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TITLE

Changes in Circulating Lymphocyte Subpopulations and Mitogen-Stimulated Response in a Rat Infusion Model of Intra-Abdominal Infection

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Changes in circulating lymphocyte subpopulations and mitogen-stimulated response in a rat infusion model of intra-abdominal infection

Lucie Martineau, PhD; Pang N. Shek, PhD

Objective: **To describe the alterations in circulating concentrations of immune cells as well as in *in vitro* mitogen-stimulated response in a recently developed rat model of intra-abdominal infection.**

Design: **Randomized, controlled study.**

Setting: **Government research facility.**

Subjects: **Male Sprague-Dawley rats.**

Interventions: **Infected animals received an intraperitoneal infusion of 6.0×10^8 colony forming units of *Escherichia coli* during 12 hrs, whereas control rats received a sterile inoculum. All experimental animals underwent laparotomy and peritoneal lavage at the end of the infusion period. Blood samples were obtained 12 hrs, 36 hrs, or 7 days after the onset of infusion. Splenocytes were concomitantly harvested and assayed for response to the mitogens phytohemagglutinin (PHA), concanavalin A (Con A), and lipopolysaccharides, as well as for production of interleukin (IL)-2.**

Measurements and Main Results: **Infected rats showed a marked leukopenia (−82% for 36 hrs), with leukocyte counts returning to normal at 7 days. They also developed a marked lymphocytopenia throughout the study; this was achieved through comparable reductions in circulating T and B cells. Con A responses in both groups were similar for 7 days. In contrast, splenocytes from infected animals showed reduced responses to lipopolysaccharides (−64%) and PHA (−30%) for 36 hrs compared with control splenocytes. IL-2 production from mitogen-stimulated splenocytes was suppressed in infected rats to 66% of that of control rats for 7 days. Suppressed PHA responses were not restored to control values in the presence of IL-2. For all of the parameters assessed, control animals showed either no significant changes or relatively fewer changes than infected rats.**

Conclusions: **This model of intra-abdominal infection is associated with changes in circulating concentrations of immune cells as well as with temporary functional defects in B and T cells, consistent with those often observed in patients with peritonitis. However, the role of IL-2 in limiting the adverse effects of infection in this experimental model seems to be limited. This model may be a useful tool in furthering our understanding of the pathophysiology of intra-abdominal infections and in assessing the efficacy of new therapeutic modalities. (Crit Care Med 2000; 28:2515–2521)**

Key Words: sepsis; animal model; bacterial infection; interleukin-2; immunosuppression; flow cytometry; *Escherichia coli*

Intra-abdominal infection and its potential complications, sepsis and multiple organ failure, cause significant morbidity and mortality in surgical patients, despite improvements in intensive care monitoring, broad-spectrum antibiotics, and aggressive surgical therapies (1, 2). In the last two decades, our understanding of the pathways, mediators, and cellular components of host defense involved in the pathogenesis of these infections has advanced considerably. Although a human model for sepsis is available (3), most of our understanding of this complex condition is based on studies using experimental models (for a review, see Ref. 4). However, some of these animal models may not accurately reflect the pathophysiology of those septic cases where the patient may be inflicted with a sustained, slow release of bacteria from an infection site.

Many experimental models of peritonitis (e.g., cecal ligation and puncture, cecal puncture or cecal laceration) mimic a focal release of bacteria leading to systemic infection (5–9). However, it is generally acknowledged that the bacterial load in these models is difficult to control because of the wide variety of strains and variability in the numbers of bacteria in the ceca of the experimental animals at the time of surgery. These models are further complicated by an unpredictable necrosis of the bowels, leading to a highly variable intestinal leakage. These drawbacks are likely responsible for the significant variation in mortality rates often observed in fecal contamination models within and/or between laboratories (10–12) and may create serious problems for evaluating potential therapies. For example, Bohnen et al. (10) reported that if a random sample of their rat cecal ligation and puncture groups was considered, it would have appeared that saline was superior to cefoxitin in treating the intra-abdominal infection, with day-to-day mortality rates ranging from 14% to 100% in the saline-treated groups and 0% to 57% in the cefoxitin-treated groups.

Our laboratory has recently developed a rat model of intra-abdominal infection, which simulates a trauma-induced, slow

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leakage of bacteria to the peritoneal cavity (13). In this model, a standardized inoculum containing *Escherichia coli* is slowly and continuously infused in the peritoneal cavity of conscious, unrestrained rats over 12 hrs. Unlike most animal models of peritonitis, the bacterial burden was mechanically removed at the end of the 12-hr infusion period, as recommended in clinical practice (1). We have shown that the lethality of our model was very consistent between groups of untreated animals infected on different days, likely resulting from the use of a prospectively quantitated bacterial dose administered under controlled experimental conditions (13). Furthermore, the infected animals exhibited many of the pathophysiological characteristics (body weight loss, fever, lactacidemia, episodic bacteremia, intraperitoneal cytokine compartmentalization) observed in patients with intra-abdominal infection. The objective of the present study was to further characterize our experimental model, by determining the alterations in circulating levels of immune cells as well as the changes in *in vitro* mitogen-stimulated response.

MATERIALS AND METHODS

Animals. Eighty-six male Sprague-Dawley rats with a mean body weight of 373 ± 6 g (mean ± SEM) were obtained from Charles River (St. Constant, Québec, Canada). The animals were housed individually, allowed to adapt to the environmental conditions (22°C, 12-hr light/dark cycle), and handled daily before undergoing surgery 7 days later. All animals had free access to standard rodent chow and water at all times during the experimental period All procedures described in this study were performed in adherence to the Canadian Council on Animal Care regulations for the use of experimental animals (14) and were reviewed and approved by the institutional animal ethics committee.

