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Role of Resident Macrophages, Peripheral Neutrophils, and Translymphatic Absorption in Bacterial Clearance from the Peritoneal Cavity

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Microbial pathogens within the peritoneal cavity are thought to encounter three categories of host defense mechanisms: (i) removal mechanisms, which occur via diaphragmatic lymphatic absorption; (ii) killing mechanisms, in which host phagocytes act as effector cells; and (iii) sequestration mechanisms due to fibrin trapping and the formation of adhesions between visceral surfaces. We sought to define and quantitate the relative role of the first two components in an experimental rat model of *Escherichia coli* peritonitis in which fibrinous adhesions do not form. Intraperitoneal challenge with $\geq 2 \times 10^8$ CFU of viable *E. coli* led to an initial decline in bacterial numbers followed by ongoing proliferation and >50% mortality. With inocula of $\leq 5 \times 10^7$ CFU, elimination of bacteria occurred after moderate initial proliferation, and no mortality ensued. Nonviable, radiolabeled *E. coli* organisms were utilized to examine bacterial clearance via translymphatic absorption and phagocytosis. Both processes were extremely rapid, serving to eliminate free bacteria rapidly within the peritoneal cavity. Although macrophages and polymorphonuclear leukocytes within the peritoneal cavity demonstrated similar phagocytic capacities, the predominance of macrophages at the time of the initial bacterial insult led to the conclusion that these cells, in addition to translymphatic absorption, represent the first line of host defenses, acting to eliminate bacteria in the incipient stages of infection.

Secondary bacterial peritonitis has long been considered a very serious disease and continues to lead to high mortality despite surgical intervention and appropriate antimicrobial therapy (11). Microbial pathogens within the peritoneal cavity are thought to encounter three categories of host defense mechanisms: (i) mechanisms designed to remove the bacterial particles from the peritoneal cavity, (ii) those that kill the bacteria in situ, and (iii) those that sequester the bacteria and prevent their export into the systemic circulation. It has been well established that removal probably operates via the diaphragmatic lymphatic lacunae and efferent diaphragmatic vessels, which lead to mediastinal lymph nodes, the thoracic duct, and eventually to the bloodstream (1, 5, 12, 26, 28). Opsonization and phagocytosis function to kill the bacteria in situ. Sequestration mechanisms are by-products of the inflammatory response. Fibrinogen-rich exudates lead to the direct trapping of bacteria by fibrin (10) as well as the formation of adhesions between the omentum and visceral surfaces, both of which serve to physically isolate invading microorganisms (11). Bacterial elimination from the peritoneal cavity is normally very efficient. If bacterial lethality-enhancing substances such as hemoglobin are not added, no net increase in viable bacteria occurs after intraperitoneal (i.p.) challenge with 10^7 CFU of *Escherichia coli* in rats. In contrast, if hemoglobin is present with a similar challenge 100 to 1,000 times more viable bacteria can be detected in the peritoneal cavity by 6 h (8).

The present study was designed to examine the ability of the rat peritoneal cavity to sterilize itself after a bacterial challenge, and to quantitate the contribution of each host defense component. No bacterial lethality promoting substances were used. Neither fibrinous exudates nor adhesions formed in this model of bacterial contamination, and fluid sequestration was not observed. The respective roles of resident peritoneal macrophages (MCs) and inflammatory polymorphonuclear leukocytes (PMNs) in peritoneal host defense were especially examined because the participation of the unstimulated resident MC population has not been well defined.

MATERIALS AND METHODS

Clearance and growth of viable *E. coli* cells within the peritoneal cavity. A clinical isolate of *E. coli*, which was initially obtained from a human infection at the University of Minnesota Hospitals, was used for all studies. The serotype of this strain is O18ab;K56/K7;−. This strain has been repeatedly used in previous studies in this laboratory (8–10). A loop sample from a stock plate culture was inoculated into 20 ml of brain heart infusion broth (Difco Laboratories, Detroit, Mich.) and incubated in a shaker bath for 18 h at 37°C; the bacteria were then washed three times in saline. Bacterial numbers were first estimated by densitometry. Serial dilutions were then performed, agar pour plates were made, and exact bacterial numbers were determined by enumeration of developed colonies.

The animal model of *E. coli* peritonitis used by this laboratory has been described previously (8, 9, 16). For these experiments, male Sprague-Dawley rats, 200 to 250 g, were injected i.p. with an inoculum of *E. coli* in 1 ml of 0.9% saline. In one group of animals, mortality was examined at 72 h. In a cohort group, animals were sacrificed at specific intervals after bacterial challenge, and 10 ml of saline was injected i.p. This dilution factor was included in all calculations. The animals were then physically shaken for 1 min to

