulation kinetics (data not shown).

Plasma TNFα was not detectable at any timepoint after recombinant human IL-1α infusion. In contrast, a monophasic increase in circulating TNFα was observed after sublethal endotoxemia, with plasma TNFα levels detectable by 1 hour and a peak level of 1000 pg/ml by 90 minutes after infusion; this was followed by a rapid decline to undetectable levels by 3 hours (Fig. 5). Similarly, live *E. coli* bacteria infusion induced a peak TNFα response of 40,000 pg/ml at 90 minutes, with a rapid return to undetectable levels by 4 hours. IL-1ra had no effect on the observed endogenous TNFα response (data not shown).

Plasma cortisol levels were significantly elevated at baseline (~ 75 and 100 μg/dL) and remained in this range throughout the course of the experiment. Subsequent administration of live *E. coli*, LPS, or IL-1 had no

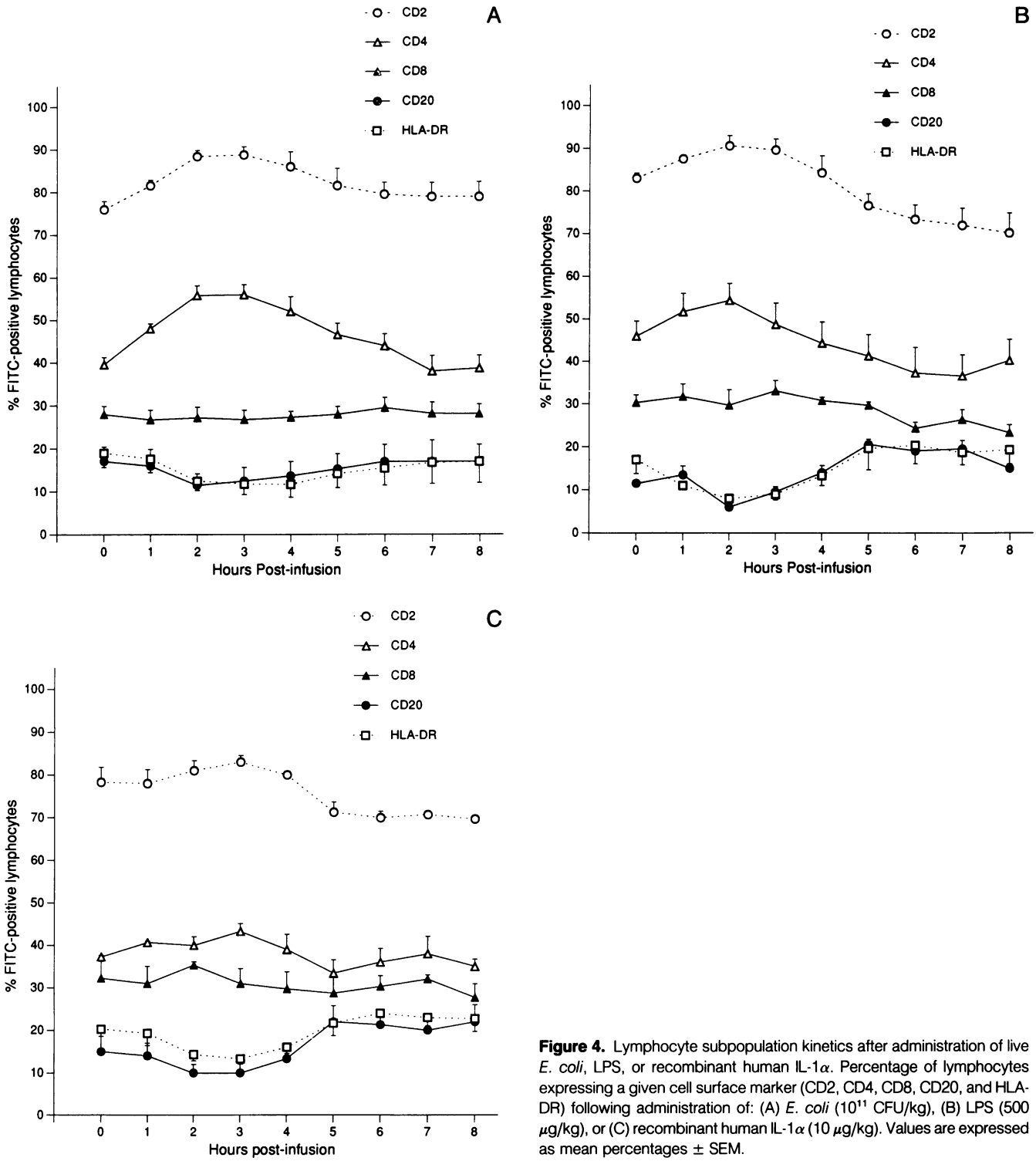

Figure 4. Lymphocyte subpopulation kinetics after administration of live *E. coli*, LPS, or recombinant human IL-1α. Percentage of lymphocytes expressing a given cell surface marker (CD2, CD4, CD8, CD20, and HLA-DR) following administration of: (A) *E. coli* (10^{11} CFU/kg), (B) LPS (500 μg/kg), or (C) recombinant human IL-1α (10 μg/kg). Values are expressed as mean percentages ± SEM.

appreciable effect on the previously elevated baseline cortisol levels. In addition, concomitant administration of IL-1ra with *E. coli,* LPS, or rhIL-1α had no apparent effect on cortisol levels (data not shown).