Intra-abdominal Infection Model The experimental procedures used to induce intra-abdominal infection in our animal model have been described in detail elsewhere (13). Briefly, all surgical procedures were performed under aseptic conditions, with the animals under general anesthesia (1.5% halothane, 1:1 O_2/NO_2). After disinfecting the abdominal and interscapular regions, a sterile self-made cannula (Silastic tubing; 0.76 mm inner diameter, 1.65 mm outer diameter; Dow Corning Medical, Mississauga, Ontario, Canada) was inserted into the abdominal cavity through a small incision. The distal end of the cannula was then tunneled subcutaneously to emerge through the skin between the scapulae and led through a stainless steel tether, which was then secured to the skin of the animal with a self-made Velcro attachment. Surgical wounds were closed with nonabsorbable 3-0 silk sutures (Johnson & Johnson, Mississauga, Ontario, Canada), and topical antibiotic (Hibitane, Ayerst Laboratories, Montréal, Québec , Canada) was applied. All cannulated animals received a subcutaneous injection of sterile saline (30 mL/kg body weight) and analgesics (buprenorphine, 0.05 mg/kg body weight, subcutaneously).

The animals were housed individually, and each tether was then connected to a fecal inoculum infusion system via a swivel to which the intraperitoneal cannula was attached (13). After 8–10 hrs of familiarization with the tether and swivel system, each cannulated rat was infused with 2 mL of either a bacterial inoculum or a control inoculum during a period of 12 hrs. The bacterial inoculum consisted of a volume of log-phase *E. coli* culture (ATCC 25922; PML Microbiologics, Mississauga, Ontario, Canada), 1.5 mL of sterile saline, and 0.5 mL of a sterile rat fecal adjuvant suspension (13). The final concentration of the bacterial inoculum was approximately 6.0×10^8 colony forming units (cfu)/mL The control inoculum was prepared similarly, substituting the same volume of sterile saline for the *E. coli* suspension in the saline/fecal inoculum.

Experimental Protocol. Intra-abdominal infection was induced in 56 rats (INF), whereas 24 animals received the control inoculum (CON). A group of six healthy, noncannulated rats (HEA) was also included in the study to provide baseline values for the different parameters measured (i.e., granulocyte, leukocyte, and lymphocyte subset counts; mitogen-stimulated lymphoproliferative responses; and splenic interleukin [IL]-2 production). At the end of the 12-hr infusion period, the cannulated animals were anesthetized, and laparotomy and peritoneal lavage were performed. Before individual housing, all surgical animals received a subcutaneous injection of sterile saline (30 mL/kg body weight) and analgesics twice daily for 72 hrs.

Animals were randomly killed at 12 hrs, 36 hrs, 60 hrs, and 7 days after the onset of infusion. Blood was collected from all animals into EDTA tubes and kept at room temperature for 2–5 hrs until immunofluorescent staining of lymphocytes was performed. The absolute number of leukocytes was determined using a Coulter Counter (model JT, Coulter Electronics, Burlington, Ontario, Canada).

Monoclonal Antibodies. All murine monoclonal antibodies (MoAb) used were IgG1 isotypes and were directly conjugated with either fluorescein isothiocyanate or phycoerythrin fluorochromes (Cedarlane Laboratories, Hornby, Ontario, Canada). OX-33 was used for enumeration of B cells (15). The T-lymphocyte population was defined by using OX-19 directed against the CD5 T-cell antigen receptor (16) T cells that have helper (T_H) functions were identified by using the MoAb OX-35 directed against the CD4 antigen (17). MoAb OX8 (anti-CD8) was used to define T-suppressor lymphocytes (T_S) (18). Natural killer (NK) cells were identified by using the 3.2.3 clone (Serotec, Toronto, Ontario, Canada). Negative controls were labeled with isotypic mouse IgG1. A titration curve was established for each MoAb in preliminary experiments to determine the optimal dilution achieving maximal cell labeling.

Immunofluorescence Staining. Lymphocytes were labeled using a modification of a staining procedure first described by Starkey et al. (19). Briefly, whole blood was incubated with an appropriate combination of optimal dilutions of subset-specific MoAb. Erythrocytes were lysed by the addition of a proprietary lysing buffer (Serotec). The cells were then centrifuged (1600 rpm, 21°C), and the cell pellet was washed with cold phosphate-buffered saline (CellWASH, Becton Dickinson, Mississauga, Ontario, Canada) to remove the excess antibody. The washed cells were recentrifuged, resuspended, and fixed in 1% paraformaldehyde in cold CellWASH. Samples were protected from light and stored at 4°C overnight for cytometric analysis the following day.

Blood Leukocyte and Lymphocyte Subsets Enumeration. Enumeration of the leukocytes was carried out on a flow cytometer (FACScan; Becton Dickinson). Information on 5,000–10,000 cells was acquired during analysis of each sample. Polymorphonuclear cells were identified by their characteristic appearance on a dot plot of forward versus side light scatter and electronically gated to exclude cell debris and aggregates. Wide light scatter gate settings were used to identify lymphocytes, to include a larger, more granulated subpopulation of activated lymphocytes that would escape analysis by gating narrowly on the predominant resting lymphocyte population (20, 21). The possibility of inclusion of some polymorphonuclear cells in the activated lymphocyte population was ruled out by our finding of comparable results in 20 random samples using both narrow and wide forward/side scatter gate settings. The absolute numbers of fluorescence-positive lymphocytes for each subset were determined by comparison with nonspecific binding of the negative control MoAb; the lymphocyte subsets were also calculated as a percentage of fluorescence-positive cells relative to total lymphocytes.