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ensure adequate mixing of peritoneal contents. A midline laparotomy was then performed, and samples of peritoneal fluid were obtained from the total of 10 ml removed from all regions of the peritoneal cavity. Serial dilutions and agar pour plates were then made, and the exact number of viable intraperitoneal bacteria was established by enumeration of developed colonies. Punch biopsy specimens (3-mm diameter) of the rat parietal peritoneum were obtained at 0, 2, 4, 6, and 8 h after bacterial challenge and were washed with saline thrice before digestion with trypsin and enumeration of developed colonies. No significant adherence of *E. coli* ($<10^2$ cells) occurred at any time point from 0 to 8 h after bacterial challenge. Also, immediately after injection of 8×10^8, 2×10^8, or 5×10^7 CFU of *E. coli* i.p., $7.24 \times 10^8 \pm 0.97 \times 10^8$, $1.92 \times 10^8 \pm 0.64 \times 10^8$, or $4.23 \times 10^7 \pm 1.20 \times 10^7$ CFU, respectively, were recoverable from the peritoneal cavity. These control experiments indicated the lack of significant bacterial adherence and adequate sampling of viable bacteria after i.p. inoculation.

Clearance of radiolabeled, nonviable *E. coli* cells from the peritoneal cavity. To study clearance of radiolabeled *E. coli* particulates from the peritoneal cavity, animals were injected i.p. with radiolabeled nonviable *E. coli* cells in 1 ml of 0.9% saline. Immediately after injection, at 0.3 and 0.6 h, and then at hourly intervals up to 8 h after bacterial challenge, animals were sacrificed, and 10 ml of saline was injected i.p. This dilution factor was included in all calculations. The animals were then physically shaken for 1 min to ensure adequate mixing of peritoneal contents, and a midline laparotomy was performed. Four 1-ml samples of peritoneal fluid were obtained from the total of 10 ml removed from all regions of the peritoneal cavity, and in two samples the amount of radioactivity was directly determined. Immediately after injection, >95% of the radiolabel was recoverable in all cases from the peritoneal fluid sample. The remaining two samples were collected in glass tubes containing EDTA at 4°C and were subjected to three cycles of low-speed centrifugation ($160 \times g$, 10 min); each cycle was followed by suspension in 0.9% saline. This procedure is effective in separating leukocytes in the pellet from other contaminants and bacteria within the supernatant (32). The amount of leukocyte-associated radioactivity in each of these samples was then determined. Control experiments were performed in which rat peritoneal MCs or PMNs and nonviable radiolabeled *E. coli* cells were combined in vitro in glass tubes containing EDTA at 4°C and similarly centrifuged; either the pellet was stained with Wright stain and observed microscopically, or the radioactivity was directly measured. These experiments demonstrated no attachment of the bacteria to the cells or phagocytosis. In addition, bacteria alone did not sediment during the low-speed centrifugation steps. Punch biopsy specimens (3-mm diameter) of the rat parietal peritoneum were obtained at 0, 2, 4, 6, and 8 h after bacterial challenge and were washed with saline thrice before gamma counting. No significant adherence of radiolabeled *E. coli* cells ($<10^6$) occurred at any time point from 0 to 8 h after bacterial challenge.

Samples (1 ml) of blood and the liver, spleen, lungs, and kidneys were obtained from each animal. The blood volume was assumed to be 10% of each animal's weight. Radioactivity in blood and organ samples was then directly measured. In a separate group of animals, the main trunk of the left thoracic duct and any right lymphatic ductal structures were isolated by supraclavicular incisions and division of the manubrium sterni and then ligated. A second group of rats underwent a sham procedure. In both groups ether anesthesia was utilized. Animals were allowed to recover 24 h before clearance studies were performed.

Preparation of ^{125}I-labeled, nonviable *E. coli* cells. A loop sample of *E. coli* cells from the stock plate culture was inoculated into 20 ml of diluted Mueller-Hinton broth (Difco). The broth was prepared by adding 25 ml of phosphate-buffered saline to 10 ml of full-strength Mueller-Hinton broth. Glucose (6.50 g) was then dissolved in 65 ml of sterile water, which was then filter sterilized; the partially diluted Mueller-Hinton broth was added to bring the total volume to 100 ml. 5-[^{125}I]iododeoxyuridine (1 mCi/ml; New England Nuclear Corp., Boston, Mass.) was added in a quantity of 0.5 ml per 20 ml of diluted Mueller-Hinton broth. Incubation took place in a shaker bath for 24 h at 37°C. A sample of the bacterial suspension was obtained, serial dilutions were performed, agar pour plates were made, and developed colonies were counted. The remainder of the culture was subjected to centrifugation ($1,600 \times g$, 10 min) and then suspended in a volume of 10% Formalin equal to the volume of the initial culture. Formalin killing of the radiolabeled bacteria was allowed to proceed for 20 min, and then the bacterial suspension was subjected to two further cycles of centrifugation. The first cycle was followed by suspension in 10% Formalin, and the next cycle was followed by suspension in a volume of 0.9% saline equal to the volume of the initial bacterial suspension. The bacterial suspension was then stored at 4°C. Complete killing was established by sterility of pour plates made at the time of use, and culture purity was assessed by examination of streaked plates obtained from samples before killing. The next day, after viable colony counting had established the exact bacterial concentration, the suspension was adjusted to 8×10^8 bacteria per ml. Petroff-Hauser chamber counts and developed colony enumeration provided similar quantitative results.