In baboons receiving recombinant human IL-1α at a dose of 0.1 μg/kg, peripheral blood mononuclear cell proliferative activity *in vitro* was increased ninefold in response to the mitogen PHA (final concentration of 2 μg/ml) by 4 hours after infusion compared to baseline proliferation before infusion of recombinant human IL-1α (Fig. 6). Eight hours after infusion, proliferative capacity had returned to near baseline levels. Similarly, in animals receiving the higher dose of recombinant human IL-1α (10 μg/kg), mononuclear cell proliferative capac-

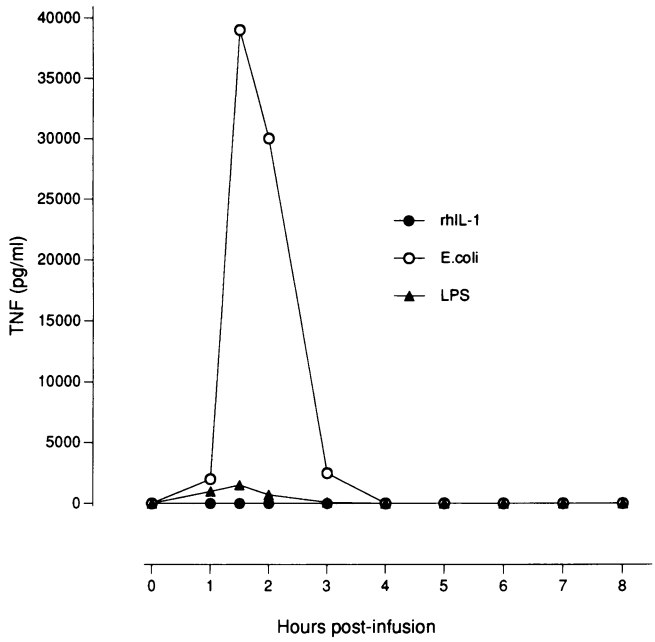

Figure 5. Kinetics of circulating TNFα after administration of lethal *E. coli* bacteremia, sublethal endotoxemia, or recombinant human IL-1α. Circulating TNFα levels are represented here after sublethal endotoxemia or an LD_{100} of live *E. coli*. Values are expressed as the mean in pg/mL of serum. TNFα levels were undetectable after administration of recombinant human IL-1α.

ity was increased fourfold over baseline values and remained near that level by 8 hours after infusion (Fig.6). In contrast to the recombinant human IL-1α effect, mononuclear cell proliferative capacity was decreased by more than five times at 8 hours after *E. coli* administration (Fig. 6). Similar trends were obtained with a higher dose of the mitogen PHA (final concentration of 5 μg/kg), although, as expected, the overall magnitude of the proliferative response was slightly greater (data not shown). In baboons receiving only human serum albumin as a control, mononuclear cell proliferative capacity did not change over the course of the experiment (data not shown). No proliferation data were obtained in animals receiving the IL-1ra.

