Morphine-Induced Degradation of the Host Defense Barrier: Role of Macrophage Injury

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The effect of morphine on the degradation of the host defense barrier in rats and mice was studied. Mice received either 3 or 11 doses of morphine. Mice receiving 11 doses of morphine showed gram-negative bacteremia and bacterial growth in samples of peritoneal fluid (PF), liver, spleen, kidneys, heart, and lungs; PF and tissue samples from only 1 control mouse showed bacterial growth, and no control mice had bacteremia. Mice receiving 11 doses also had suppressed bone marrow macrophage colony formation. Monocytes and peritoneal macrophages harvested from morphine-treated mice showed greater injury than did those from control mice. Pretreatment of mice with naloxone inhibited morphine-induced macrophage injury and degradation of the host defense barrier. In in vitro studies, morphine attenuated the killing of bacteria phagocytosed by macrophages and also facilitated their escape. This study indicates that morphine-induced monocyte and macrophage injury may be linked to degradation of the host defense barrier.

Opiate addiction has been the scourge of society for many years and remains a difficult problem to solve. Heroin addiction is one of the most difficult addictions to treat. Moreover, opiates are increasingly used in medicine to alleviate pain; morphine, an active metabolite of heroin, is an important analgesic for patients with terminal cancer and/or in the postoperative states. The effect of opiates on immune function has been investigated for many years. Although first described by Cantacuzene, as early as 1898, the mechanism and implications have remained the subject of various investigations [1–5]. Morphine reportedly promotes the translocation of intestinal bacteria to the abdominal viscera and peritoneal cavity [6]. However, whether morphine administration can cause bacteremia and systemic dissemination of bacteria through the blood has not yet been reported.

Opiate addicts are prone to various bacterial and viral infections [7]. In animal experimental models, morphine not only induces the degradation of the host defense barrier but also augments the effects of lipopolysaccharide [8, 9]. However, the mechanisms leading to these demonstrable consequences have remained largely speculative [10]. Other reports from our laboratory (Renal Research laboratory, Long Island Jewish Medical Center, New Hyde Park) showed that morphine enhances apoptosis of murine macrophages in a dose-dependent manner [2]. Moreover, human monocytes treated with morphine also show evidence of enhanced apoptosis [2].

We hypothesized that morphine-induced entry of bacteria into the peritoneal cavity and peritoneal macrophage injury are linked. Similarly, emergence of bacteria in the blood and apoptosis and necrosis of circulating monocytes may also be linked. We propose that morphine-induced macrophage injury (apoptosis and necrosis) not only attenuates the killing of phagocytosed bacteria but also allows them to escape from macrophages, with the potential for disseminated infection to result. In the present study, we examined the degradation of the host defense barrier in rats and mice after morphine administration. We also examined the role of morphine-induced macrophage injury on the survival and escape of phagocytosed bacteria.

Materials and Methods

Mice and rats. Female FVB/N mice were housed in groups of 4 in a laminar-flow facility (Small Animal Facility, Long Island Jewish Medical Center). Male rats were housed in groups of 4 in our facility. Rodent diet 5001 (PMI Nutrition International) and fresh water were available ad libitum. The pathogen-free status of each mouse and rat was determined by culture of peritoneal fluid (PF) and blood for any bacterial growth before the start of the experiments.

Colonies and bacterial count. Biological fluids (PF, blood, minced tissues) were diluted in a volume of 5 mL of sterile normal saline, and 1 μL of each type of fluid from each animal was collected with a calibrated disposable inoculating loop (Difco Disposable Inoculating Loops; Becton Dickinson) and inoculated onto blood agar plates. After 24 h, the number of colonies in each plate was counted. Each colony contained 1000 bacteria/mL. Bacterial colonies were identified by a commercial semiautomated system (Vitek 2, bioMérieux).
Experimental protocols. Twenty-two FVB/N mice (weighing 25 g each) were used in 2 sets of experiments (each set included 11 mice in 2 groups: 6 mice received morphine, and 5 mice received normal saline). Mice received 11 doses of either normal saline (control) or normal saline containing morphine sulfate (40 mg/kg of body weight; National Institute on Drug Abuse); doses were administered subcutaneously (sc) under sterile conditions every 8 h. At the end of the scheduled period, samples of blood and PF were obtained under sterile conditions and inoculated by a 1-µL calibrated inoculating loop onto agar plates. Macrophages were isolated from the peritoneal cavities of mice, as described elsewhere [2], and evaluated for the occurrence of apoptosis by staining with Hoechst 33342 (H-33342; Molecular Probes) and propidium iodide (Sigma Chemical) [2, 11].

Liver, spleen, kidneys, heart, and lungs were harvested (after administration of sodium pentothal anesthetic, 40 mg/kg of body weight) under sterile conditions from both sets of control (4 mice from each set) and morphine-treated mice (4 mice from each set) and cultured for bacterial growth. Extreme precautions were observed to avoid any spillover from the peritoneal cavity to the lungs (lungs are separated from abdominal viscera by a sterile barrier).