Serial dilutions of the killed, radiolabeled bacterial suspension were then made, and 1-ml samples were counted in a 200-sample gamma counter (Biogamma II; Beckman Instruments, Inc., Fullerton, Calif.); a standard calibration curve relating gamma counts per minute to the number of radiolabeled bacteria was then established. Stability of this label was assessed by incubation of 2×10^8 ^{125}I-labeled, nonviable *E. coli* cells with 5×10^7 rat peritoneal MCs or peripheral PMNs. Samples were rotated and centrifuged at $1,600 \times g$ for 10 min, and the radioactivity in the supernatant was determined at various time intervals. At 4 h, 3.8 ± 0.05% (MC) and 2.4 ± 0.8% (PMN) release of ^{125}I occurred; by 24 h, only 11.8 ± 1.5% (MC) and 9.4 ± 3.4% (PMN) release took place. These results indicated that label degradation and release after phagocytosis did not significantly affect clearance studies.

Quantitation of host peritoneal leukocyte populations. Animals were injected i.p. with 2×10^8 nonviable *E. coli* cells in 1 ml of 0.9% saline. These bacteria were prepared in a similar fashion to the 5-[^{125}I]iododeoxyuridine–labeled *E. coli* cells with the exception of omission of the 5-[^{125}I]iododeoxyuridine label from the growth medium. Sterility and purity were similarly assessed. Animals were prepared for sample collection as described above. The 10-ml dilution factor was included in all calculations. Four 1-ml samples of peritoneal fluid were obtained in glass tubes containing EDTA at 4°C. Cell counts were directly performed with a cell counter (TOA Medical Electronics Co. Ltd., Kobe, Japan). Portions of the two remaining samples from each animal were centrifuged at $160 \times g$ for 10 min, layered onto glass slides in duplicate, allowed to adhere for