DISCUSSION

The current study sought to examine the role of IL-1α as a possible mediator in the induction of peripheral blood leukocyte kinetic changes during states of systemic sepsis and endotoxemia by using two approaches: (1) intravenously infusing live *E. coli* or LPS into healthy baboons and then attempting to replicate the observed changes in leukocyte kinetics induced by the administration of exogenous recombinant human IL-1α; and (2) employing recently described[21,22] IL-1ra to further investigate the role of IL-1 in these changes. IL-1ra is a naturally occurring 17-kD protein produced endogenously by cells of monocyte/macrophage lineage in response to *in vitro* stimulation with antigen–antibody complexes.[22,29] It binds specifically to the type I, IL-1 receptor found predominantly on T-lymphocytes, endothelial cells, tissue macrophages, and hepatocytes, but binds with less affinity to the type II, IL-1 receptor found principally on granulocytes.[30,31] The binding affinity of IL-1ra to the type I, IL-1 receptor has been shown to be similar to that of either IL-1α or IL-1β.[31] Because IL-1α and IL-1ra have the same molecular weight and bind with equal affinity to the type I, IL-1 receptor, administration of a 1000-fold molar excess of IL-1ra simultaneously with recombinant human IL-1α, as in this study, effectively inhibits binding of IL-1α to its type I receptor. Currently, IL-1ra has been shown to have no agonist activity either *in vitro*[29] or *in vivo*.[23] A recently cloned, purified recombinant form of human IL-1ra having the same biological activity as the naturally occurring form[29] was used in the current study.

The experiments reported herein demonstrate that regardless of the initiating stimulus, whether live *E. coli* or LPS, strikingly similar changes in peripheral blood leukocyte kinetics occur, implicating the LPS component of gram-negative bacteria as the active moiety responsible for this effect. After either LPS or *E. coli* administration, a precipitous decline in circulating granulocytes, monocytes, and lymphocytes was observed. In the less

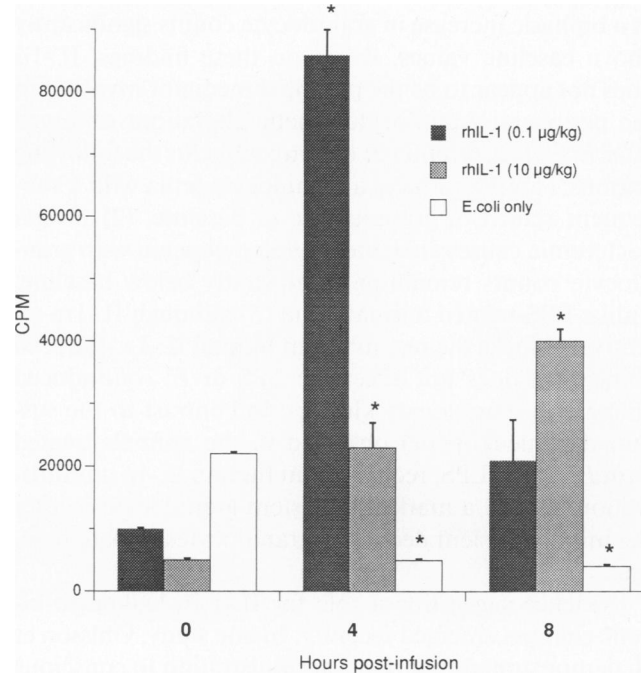

Figure 6. *In vitro* mononuclear cell proliferative capacity after administration of *in vivo* IL-1α or live *E. coli*. Animals were treated *in vivo* with either two different doses of IL-1α (10 or 0.1 μg/kg) or an LD_{100} of live *E. coli*. PHA-stimulated (2 μg/mL) proliferative capacity was assessed at baseline (t = 0 hours) and at 4 and 8 hours after infusion. Values are expressed as mean ± SEM counts/min. *$p < 0.01$ *versus* baseline.