Splenocyte Mitogenic Responses. Because of technical considerations, spleens were removed from all animals except for those killed at 60 hrs. Spleens were teased apart under sterile conditions, and cell suspensions were prepared from individual rats. Cell suspensions were washed three times in serum-free medium (RPMI 1640 with 2 mM glutamine, 50 μM mercaptoethanol, 10 mM HEPES buffer, and 1% antibiotic-antimycotic solution; GIBCO, Mississauga, Ontario, Canada). Viability of the cells was assessed using the trypan

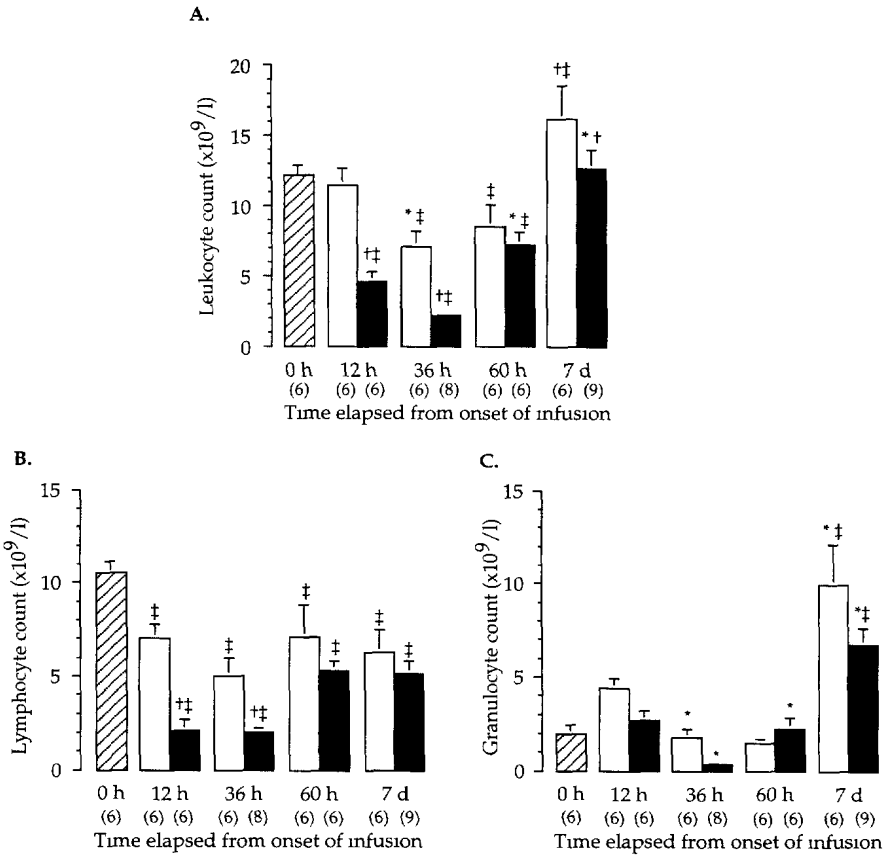

Figure 1. Changes in leukocyte (*A*), lymphocyte (*B*), and granulocyte (*C*) counts in infected (*filled bars*) and control rats (*unfilled bars*) during the study period. *Dashed bars* represent baseline values from healthy, unmanipulated rats. The number of experimental animals randomly killed at a given time period is indicated in parentheses. †Significantly different from control rats ($p < 05$); ‡significantly different from healthy rats; *significantly different from previous time period ($p < .05$)

blue dye exclusion technique. Cells were counted and cultured in sterile U-bottomed 96-well microtiter plates at 2×10^5 viable cells/well in 100 μL of medium supplemented with 5% fetal calf serum (complete medium; GIBCO). Various concentrations of phytohemagglutinin (PHA), concanavalin A (Con A), or lipopolysaccharide (LPS) (*E. coli* 026.B6) (Sigma Chemical, St. Louis, MO) were added (100 μL) to the cell suspensions to establish the optimal concentration of each mitogen that maximized the incorporation of tritiated thymidine (37 mBq/mmol; Amersham, Mississauga, Ontario, Canada). Cultures were incubated at 37°C in 5% CO_2 for 48 hrs and then pulsed with 20 μL of tritiated thymidine (1 μCi/well) for 18 hrs. Cells were harvested onto glass microfiber filter papers, using a semiautomated cell harvester (PHD, Cambridge Technology, Cambridge, MA), and incorporation of tritiated thymidine was determined by liquid scintillation counting (Beckman Instruments, Mississauga, Ontario, Canada). Percent suppression was calculated as: 100*(mean net disintegrations/min [DPM] for each INF − mean net DPM for all CON)/mean net DPM for all CON.

Effect of in Vitro *IL-2 on PHA Responses.* Recombinant IL-2 (Serotec) was diluted with sterile 5% dextrose at a concentration of 100 units/mL in 20 μL Splenocyte cultures were incubated with PHA for 48 hrs in the presence or absence of IL-2. Five percent dextrose was used in equivalent amounts as a control for the addition of IL-2.

Splenic Production of IL-2. Splenocytes from individual rats were harvested, washed, counted as above, and adjusted to 2×10^5 viable cells/well in 100 μL of complete medium. These cells were incubated with PHA for 48 hrs. Supernatants were carefully removed and frozen at −70°C until the assay was performed. IL-2 levels were measured using commercially available enzyme-linked immunosorbent assay kits (MediCorp, Montréal, Québec, Canada) specific for rat IL-2. The sensitivity of the assay was 10 pg/mL. The within-assay variation was <5% (coefficient of variation)

Statistics. Within each group, data were analyzed using the Kruskal-Wallis test. When the P ratio proved significant ($p < 05$), either the Dunnett's or Dunn's test was used to compare the individual time points (i.e., 12 hrs, 36 hrs, 60 hrs, and 7 days) with baseline values for each of the parameters measured (i.e., levels of granulocytes, leukocytes, and lymphocyte subsets, as well as mitogen-stimulated lymphoproliferative responses and splenic IL-2 production). Dunnett's or Dunn's test was also used to compare intergroup differences for each of those parameters. All data are expressed as mean ± SEM.