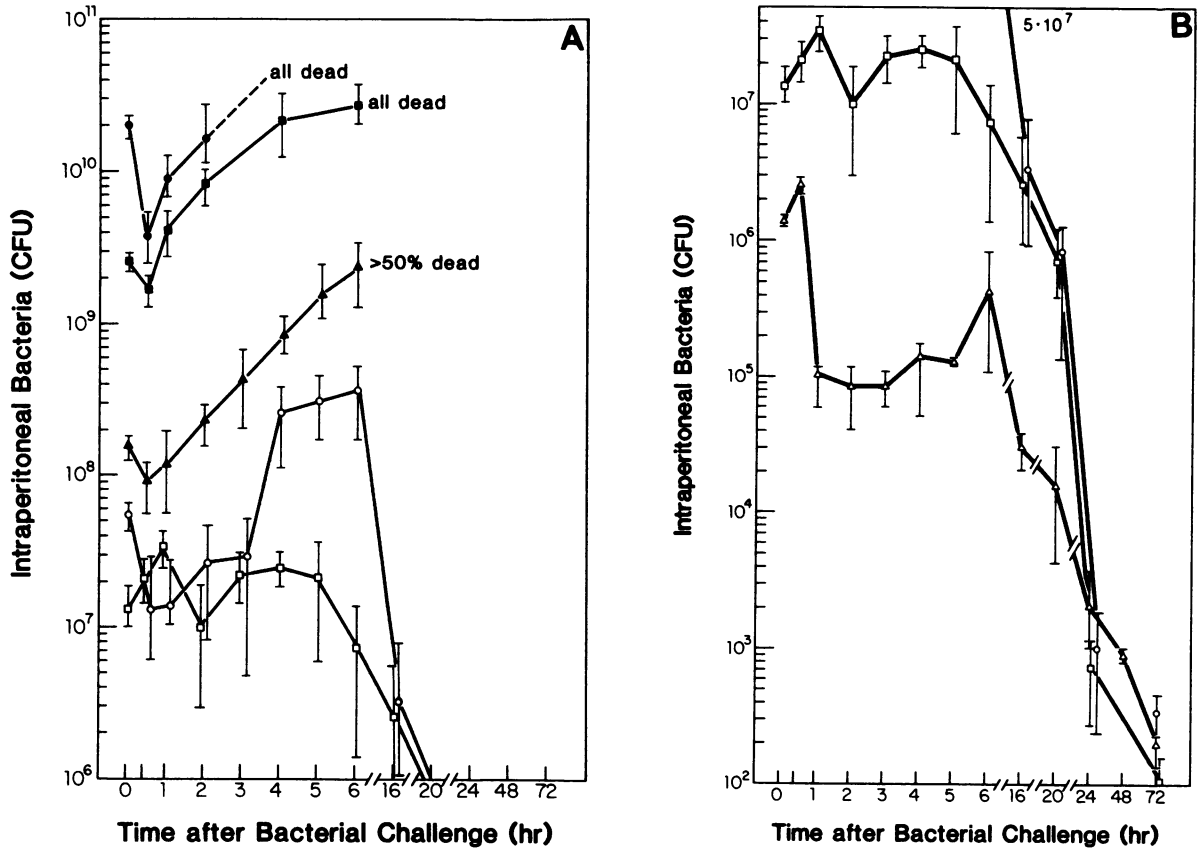

FIG. 1. Kinetics of bacterial proliferation within the rat peritoneal cavity after i.p. inoculation of *E. coli* at initial inoculum sizes $>10^7$ CFU (A) and 10^7 and 10^6 CFU (B). Panel B also shows later time points from animals receiving an inoculum of 5×10^7 CFU of *E. coli*. Each point represents the mean i.p. CFU ± standard deviation from experiments in which 10 animals per point were studied.

5 min, and stained with Wright stain. Differential counts were then performed with light microscopy. One hundred random cells were counted per slide. The mean total number of i.p. leukocytes as well as total MC and PMN subpopulations were calculated and expressed as cells $\times 10^7 \pm$ standard deviation.

Assessment of phagocytosis. Peritoneal fluid was obtained as described above for quantitation of leukocyte populations, layered directly onto glass cover slips, and allowed to adhere for 5 min. A solution of acridine orange dye (0.2 mg/ml; J. T. Baker Chemical Co., Phillipsburg, N.J.) was prepared each day and layered on, and staining was allowed to proceed for 45 s. At the end of the staining period, the dye was gently decanted off the cover slip, which was gently washed once with 0.9% saline and then placed on a glass slide with the stained side down. The number of intracellular bacteria in both MCs and PMNs was then determined by UV fluorescent microscopy. MCs were readily distinguished from PMNs on the basis of nuclear morphology. Intracellular bacteria were also readily distinguished from lysosomal granules by their larger rod-shaped form. A total of 100 random cells per slide were counted, and the average number of intracellular bacteria per either MC or PMN was expressed as the phagocytic index (PI). This value reflected the average phagocytic capacity of each leukocyte cell population. A derived value, termed the phagocytic coefficient (PC), of MCs or PMNs was also calculated with the following formula: PO = PI × total number of cells. This value was used to assess the relative contribution of each leukocyte subpopulation on the basis of both phagocytic capacity as well as overall cell numbers.

Statistics. Student's *t* test and standard tables were used to compare all data points at each time point.

RESULTS

Mortality and clearance of viable *E. coli* cells from the peritoneal cavity. The 50% lethal dose of *E. coli* O18:K56/K7:− at 72 h after i.p. injection in rats was determined by the method of Reed and Muench (23) and was found to be $1.5 \times 10^8 \pm 0.2 \times 10^8$ CFU (30 animals per group). This model of experimental peritonitis does not lead to fibrin deposition or adhesions that are associated with bacterial sequestration. Figure 1 shows the bacterial counts remaining in the peritoneal cavity after inoculation of different quantities of viable *E. coli* cells. Initial inocula of $\geq 2 \times 10^8$ CFU led to only a minimal initial decrease in bacterial numbers, followed by rapid proliferation. After inoculation of 5×10^7 CFU of *E. coli* there was an initial decrease, followed by an increase in bacterial numbers. After 6 h bacterial numbers again declined. After inoculation of 10^7 or 10^6 CFU, bacterial numbers immediately declined and remained low.

Peritoneal clearance, leukocyte association, and blood and organ uptake of nonviable, radiolabeled *E. coli* cells. Clearance and leukocyte association were studied by using initial inocula of either 5×10^7, 2×10^8, or 8×10^8 radiolabeled, nonviable *E. coli* cells. Both host defense components were extremely rapid and efficient, serving to reduce the bacterial inoculum by >1 $\log_{10}$ within 2 h in each case. Figure 2A