severe model of sublethal endotoxemia, granulocyte counts gradually returned to baseline. In the more severe lethal *E. coli* model, granulocyte counts remained significantly below baseline levels. This difference in response may be due, in part, to continuous release of LPS as live bacteria that are gradually cleared from the circulation by the reticuloendothelial system and destroyed over time in the *E. coli* bacteremic baboons. These results are consistent with the findings of recent studies in which normal human subjects received a small bolus of purified LPS intravenously. LPS elicited an acute granulocytopenia, lymphopenia, and monocytopenia followed by a significant granulocytosis.[7,8] Recently, Wakabayashi et al. demonstrated that an infusion of live *E. coli* into rabbits yielded similar findings in that an acute 60% decline in circulating leukocytes was observed by 1 hour after infusion and remained persistently decreased throughout the duration (5 hours) of the experiment.[20] This observed leukopenia was partially attenuated by pretreatment with IL-1ra; in the current study, however, IL-1ra failed to demonstrate an apparent effect on leukocyte kinetics during *E. coli* bacteremia. The failure of IL-1ra to attenuate the observed leukopenia in the *E. coli* bacteremic baboons may be accounted for, in part, by differences in model severity since the decrease in circulating leukocytes was of much greater magnitude (90% of baseline) than the decline observed in the rabbit model (60% of baseline).

Administration of IL-1α (at either dose) alone resulted in a biphasic increase in granulocyte counts significantly above baseline values. Based on these findings, IL-1α does not appear to be the principal mediator involved in the peripheral granulocyte kinetic alterations observed after *E. coli* bacteremia or endotoxemia for the following reasons: (1) LPS induces a granulocytopenia with a subsequent return of granulocytes to baseline, (2) *E. coli* bacteremia causes an acute granulocytopenia with granulocyte counts remaining significantly below baseline, unlike LPS-treated animals; and (3) although IL-1ra effectively blocks the recombinant human IL-1α–induced changes, it does not affect the LPS or *E. coli*-induced changes in granulocyte kinetics. In contrast to the sustained panleukopenia observed in the animals treated with *E. coli* or LPS, recombinant human IL-1α administration elicited a marked persistent granulocytosis after the initial transient decline in granulocytes back to baseline.

Evidence suggesting a role for IL-1 in leukocyte kinetic changes emerged recently. In one study, Ohlsson et al. demonstrated that IL-1β administration to conscious rabbits results in an early profound leukopenia followed by a significant leukocytosis.[4] Furthermore, the early leukopenia was completely attenuated by pretreatment with IL-1ra. Similarly, Ulich et al. have shown that after either IL-1α or IL-1β administration to healthy animals, a significant sustained granulocytosis and lymphopenia was observed.[17]

The acute monocytopenia induced by either *E. coli* or LPS administered to animals appears to be identical in its time course and magnitude of effect. Although recombinant human IL-1α induces a similar acute monocytopenia, a subsequent return of monocyte counts to baseline occurs within 8 hours. In addition, although simultaneous administration of IL-1ra with recombinant human IL-1α completely blocked the profound monocytopenia observed with recombinant human IL-1α treatment alone, it had no apparent effect on the *E. coli* or LPS-induced monocytopenia, suggesting that perhaps LPS-induced mediators other than IL-1α may exert a predominating effect on the sustained monocytopenia during endotoxemia and sepsis.

After either *E. coli,* LPS, or recombinant human IL-1α administration, an acute precipitous lymphopenia was observed. In addition, the magnitude and time course of this lymphopenia in these three treatment groups is roughly identical. Co-administration of IL-1ra with *E. coli,* LPS, or recombinant human IL-1α has no apparent effect on lymphocyte kinetics, suggesting that the *E. coli* and LPS-induced lymphopenia may be mediated by IL-1 through a non-type I, IL-1 receptor mechanism.