RESULTS

Lethality of Peritonitis Model. All control animals (n = 24) survived throughout the 7-day study period. Most deaths resulting from the sequelae of infection (i.e., animals died spontaneously, n = 14; or were humanely euthanized, n = 9) occurred within 24–48 hrs after the onset of bacterial infusion, as reported previously (13, 22). Because the remaining infected animals (n = 33) were killed randomly at different times (i.e., 12 hrs, 36 hrs, 60 hrs, and 7 days) after the onset of bacterial infusion, the overall 7-day mortality rate cannot be calculated from our data. However, we have previously shown that under similar experimental conditions, the dose of *E. coli* used in the present study corresponded to the LD_{50} in our model (13, 22). It is also noteworthy that the infected rats that were randomly killed at 12 hrs and 36 hrs likely included animals that might have survived for 7 days as well as others that might have died of the sequelae of infection; thus, the changes in circulating lymphocyte subpopulations and mitogen-stimulated response of INF described below are likely to represent those of a "mixed" population of infected animals.

Leukocyte and Granulocyte Levels. There was no definite population of monocytes in most of the samples analyzed by flow cytometry; differential cell counts determined by light microscopy also indicated that the number of monocytes was very low (<1%) during the 7-day study period. This finding confirms previous reports that monocytes represent only a small percentage of peripheral blood leukocytes in rodent samples (21). Thus, the absolute number of lymphocytes and polymorphonuclear cells relative to total leukocytes might be only slightly overestimated by our assumption that the changes in the monocyte population were negligible during the 7-day study period.

Changes in leukocyte levels in CON and INF throughout the experiment are presented in Figure 1. INF initially developed a marked leukopenia (−82% at 36

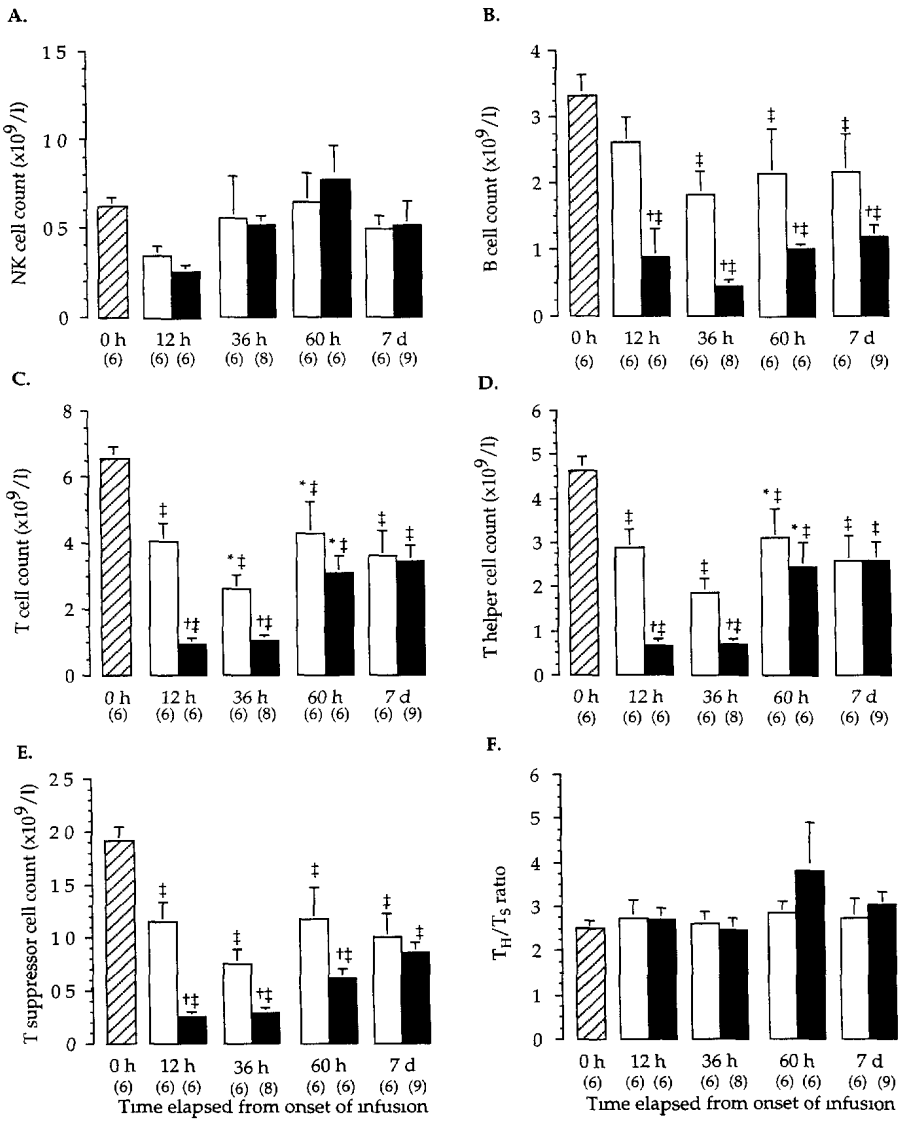

Figure 2. Changes in concentrations of lymphocyte subsets in infected (*filled bars*) and control rats (*unfilled bars*) during the study period *Dashed bars* represent baseline values from healthy, unmanipulated rats The number of experimental animals randomly killed at a given time period is indicated in parentheses. †Significantly different from control rats ($p < .05$); ‡significantly different from healthy rats, *significantly different from previous time period ($p < .05$). NK, natural killer cells, T_S, T-suppressor cells, T_H, T-helper cells

hrs; $p < .05$), with leukocyte counts progressively returning to values comparable to those of HEA at 7 days (Fig. 1*A*). These effects were largely achieved through a pronounced lymphocytopenia (−81% at 36 hrs; $p < .05$) throughout the 7-day period (Fig. 1*B*). Polymorphonuclear cell levels were decreased in INF at 36 hrs ($p < .05$), but a 3-fold increase was observed at 7 days (Fig. 1*C*; $p < .05$). CON showed no changes in the number of polymorphonuclear cells and relatively smaller reductions in the number of lymphocytes for 36 hrs than those observed for INF (Fig. 1). However, CON showed a significant leukocytosis (~35% increase; $p < .05$) at 7 days, largely the result of a four-fold increase in polymorphonuclear cell levels above normal values (Fig. 1*C*).