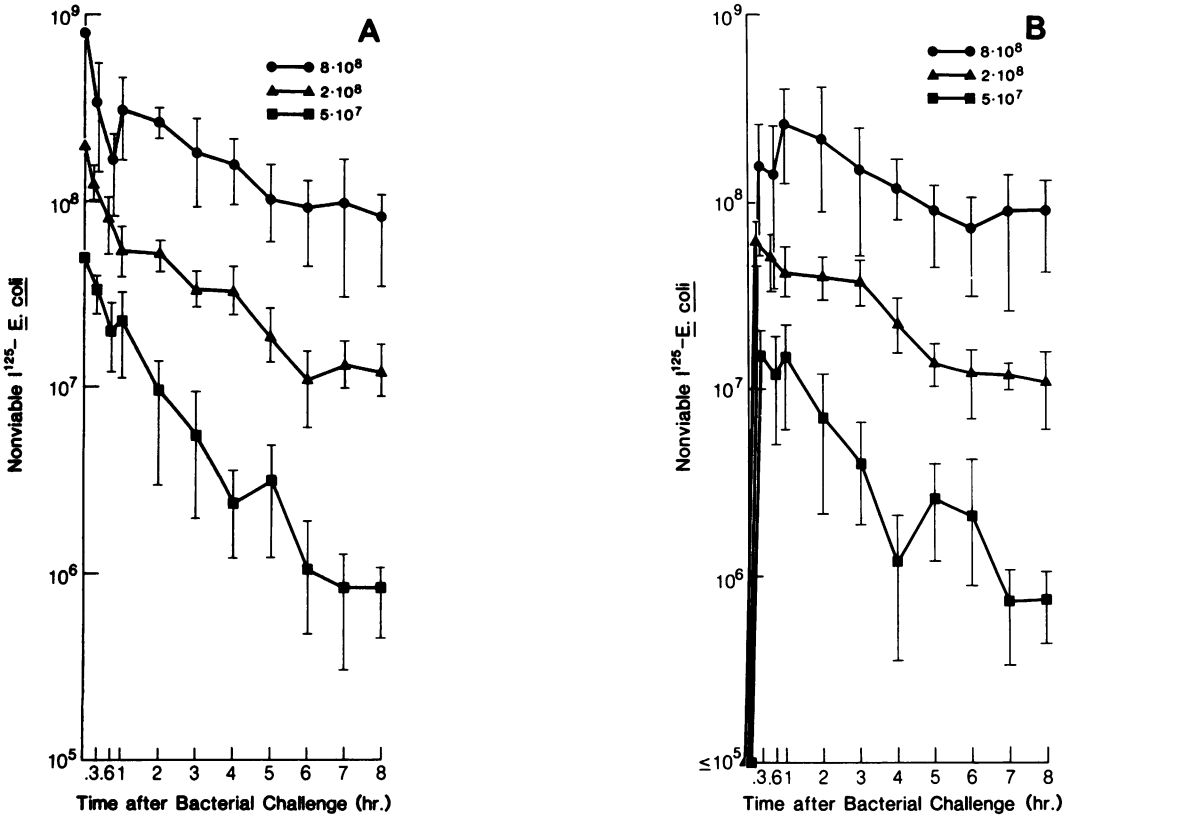

FIG. 2. Kinetics of bacterial removal via absorption or phagocytosis within the rat peritoneal cavity. Animals received an initial challenge of either 5×10^7, 2×10^8, or 8×10^8 nonviable, ^{125}I-labeled *E. coli* cells i.p. (A) Total number of *E. coli* cells within the peritoneal cavity at successive time points after bacterial challenge. This value includes leukocyte-associated radiolabel. (B) Leukocyte uptake of bacterial radiolabel. Each point represents the mean ± standard deviation from experiments in which 10 animals per point were studied.

represents the total number of bacteria remaining within the peritoneal cavity. Figure 2B represents the number of bacteria associated with peritoneal luekocytes. Blood and organ uptake coincided in quantitative fashion with disappearance of radiolabel within the peritoneal cavity (Fig. 3). An initial rapid decline in i.p. radiolabel was noted; this coincided with a peak in leukocyte, blood, and organ label uptake. Decline of leukocyte, blood, organ, and total body radiolabel occurred thereafter (Fig. 2 and 3).

TABLE 1. Role of clearance and phagocytosis in i.p. host defense during rat *E. coli* peritonitis[a]

No. of *E. coli* cells injected[b]	Time (h) after challenge	% Clearance[c]	% Phagocytosis
5×10^7	0.3	31.5	29.9
	1	64.0	29.3
	2	81.2	14.1
	6	97.7	2.3
2×10^8	0.3	38.5	31.7
	1	78.0	21.0
	2	77.3	20.0
	6	94.4	5.6
8×10^8	0.3	56.4	30.0
	1	59.3	33.0
	2	66.2	27.5
	6	88.0	8.9

[a] Mean percent values are shown (standard deviations are shown in Fig. 2); each value represents the percent contribution toward diminution of the total number of ^{125}I-labeled bacteria. Ten animals were used per time point per group.

[b] Nonviable, ^{125}I-labeled cells were injected.

[c] Clearance represents the removal of radiolabed bacteria from the rat peritoneal cavity and may represent translymphatic absorption of both unphagocytized bacteria and phagocytized bacteria within lymphocytes.

Thoracic duct ligation produced a marked diminution in bacterial clearance from the peritoneal cavity. By 2 h after injection of 2×10^8 radiolabeled *E. coli* cells, $1.5 \times 10^8 \pm 0.7 \times 10^8$ nonviable organisms were still present in the peritoneal cavity of thoracic duct-ligated animals, whereas $6.9 \times 10^7 \pm 0.6 \times 10^7$ *E. coli* organisms remained in the peritoneal cavity of sham-operated animals; the latter figure was similar ($P > 0.1$) to values in control animals that had had no operation (Fig. 2A).