It remains unclear whether LPS exerts its effects on leukocyte kinetics directly, or alternatively through induction of other mediators. After *in vivo* administration of LPS, a readily detectable plasma cytokine response ensues, including detectable circulating TNFα. Both LPS and *E. coli* infusion in this model elicited an acute, transient, circulating TNFα response that temporally coincides with the rapid decline in all circulating leukocytes within 2 hours of LPS or *E. coli* administration. Recent evidence implicates TNF in the observed early leukopenia because TNF induces a profound granulocytopenia 15 minutes after administration in humans.[32] *In vitro* data have demonstrated that both LPS and TNFα induce rapid upregulation of preformed integrin protein molecules on the surface of leukocytes that promote adhesion of these cells to the vascular endothelium, allowing for the possibility of selective sequestration of leukocytes from the peripheral circulation.[33,34]

Bacterial endotoxin elicits an endogenous adrenocortical response resulting in acute elevation of plasma cortisol levels. However, in these models, markedly elevated baseline plasma cortisol levels were observed several hours before recombinant human IL-1α, LPS, or *E. coli* administration as a result of anesthesia-induced stress. Furthermore, administration of recombinant human IL-1α, LPS, or *E. coli* had no apparent effect on subsequent plasma cortisol levels, suggesting that a maximal adrenocortical cortisol response already existed. Although previous reports have indicated that the adrenocortical re-

sponse to endotoxin exposure may have an important role in peripheral blood leukocyte dynamics,[7] the glucocorticoid response in this model does not appear to be a factor in the acute leukocyte kinetic alterations observed here because these changes occurred in the presence of elevated background levels of plasma cortisol.

Acute, transient increases in the percentages of total T-lymphocytes (CD2+) were consistently seen after either LPS, *E. coli,* or recombinant human IL-1α administration. These increases are accounted for, in part, by slight increases in helper/inducer (CD4+) lymphocytes, while percentages of suppressor/cytotoxic lymphocytes (CD8+) remained relatively constant. A concomitant decrease in the percentage of B-lymphocytes (CD20+ or HLA-DR+) was also observed, which is consistent with the fact that most circulating lymphocytes are either T- or B-lymphocytes and a decline in the percentage of one class would result in an increase in the percentage of the other. Although previous reports indicate that LPS administration to human subjects causes a decrease in the percentages of CD3+ T-cells, which can be accounted for almost entirely by a concomitant decrease in the percentage of CD4+ helper/inducer T- cells,[7] others have reported no changes in T-cell percentages after LPS administration.[10] The failure of IL-1ra to have an influence on these changes strongly suggests that they are not IL-1–mediated events. The immunologic significance of these transient perturbations in lymphocyte subpopulations remains unclear; however, functional defects are present (see below).

IL-1 was initially described as a co-mitogen for the proliferation of T-lymphocytes. Consistent with its known immunologic effects, proliferation data from the current study indicate that *in vivo* exposure to recombinant human IL-1α results in augmented *in vitro* proliferative capacity. In contrast, *in vivo* exposure to an overwhelming dose of live *E. coli* bacteria diminishes *in vitro* lymphocyte proliferative capacity, consistent with functional immunologic data from normal human subjects given LPS.[9]

Regardless of whether the initiating stimulus is live *E. coli* or LPS, remarkably similar patterns of hematologic change characterized by a sustained granulocytopenia, lymphopenia, and monocytopenia strongly implicate LPS as the causative factor in these changes. Further, direct administration of recombinant human IL-1α elicits a biphasic granulocytosis, a lymphopenia, and a transient monocytopenia. Comparison of patterns of hematologic change demonstrates that *in vivo* recombinant human IL-1α administration can replicate some of the numerical leukocyte changes (*i.e.,* lymphopenia and monocytopenia) observed in endotoxemia and sepsis. However, the failure of IL-1 receptor blockade to significantly alter leukocyte kinetics when co-administered with LPS or *E. coli* suggests that other LPS-induced mediators, such as TNFα, can elicit similar patterns of hematologic change through mechanisms independent of IL-1.

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