Changes in Concentrations of Lymphocyte Subsets. Figure 2 summarizes the changes in NK, B-, and T-lymphocyte counts in all experimental animals during the study. The number of NK cells remained unchanged in both INF and CON throughout the experiment (Fig. 2*A*). In contrast, B-lymphocyte levels were significantly depressed (by 47–76%; $p < .05$) during the 7-day study period in INF compared to CON (Fig. 2*B*). Although the number of B cells in CON was comparable to that of HEA at 12 hrs, a significant decrease (38%; $p < .05$) was observed 24 hrs after completion of the laparotomy procedures in CON, a reduction that persisted for the remainder of the study.

T-cell counts were depressed ($p < .05$) in both CON and INF for 7 days compared with HEA (Fig. 2*C*). However, this reduction was three to four times greater ($p < .05$) in INF than CON for 36 hrs. The changes in T_H and T_S lymphocyte levels paralleled those of T-cell counts in both INF and CON (Fig. 2, *D* and *E*). The T_H/T_S ratios were maintained at normal values throughout the experiment (Fig. 2*F*).

Effect of Intra-abdominal Infection on the Mitogen-Stimulated Response. Changes in lymphoproliferative responses of splenocytes in infected rats compared with those of control animals are depicted in Figure 3. LPS- and PHA-induced splenocyte proliferation in INF was inhibited ($p < .05$) by approximately 28% compared with that of CON at 12 hrs (Fig. 3). This inhibition of PHA-induced splenocyte proliferation in INF was maintained at 36 hrs ($p < .05$), but the LPS-stimulated mitogenic response in INF was further reduced ($p < .05$) to 64% of that of CON (Fig. 3). The LPS- and PHA-stimulated mitogenic responses of INF were restored by day 7 to levels comparable to those of CON.

The LPS-induced mitogenic responses of CON and INF at 7 days were comparable to that of HEA (data not shown; 45,040 ± 6,311 DPM vs. 45,366 ± 2,709 DPM). However, the PHA-induced responses of splenocytes from CON and INF at 7 days remained significantly lower ($p < .05$) than those of HEA (Fig. 4). The Con A-induced response in INF (558,059 ± 51,124 DPM) was not affected compared with CON (547,909 ± 60,242 DPM) or HEA (data not shown; 525,513 ± 26,966 DPM) throughout the 7-day study. Interestingly, there was no significant effect of adding IL-2 to the cell culture on the PHA-stimulated mitogenic responses of splenocytes during the study (Fig. 4).

Figure 5 depicts the changes in IL-2 production from PHA-stimulated splenocytes. Although there was no change in IL-2 production in CON (1.3 ± 0.2 ng/mL) compared with HEA (1.4 ± 0.1 ng/mL) throughout the 7-day study, this parameter was persistently reduced by ~35% ($p < .05$) in INF.

DISCUSSION

The focus of the present study was to establish baseline data in a recently developed rat model of Gram-negative intra-abdominal infection, which simulates a trauma-induced, slow bacterial release from the gut (13). The bacterial insult was found to be associated with defective immune cell reactivities, as well as a marked peripheral leukopenia and profound lymphocytopenia, the latter resulting from concomitant reductions in most lymphocyte subsets. It should be stressed that the data presented reflect a natural course of intra-abdominal infection, in the absence of standard supportive therapy normally provided in a clinical setting. Nevertheless, these data are consistent with changes in immune cells often observed in patients with intra-abdominal infections (23–25).

The granulocyte kinetic observed in the infected rats is in agreement with results from a well-established peritonitis model, cecal ligation and puncture (8). Furthermore, *E. coli* endotoxin has been shown to induce an efflux of activated granulocytes from peripheral blood and bone marrow to the lungs and liver in septic rabbits (26). An abnormal granulocyte level in the vascular compartment generally provides a good indication of a major microbial insult to the body, such as in the case of intra-abdominal infection.

The peripheral lymphocytopenia observed in our study can be the result of a variety of factors (e.g., decreased lymphocyte production in one or more organs, perturbed migration to/from one or more organs, or a shortened life span of lymphocytes). Thus, the enumeration of peripheral blood lymphocytes provides a view of only one of many lymphoid compartments involved. Indeed, lymphocyte subpopulation alterations after trauma and burn injuries, with or without infection, have been shown to differ depending on the lymphoid compartment examined (27, 28). Unfortunately, technical difficulties prevented the measurement of splenic lymphocyte subpopulations in the present study. Nevertheless, this report is the first, to our knowledge, to assess the peripheral blood lymphocyte kinetics during experimental intra-abdominal infection.