In Table 1 the relative contribution to the host defense of each of these components is listed. At each bacterial concentration, leukocyte uptake of bacterial radiolabel was rapid, and clearance continued after maximal phagocytosis had occurred. Similar results for i.p. clearance, leukocyte uptake, and blood and organ uptake were obtained by utilizing either radiolabeled, nonviable *Bacteroides fragilis* (VPI 9032) cells or *E. coli* O111:B4 cells as the challenge organisms (7a; $P > 0.1$ compared with the *E. coli* strain utilized in these studies).

Total peritoneal leukocyte, PMN, and MC numbers in the peritoneal cavity after challenge with viable or nonviable *E. coli* cells. After injection of 2×10^8 ^{125}I-labeled *E. coli* cells, the MC population did not change significantly (Fig. 4). Total peritoneal leukocyte numbers, however, did increase beginning 1 to 2 h after the i.p. introduction of viable or nonviable *E. coli* cells. This increase was almost entirely due to an

increase in PMNs (Fig. 4). No significant differences were noted when either 5×10^7, 2×10^8, or 8×10^8 viable or nonviable *E. coli* cells were utilized (data not shown).

MC and PMN phagocytic indices. Preparations stained with Wright stain and acridine orange provided differential counts at all time points after bacterial challenge which were not statistically dissimilar from one another after challenge with either 5×10^7, 2×10^8, or 8×10^8 viable or nonviable *E. coli* cells ($P > 0.1$; data not shown). Evaluation of the phagocytic index suggested that each cell population possessed similar phagocytic capabilities in this system (Table 2). A more accurate indication of the respective role of each leukocyte subpopulation was obtained by calculating the phagocytic coefficient (Fig. 5). At all early time points after bacterial challenge, over 50% of intracellular bacteria were found within MCs. Intracellular bacteria were no longer visible in rod form after 2 h; they were indistinguishable from the intracellular granules and lysosomes.

DISCUSSION

The means by which the host is able to combat bacterial infection within the peritoneal cavity has been of interest to investigators for over a century (2). From the outset, it was clear that bacterial removal and phagocytosis are important host defenses (1, 2). The relative quantitative role of these various processes, however, has been poorly determined. The goal of these present studies was to more precisely define the various components of peritoneal host defense and to quantitate the role of each component in the host-microbe interaction during experimental peritonitis. Of special interest was the relative role of the neutrophil and resident MCs in bacterial phagocytosis because of the known delay in peritoneal leukocytosis.

Early studies utilizing the i.p. injection of particulate dyes or graphite demonstrated the existence of some form of

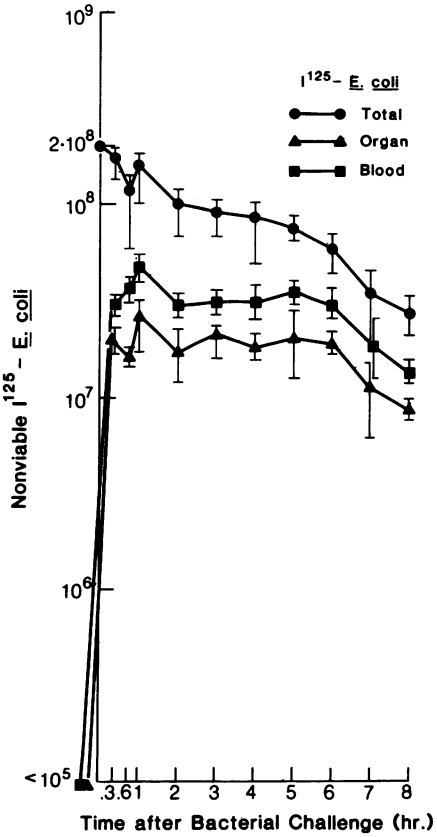

FIG. 3. Kinetics of blood and organ uptake of bacteria cleared from the peritoneal cavity. Animals received 2×10^8 nonviable, ^{125}-labeled E- coli cells i.p. Total body radiolabel, which includes i.p., blood, and organ label, is also shown. Each point represents the mean ± standard deviation from experiments in which six animals per point were studied.

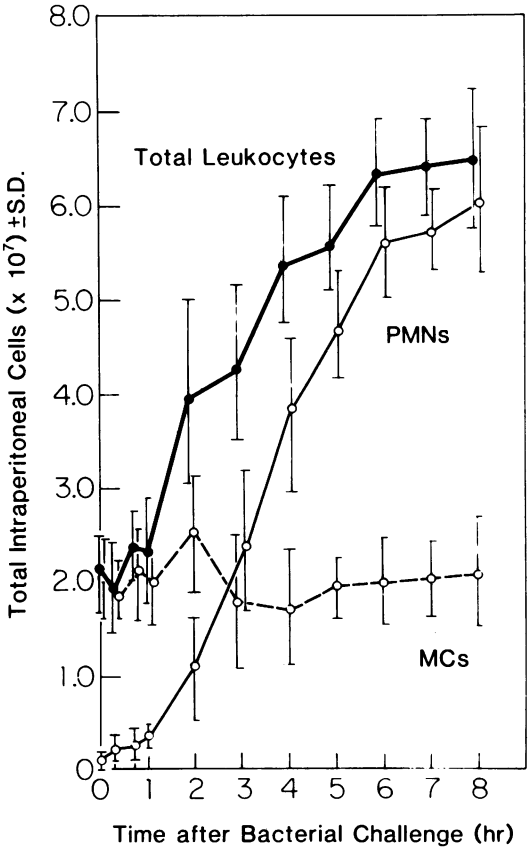

FIG. 4. Alterations in leukocyte populations within the rat peritoneal cavity after i.p. challenge with 2×10^8 nonviable *E. coli* cells. The MC population remained relatively constant in number, whereas the PMN influx became marked after 1 h. Each point represents the mean ± standard deviation from experiments in which 10 animals per point were studied.