To study the effect of a shorter regimen of morphine administration, 4 mice received 3 doses of normal saline, and 4 mice received 3 doses of morphine (40 mg/kg of body weight). At the end of the scheduled periods, samples of blood and PF were collected under sterile conditions and seeded onto agar plates. To determine whether morphine has any species-specific effect, we also studied the effect of morphine on the degradation of the host defense barrier in rats. Sprague-Dawley rats (weighing 100 g each) received 3 doses of either normal saline (control group of 3 rats) or normal saline containing morphine sulfate (40 mg/kg of body weight, administered sc under sterile conditions to 3 rats) every 8 h. Subsequently, samples of blood and PF were collected under sterile conditions and seeded onto agar plates. In addition, liver, spleen, kidneys, heart, and lungs were harvested to be tested for bacterial growth.

Evaluation of the role of opiate receptors in morphine-induced degradation of the host defense barrier. To determine the role of opiate receptors in the morphine-induced degradation of the host defense barrier, we studied the effect of naloxone, an opiate receptor antagonist, on rats receiving morphine. Rats (in groups of 4) received 11 doses of normal saline, morphine (40 mg/kg of body weight), or naloxone (Sigma; 4 mg/kg of body weight, administered 30 min before morphine) and morphine (40 mg/kg of body weight), given at 8-h intervals. Mice (n = 4) were treated under identical conditions. On day 4 (after the first dose of morphine), samples of blood and PF were collected and examined for bacterial growth. Peritoneal macrophages were harvested and observed for apoptosis.

Evaluation of the effect of morphine on survival and escape of macrophage-phagocytosed bacteria. To study the effect of morphine on the killing and containment of bacteria by macrophages, we incubated murine macrophages (ATCC J774.16; 10⁶ cells/petri dish) with opsonized clinical isolates of *Escherichia coli* (10⁶ microorganisms/mL) for 1 h at 37°C. At the end of the incubation, cells were repeatedly washed, trypsinized, and washed again to remove any bacteria on the surface of macrophages. Three sets of experiments were done.

Equal numbers of macrophages containing phagocytosed bacteria (MCPB) were reincubated in medium (RPMI 1640 medium, 10% fetal calf serum [FCS]; Gibco) containing either vehicle (control) or morphine (10⁻⁶ and 10⁻⁸ M) for 240 min. At the end of the incubation period, to determine the level of escape of phagocytosed bacteria, samples of supernatants from control and morphine-treated cells were collected at 1, 2, 3, and 4 h; prepared in different dilutions (1:0, 1:10, and 1:100); and inoculated (1 µL) with a calibrated inoculating loop onto agar plates. To determine the survival level among the phagocytosed bacteria, aliquots of trypsinized macrophages were collected at variable periods (1, 2, 3, and 4 h), homogenized, prepared in different dilutions (1:0, 1:10, and 1:100), and inoculated (1 µL) by calibrated inoculating loop onto agar plates. Agar plates were incubated at 37°C and observed for growth of bacterial colonies after a 24-h incubation. Three sets of experiments were done.

Evaluation of the effect of morphine on bone marrow macrophage colony formation. To determine the effect of morphine on bone marrow macrophage colony formation, 3 doses of either normal saline (0.5 mL; control) or normal saline containing morphine sulfate (0.5 mL; 40 mg/kg of body weight administered sc under sterile conditions) were given at 8-h intervals to 5 mice. In another set of experiments, 5 mice received 11 doses of either normal saline (control) or morphine at 8-h intervals. Mice were euthanized at the end of the treatment periods, femurs were removed under sterile conditions, and bone marrow was flushed from the cut ends by injection of Iscove’s modified Dulbecco’s medium (IDDM) with a 30-gauge needle. Nucleated cells (10⁶) were harvested and suspended in 0.3% agarose prepared in IDDM containing 20% FCS and seeded in 100-mm petri dishes with grids. After cells were treated with 2.5 ng/mL of recombinant mouse macrophage colony-stimulating factor (M-CSF; Sigma) for 5 days, colonies were counted.

Apoptosis studies. To determine the occurrence of apoptosis and necrosis in monocytes and macrophages, we used H-33342 and propidium iodide stains. H-33432 stains the nuclei of live cells and identifies apoptosed cells by increased fluorescence; propidium iodide stains only necrosed cells [2]. Equal numbers of monocytes and macrophages were plated in petri dishes in RPMI 1640 medium containing 10% FCS. Once attached, aliquots of methanol containing H-33432 (final concentration, 1 µM/mL) were added and incubated for 10 min at 37°C. Subsequently, cells (without a wash) were placed on ice, and propidium iodide (final concentration, 1 µg/mL) was added to each well. Cells were incubated with the dye for 10 min on ice, protected from light, and examined under UV light by use of a Hoechst filter (Nikon). The percentages of live, apoptosed, and necrosed cells in 8 random fields were recorded by 2 observers who were unaware of the experimental conditions. Because apoptosed monocytes and macrophages are immediately removed from the site of injury, it was difficult to assess the extent of monocyte and macrophage injury from examination of the harvested samples of blood, PF, and bone marrow aspirates. Therefore, equal numbers of cells from the harvested samples were incubated in RPMI 1640 medium containing 20% FCS for 16 h. Subsequently, cells were stained with H-33432...
and propidium iodide, and the extent of macrophage injury was determined.