The mechanisms responsible for the observed alterations in lymphocyte subsets in the septic animals were not investigated in the present study. However, Hawes et al. (29) showed that intravascular infusion of *E. coli* or LPS induces strikingly similar reductions in lymphocyte subset levels, pointing to the important role of the endotoxin component of Gram-negative bacteria in eliciting this response. LPS levels in the different inocula were not measured in the present study. However, we have observed that these levels were several orders of magnitude greater in a bacterial than in a sterile inoculum [unpublished data]; infusion of the latter inoculum resulted in considerably less alteration of the levels of immune cells during the 12-hr infusion period. The contributory role of IL-1 was also suggested in studies showing that the moderate leukopenia observed in *E. coli* bacteremic rabbits was partially abolished by pretreatment with IL-1 receptor antagonist (30). However, this therapeutic agent failed to attenuate this response in septic baboons in which the magnitude of the leukopenia was comparable to that observed in our infected rats (29). We have previously observed that plasma levels of IL-1 were undetectable in our infected rats, but the intraperitoneal IL-1 levels were markedly increased (31). Finally, the lymphocytopenia after major surgery, endotoxin infusion, or *E. coli* infection has been partly explained by increased levels of corticosteroids and noradrenaline that cause a margination of circulating lymphocytes to the liver and spleen (26, 32).

In most humans studies, the overall lymphocytopenia resulting from various infections or following administration of bacterial endotoxin was accompanied by marked reductions in total numbers of B, NK, and T cells in peripheral blood (24, 25, 33). Although such reductions were also observed in our infected animals, T-helper (T_H) and T-suppressor (T_S) levels were similarly altered so that the T_H/T_S ratio was maintained. Several studies have shown that septic patients who had a poor prognosis or died of their septic complications exhibited depressed T_H/T_S ratios (24, 25). This possibility cannot be ruled out, because this parameter was not assessed serially in any of the experimental animals. However, T_H/T_S ratios from three infected rats that were humanely euthanized tended to be lower (1.71 ± 0.13) than those of the infected rats that survived the 7-day study period (3.03 ± 0.31; $p = .07$). Furthermore, the euthanized rats also showed significantly ($p < .05$) lower T-helper ($0.70 \pm 0.17 \times 10^9$/L vs. $2.59 \pm 0.40 \times 10^9$/L) and T-suppressor ($0.41 \pm 0.06 \times 10^9$/L vs. $0.85 \pm 0.11 \times 10^9$/L) levels than those of the 7-day survivors. The latter observation is in agreement with the suggestion that the magnitude of the absolute changes in T-cell subsets in trauma patients is more important than their relative change to identify a patient's susceptibility to immune dysfunction and infection (34).

In the present study, low levels of T-lymphocyte subsets were associated with functional defects in T cells, as suggested by the depressed PHA-induced mitogenic responses and production of IL-2 from splenocytes. However, the greater abso-

This model of intra-abdominal infection is associated with changes in circulating concentrations of immune cells as well as with temporary functional defects in B and T cells, consistent with those often observed in patients with peritonitis.

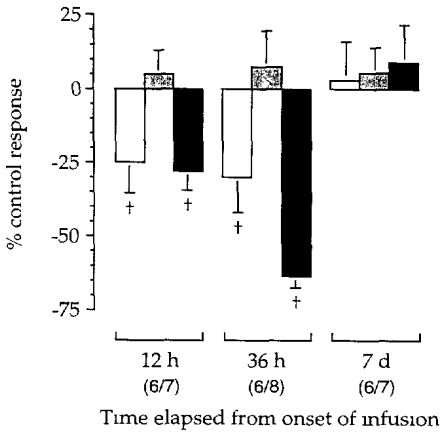

Figure 3. Changes in phytohemagglutinin (*unfilled bars*), concanavalin A (*shaded bars*), and lipopolysaccharide (*filled bars*) responses of splenocytes in infected rats compared with those of control animals at various times after the onset of infusion. The number of experimental animals randomly killed at a given time period is indicated in parentheses †Significantly different from control rats ($p < .05$).

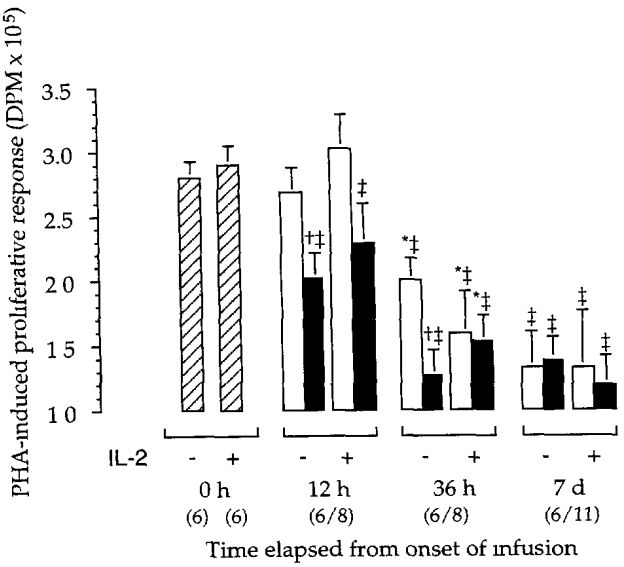

Figure 4. Effect of *in vitro* interleukin-2 (*IL-2*) on phytohemagglutinin (*PHA*) proliferative response after intra-abdominal infection (*filled bars*) or sham-operation (*unfilled bars*) during the study period. *Dashed bars* represent baseline values from healthy, unmanipulated rats. The number of spleens assayed for each group at a given time period is indicated in parentheses (control/infected). †Significantly different from control rats ($p < .05$), ‡significantly different from healthy rats; *significantly different from the previous time period ($p < .05$). *DPM*, disintegrations/min