TABLE 2. Phagocytic indices of MC and PMN cell populations after inoculation of rats with 2×10^8 nonviable *E. coli* cells i.p.

Time (min) after challenge	Phagocytic index[a]	
	MC	PMN
3	0.02 ± 0.03	0.04 ± 0.03
20	3.58 ± 1.22	2.94 ± 0.77
40	3.29 ± 0.53	2.74 ± 0.78
60	3.81 ± 0.99	2.81 ± 1.06
90	1.63 ± 0.31	1.20 ± 0.23
120	1.81 ± 0.61	0.66 ± 0.24

[a] The phagocytic index is the average number of intracellular bacteria per MC or PMN. The values represent the means ± standard deviation of four separate experiments. $P > 0.1$ at all time points, comparing MC and PMN phagocytic indices.

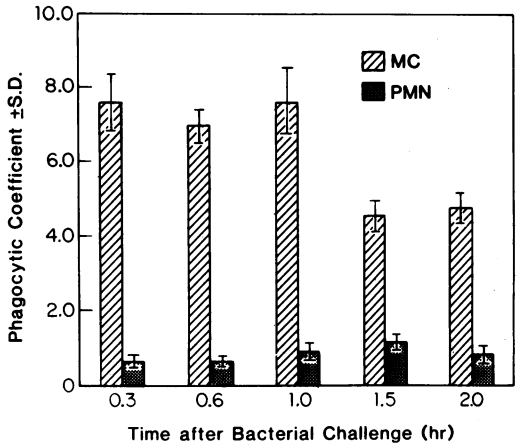

FIG. 5. Relative phagocytic role of peritoneal luekocyte populations during rat peritonitis. The phagocytic coefficient is the phagocytic index multiplied by the total number of MCs or PMNs. MCs accounted for the majority of phagocytosis after i.p. challenge with 2×10^8 nonviable *E. coli* cells ($P < 0.01$ at all time points). Each point represents the mean ± standard deviation from experiments in which eight animals per point were studied.

peritoneal absorptive mechanism. Steinberg demonstrated that bacteria could be observed in the thoracic duct lymph as early as 6 to 10 min after i.p. injection in dogs, whereas the blood remained sterile, indicating the rapidity with which some particles can be absorbed (26). By 30 to 40 min, bacteria were present in both lymph and blood. Courtice et al. (5) examined the peritoneal clearance of rat, rabbit, and human erythrocytes (7- to 8-μm diameter) in the right and left thoracic duct effluents of cats. Most of the erythrocytes emerged into the right lymph duct without change or lysis, beginning 15 to 20 min after their introduction i.p. Higgins et al. (12) demonstrated that i.p. graphite could be traced into the thoracic duct effluent of dogs both free and in phagocytic cells, although it was not clear whether these cells were of peritoneal or thoracic lymph node origin. They also noted that removal of the omentum had no effect on the appearance of graphite in the thoracic lymph duct, whereas phrenic neurectomy, which produced diaphragmatic atrophy, led to increased translymphatic absorption.

The anatomic correlate of this particle removal system has been demonstrated to be the lymphatic system resting exclusively over the diaphragmatic mesothelium. Inspiratory and expiratory diaphragm movements appear to open and close specialized stomata that provide access to lymphatic lacunae from the peritoneal cavity. These lacunae eventually lead to larger intrathoracic lymphatics, which in turn enter the thoracic ducts, which drain into the bloodstream (1, 5, 14, 26, 28). Allen and Weatherford (1) were able to calibrate the size of these structures by use of polystyrene beads. They found in rats that the stomata and lacunae would accept particles as large as 24 μm. Tsilibary and Wissig (28) extensively studied the ultrastructural characteristics of the diaphragmatic lymphatic system by use of scanning electron microscopy and reached similar conclusions regarding their nature.

The present studies indicate that an inoculum of nonviable *E. coli* cells is removed from the peritoneal cavity with great rapidity, and that thoracic duct ligation markedly inhibits this clearance mechanism. These data are consistent with previous studies that demonstrate that particulates leave the peritoneal cavity via lymphatic absorption and demonstrate the efficiency of this host defense system when substances that lead to i.p. sequestration are not present and bacterial proliferation does not occur. The lymphatic system would appear to act as a pump to remove particulates suspended in a fluid medium, to maintain the pristine nature of the peritoneal cavity. In contrast, current evidence suggests that bacterial clearance via the lymphatic system is quantitatively negligible from contaminated soft tissues (7). The peritoneal cavity thus seems to possess a unique defense mechanism.

The rapid removal of bacteria may effectively reduce the inoculum size within the peritoneal cavity, but could conceivably have severe effects on the host due to the septicemia produced. It is not clear from these studies whether a tradeoff occurs in terms of overall host survival by removal of a portion of the bacterial inoculum from the peritoneal cavity, concomitantly exposing the host to the systemic effects of gram-negative bacteremia. Systemic reticuloendothelial clearance of circulating microorganisms also occurs very rapidly, and it may be that the benefits achieved by the initial septicemia with concomitant systemic clearance confer net survival advantage when compared with the alternative, in which bacteria remain and proliferate within the peritoneal cavity.

It must be recognized, however, that the experimental system studied here did not include blood, blood proteins such as fibrinogen, or intestinal contents, all of which are known to foster peritoneal infection. Also, although the use of nonviable radiolabeled bacteria allowed a more precise examination of host peritoneal defenses, this particular aspect of our studies examined a static system in which bacterial proliferation did not occur. The results obtained must therefore be extrapolated to hypothesize what takes place in naturally occurring infections.

Concurrent with the rapid removal of bacterial particulates from the peritoneal cavity, bacteria are also immediately taken up by peritoneal phagocytes. This cannot be quantified when viable bacteria are injected, but with a constant number of nonviable *E. coli* cells, phagocytosis appears to be responsible for removing approximately one-third of the inoculum within minutes (Table 1). Clearance then continues, perhaps removing both free bacteria and bacteria within phagocytes. Our clearance measurements do not, in fact, distinguish between the translymphatic absorption of unphagocytized and phagocytized microorganisms. That peritoneal macrophages can appear within the thoracic duct has been demonstrated by Roser (24), and it is not inconceivable that those that have phagocytized bacteria would act similarly. This mechanism may represent an extremely important aspect of peritoneal host defense, serving to clear both microorganisms and cells that have achieved maximal phagocytic capacity. Radiolabeled bacteria then enter the bloodstream and various organs. The subsequent decline in organ and total body radiolabel may occur on the basis of label destruction at the tissue level.

In the one system, utilizing nonviable *E. coli* cells, the majority of phagocytosis is completed long before the influx of PMNs. This is clearly different from the fate of bacteria in other tissues, in which bacterial elimination seems to await the acute inflammatory response, but true quantitative analysis of bacterial viability in various tissues has not been carried out. Despite these defenses, viable free bacteria go on to proliferate at least briefly in this environment at the inoculum sizes studied. These data confirm the finding of Cohn (3) that murine peritoneal host defenses are strictly limited by the size of the inoculum and the phagocytic response present at any given time. The first line of host

defense appears to be the capacity of the peritoneal MCs and the diaphragmatic lymphatic system to inactivate and eliminate invading microorganisms. The acute inflammatory response with PMN influx is delayed and probably serves to eliminate those bacteria that have eluded the first line of defense.

Previous studies have clearly demonstrated that unstimulated MCs from the peritoneal cavity have substantial phagocytic ability (4, 13, 17, 18, 20, 25, 27). These cells are thought to be derived either from blood monocytes (22) or from distinct bone marrow stem cells (6), although some investigators have considered the possibility of a mesothelial cell origin (33). It is possible, however, that the mesothelial cell is simply a differentiated MC. Division of peritoneal MCs is very slow, and some authors have provided evidence demonstrating that it may occur only once, if at all (29). Under the conditions studied here, both MCs and PMNs within the peritoneal cavity seem to provide similar phagocytic capacity, although the relative ratio of the two phagocytic populations is such that MCs initially account numerically for the larger quantity of bacteria phagocytized. Peterson et al. (21) compared the ability of peripheral blood monocytes and PMNs to phagocytize bacteria. PMNs were more active under direct comparison. Other work by these investigators (30, 31) has demonstrated the phagocytic capacity of both human alveolar and peritoneal MCs to be great. In our system no PMNs were initially present; by the time PMNs did arrive, the bacterial load was much reduced by the MC population.

Substances known to increase the phagocytic capacity of MCs in vitro (zymosan, muramyl dipeptide, glucan) have been shown to increase resistance of animals to i.p. contamination (15, 19, 34). Although it is frequently assumed that a cause-and-effect relationship exists, the actual effect of these agents on peritoneal MC bacterial phagocytosis has not been studied. The present studies provide evidence that the unstimulated peritoneal MC is indeed an important phagocytic effector cell in peritoneal host defense, and we hypothesize that stimulation of this cell population may, indeed, augment host defense against bacterial pathogens. Furthermore, precise quantitation and mathematical modeling of the various components of their host defense system should allow better characterization of the effect of any stimulatory agent on the overall system.

ACKNOWLEDGMENTS

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