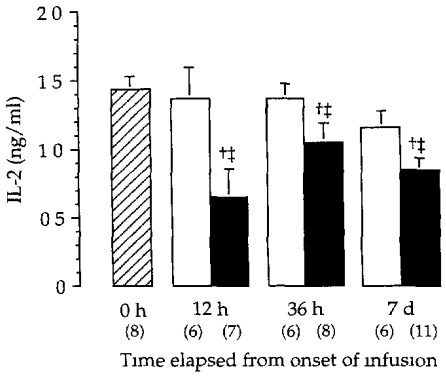

Figure 5. Interleukin-2 (*IL-2*) production by splenocytes from infected (*filled bars*) and control rats (*unfilled bars*) during the study period. *Dashed bar* represents baseline values from healthy, unmanipulated rats The number of experimental animals randomly killed at a given time period is indicated in parentheses. †Significantly different from control rats ($p < .05$); ‡significantly different from healthy rats; *significantly different from previous time period ($p < .05$)

lute mitogenic response of splenocytes after stimulation with Con A than PHA (i.e., two T-cell mitogens), taken together with the apparent lack of T-cell defects of Con A-stimulated splenocytes, would suggest that the nature of immune dysfunction in T cells varies between the different T-cell subsets (i.e., T-helper and T-suppressor cells) in our experimental model of intra-abdominal infection. Our data are in contrast to results from experiments performed in a murine model of cecal ligation and puncture (CLP) (35). Indeed, both PHA and Con A responses were equally suppressed 4 days after CLP. Furthermore, suppressed PHA responses after CLP were restored to normal with *in vitro* IL-2, suggesting that IL-2 may have a role in limiting the adverse effects of sepsis in that murine model of experimental intra-abdominal infection (35). Our results would suggest that a lack of IL-2-responsive cells or an alteration in cell phenotype may have a role in suppressing the immune response after intra-abdominal infection.

Marked functional defects in T cells were observed in our infected rats as early as 12 hrs after the onset of bacterial infusion. In contrast, T-cell responses to PHA were increased in mice 1 day after undergoing CLP (35). Although this discrepancy may be partly related to differences in the severity of sepsis (i.e., 20% vs. 50–55% mortality in our study), it could also be the result of differences in the mode of induction or the nature (i.e., polymicrobial vs. monomicrobial) of the intra-abdominal infection. Our finding of a return of the PHA-stimulated mitogenic response to control values after 7 days suggests that the potential of bacteria to suppress the cell-mediated immunity is short-lived. However, it is noteworthy that our control animals showed significant reductions in lymphocyte numbers and mitogenic responses at 7 days. These data are in agreement with those reporting that midline laparotomy produces significant and persistent (at least 3 days) impairments in both cell-mediated immunity and macrophage functions (36, 37). Although the value of peritoneal lavage in reducing the bacterial load in clinical peritonitis is well established (1), our data support the use of less invasive surgical procedures. To this effect, laparoscopy has been recently shown to induce a lower systemic inflammation than that observed after laparotomy in experimental peritonitis (38).

In the present model, intra-abdominal infection induced not only a marked reduction in circulating B-cell levels, but also significant functional defects in those lymphocytes, as suggested by the marked suppression in the LPS-induced mitogenic response. These data, taken together with the reduced production of IL-2 (a cytokine known to potentiate the antibody-producing response of B cells) from LPS-stimulated splenocytes, suggest that the infection conditions in our model may be associated with an impaired humoral immunity. However, we acknowledge that the responses observed in the blood and spleen may not reflect those of resident cell populations in the peritoneum. To our knowledge, there is little or no information on the humoral immunity in either experimental or clinical intra-abdominal infection. For example, Baker (39) has shown that the antibody-producing response of B cells of trauma and burned patients with severe sepsis is profoundly depressed compared with that of patients who did not develop sepsis. Whether or not the functional defects that we observed in our infected rats represent intrinsic B-cell abnormalities or are the result of an abnormal regulation of T-cell or B-cell function cannot be determined from our data. Alternatively, the decreased responsiveness of B cells to *in vitro* LPS in the infected rats may be partly the result of LPS tolerance, induced by their prior exposure to the *E. coli* endotoxin in the bacterial inoculum.

In summary, our experimental model of intra-abdominal infection is associated with changes in circulating concentrations of immune cells and *in vitro* mitogen-stimulated response, consistent with those often observed in patients. Thus, this model may be a useful tool in fur-

thering our understanding of the pathophysiology of intra-abdominal infections and in assessing the efficacy of new therapeutic modalities.

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REFERENCES

1. Bartlett JG: Intraabdominal sepsis *Med Clin North Am* 1995; 79:599–617
2. Bone RC Gram-negative sepsis: Background, clinical features, and intervention. *Chest* 1991; 100:802–808
3. Suffredini AF: Endotoxin administration to humans A model of inflammatory responses relevant to sepsis, *In:* Mediators of Sepsis. Update in Intensive Care and Emergency Medicine Vol. 16. Lamy M, Thijs LG (Eds). Berlin, Springer-Verlag, 1992, pp 13–30
4. Deitch EA· Animal models of sepsis and septic shock· A review and lessons learned. *Shock* 1998, 9.1–11
5 Wichterman KA, Baue AE, Chaudry IH: Sepsis and septic shock: A review of laboratory models and a proposal. *J Surg Res* 1980; 29:189–201
6. Lang CH, Bagby GJ. Bornside GH, et al. Sustained hypermetabolic sepsis in the rat: Characterization of the model *J Surg Res* 1983; 35:201–210
7. Martinell SFA, Haglund U, Myrvold H: Peritonitis and septic shock: An evaluation of two experimental models in the rat *Eur Surg Res* 1985; 17:160–166
8. Lundblad R, Sandven P, Giercksky KE: The physical nature of a large bowel perforation predicts severity of the subsequent inflammatory response. *Shock* 1995; 3:455–461
9 Almdahl SM, Nordstrand K, Melby K, et al. Faecal peritonitis in the rat: An experimental model for evaluation of surgical and adjuvant therapies. *Acta Chir Scand* 1985; 151: 213–216
10. Bohnen JMA, Matlow AG, Mustard RA, et al: Antibiotic efficacy in intraabdominal sepsis: A clinically relevant model. *Can J Microbiol* 1988; 34:323–326
11. Alexander JW, Gonce SJ. Miskell PW, et al: A new model for studying nutrition in peritonitis. The adverse effect of overfeeding. *Ann Surg* 1989; 209 334–340
12. Alexander HR, Sheppard BC, Jensen JC, et al: Treatment with recombinant human tumor necrosis factor-alpha protects rats against the lethality, hypotension, and hypothermia of gram-negative sepsis. *J Clin Invest* 1991; 88·34–39
13. Martineau L, Shek PN· A sustained release bacterial inoculum infusion model of intraabdominal infection in conscious rats. I. Bacteriology, metabolism, and histopathology. *Shock* 1996; 5:446–454
14. Canadian Council on Animal Care: Laboratory rats. *In* Guide to the Use and Care of Experimental Animals. Vol. 2 Canadian Council on Animal Care (Ed) Ottawa, National Library of Canada, 1984, pp 175–189
15. Woollett GR, Barclay AN, Puklavec M, et al: Molecular and antigenic heterogeneity of the rat leukocyte-common antigen from thymocytes and T and B lymphocytes. *Eur J Immunol* 1985; 15·168–173
16. Dallman MJ, Thomas ML, Green JR: MRC OX-19: A monoclonal antibody that labels rat T lymphocytes and augments in vitro proliferative responses. *Eur J Immunol* 1984; 14· 260–267
17. Jeffries WA, Green JR, Wiliams AF: Authentic T helper CD4 (W3/25) antigen on rat peritoneal macrophages. *J Exp Med* 1985; 162: 117–127
18. Brideau RJ, Carter PB, McMaster WR, et al: Two subsets of rat T lymphocytes defined with monoclonal antibodies. *Eur J Immunol* 1980; 10:609–615
19. Starkey PM, Sargent IL, Redman CWG: Cell populations in human early pregnancy decidua· Characterization and isolation of large granular lymphocytes by flow cytometry. *Immunology* 1988; 65 129–134
20. Hansbrough JF, Gadd MA Temporal analysis of murine lymphocyte subpopulations by monoclonal antibodies and dual-color flow cytometry after burn and nonburn injury. *Surgery* 1989; 106.69–80
21. Hansbrough JF, Soderberg C, Field TO, et al: Analysis of murine lymphocyte subpopulations by dual-color flow cytometry: Technical considerations and specificities of monoclonal antibodies directed against surface markers. *J Surg Res* 1988; 44:121–136
22. Martineau L, Shek PN: Efficacy of liposomal antibiotic therapy in a rat infusion model of *E. coli* peritonitis. *Crit Care Med* 1999, 27. 1153–1158
23. Holzheimer RG, Cavaillon JM, Mosca F, et al: Clinical and basic science aspects of immune pathogenesis and peritonitis. *Med Microbiol Lett* 1996; 5.386–393
24. Dahn MS, Whitcomb MP, Lange MP, et al Altered T-lymphocyte subsets in severe sepsis. *Am Surg* 1988, 54:450–455
25. Williams RC, Koster FT, Kilpatrick KA: Alterations in lymphocyte cell surface markers during various human infections. *Am J Med* 1983; 75 807–816
26. Toft P, Lillevang ST, T¿nnesen E, et al: The redistribution of granulocytes following *E. coli* endotoxin induced sepsis. *Acta Anaesthesiol Scand* 1994; 38 852–857
27. Burleson DG, Mason AD, Pruitt BA Lymphoid subpopulation changes after thermal injury and thermal injury with infection in an experimental model. *Ann Surg* 1988; 207· 208–212
28 Organ BC, Antonacci AC, Chiao J, et al: Changes in lymphocyte number and phenotype in seven lymphoid compartments after thermal injury. *Ann Surg* 1989; 210:78–89
29. Hawes AS, Fischer MA, Van Zee KJ, et al Comparison of peripheral blood leukocyte kinetics after live *Escherichia coli*, endotoxin, or interleukin-1 administration. *Ann Surg* 1993, 218:79–90
30. Wakabayashi G, Gelfand JA, Burke JF, et al. A specific receptor antagonist for interleukin-1 prevents *Escherichia coli*-induced shock in rabbits. *FASEB J* 1991, 5:338–343
31. Martineau L, Shek PN· Peritoneal cytokine levels and survival outcome in an experimental bacterial infusion model of peritonitis. *Crit Care Med* 2000, 28·788–794
32 Toft P, Hokland M, Hansen TG, et al Changes in lymphocyte subpopulations and adhesion/activation molecules following endotoxemia and major surgery. *APMIS* 1995; 103:261–266
33. Hoyt DB, Ozkan AN, Ninnemann JL, et al. Trauma peptide induction of lymphocyte changes predictive of sepsis. *J Surg Res* 1988; 45:342–348
34. Cheadle WG, Pemberton RM, Robinson D, et al· Lymphocyte subset responses to trauma and sepsis. *J Trauma* 1993; 35 844–849
35. Gough DB, Jordan A, Mannick JA, et al: Impaired cell-mediated immunity in experimental abdominal sepsis and the effect of interleukin-2. *Arch Surg* 1992; 127:859–863
36. Redmond HP, Hofmann K, Shou J, et al Effects of laparotomy on systemic macrophage function. *Surgery* 1992, 111:647–655
37 Zellweger R, Ayala A, Zhu X-L, et al: Effect of surgical trauma on splenocyte and peritoneal macrophage immune function. *J Trauma* 1995; 39:645–650
38. Jacobi CA, Ordermann J, Zieren HU, et al: Increased systemic inflammation after laparotomy vs laparoscopy in an animal model of peritonitis. *Arch Surg* 1998, 133 258–262
39 Baker CC: Immune mechanisms and host resistance in the trauma patient *Yale J Biol Med* 1986; 59:387–393

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