Evaluation of the effect of bacterial leakage on macrophage injury in nondrugged mice. To determine whether the morphine-induced bacterial leakage in the peritoneal cavity might have led to peritoneal macrophage apoptosis, we evaluated the effect of leakage of bacteria in the peritoneal cavity on macrophage injury in nondrugged mice. Aliquots (0.1 mL) of either normal saline or normal saline containing 10^4 or 10^7 bacteria/mL (E. coli) were administered to mice (in groups of 4). Then, 24 h later, blood monocytes and peritoneal macrophages were harvested from mice and evaluated for injury.

Statistical analysis. For comparison of mean values between groups, we used the unpaired Student’s t test. To compare values between multiple groups, analysis of variance was applied, and a Bonferroni multiple comparison test was used to calculate P values. All data are mean ± SEM, unless otherwise indicated. Statistical significance was defined as P < .05.

Results

Effect of morphine on degradation of the host defense barrier. All mice that received 11 doses of morphine showed gram-negative bacteremia, whereas all control mice had sterile blood cultures. All mice receiving 11 doses of morphine also showed bacterial growth in PF and in samples from liver, spleen, kidneys, heart, and lungs. Samples of lung, spleen, and liver tissue from only 1 control mouse tested positive for bacteria. Figure 1 shows the bacteria counts grown from each sample. Identification of bacteria from 2 sets of mice treated with morphine revealed gram-negative bacteria (E. coli, Proteus mirabilis, and Serratia species; table 1); samples from the single control mouse with positive results showed gram-positive bacteria (Staphylococcus epidermidis). Interestingly, mice that received 3 doses of morphine showed neither bacteremia nor bacterial entry into the peritoneal cavity.

Table 1. Identification of bacterial species from various sources in mice and rats.

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Mice (n = 8)</th>
<th>Rats (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PF</td>
<td>Blood</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Serratia species</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

NOTE. Data are no. of mice or rats. Mice (2 sets, 4 mice in each set) and rats (1 set, 4 rats) received 11 doses of morphine, administered at 8-h intervals. Subsequently, peritoneal fluid (PF), blood, and tissues (liver, spleen, kidney, heart, and lung) were harvested and inoculated (1 μL, using a calibrated inoculating loop: Becton Dickinson) onto agar plates. After 24 h, bacterial count and identification of bacterial species were carried out.
Samples from all rats that received 11 doses of morphine grew gram-negative bacteria on culture; samples from the single control rat with positive results grew a few colonies of gram-positive cocci. All rats that received 11 doses of morphine showed bacterial growth in samples of blood, PF, liver, spleen, kidneys, heart, and lungs (table 1). Figure 2 shows the number of bacteria grown from each sample; gram-positive colonies were found in samples of spleen, liver, and lung from only 1 control rat.

**Effect of naloxone on degradation of the host defense barrier.** Only 1 of 4 rats treated with naloxone showed the emergence of bacteria both in blood and in the peritoneal cavity after morphine administration, and no naloxone-treated mice showed a breach in the host defense barrier in response to morphine treatment. All rats and mice treated with 11 doses of morphine alone showed bacteremia and bacterial growth in PF samples. No control rats or mice showed bacteremia or entry of bacteria into the peritoneal cavity.

**Effect of morphine on colony formation by bone marrow macrophages.** Five mice received either 3 or 11 doses of normal saline (5 mL) or normal saline containing morphine sulfate, administered at 8-h intervals. Subsequently, bone marrow macrophages were harvested and treated with recombinant M-CSF for 5 days before colonies were counted. Bone marrow macrophages isolated from both control mice and mice treated with 3 doses of morphine responded to M-CSF (19.7 ± 1.1 vs. 26.8 ± 0.4 colonies, respectively). However, bone marrow macrophages isolated from mice treated with 11 doses of morphine showed an attenuated response to M-CSF, compared with that of control colonies (9.5 ± 0.5 vs. 29.7 ± 1.1 colonies, respectively; \( P < .001 \), vs. mice treated with 3 doses). These results indicate that long-term administration of morphine in mice may compromise the growth of bone marrow macrophages.

**Apoptosis studies.** Morphine-treated mice showed greater injury of blood monocytes than did control mice (\( P < .001 \); apoptosis in control mice, 3.1% ± 0.5% apotosed cells/field, vs. morphine-treated mice, 17.9% ± 0.8% apotosed cells/field; necrosis in control mice, 0.3% ± 0.3% necrosed cells/field, vs. morphine-treated mice, 8.9% ± 0.5% necrosed cells/field). Mice that received 3 doses and mice that received 11 doses of morphine showed greater injury of peritoneal macrophages than did control mice (table 2).

Monocytes isolated from rats that received 11 doses of morphine showed greater injury than those from control rats (\( P < .001 \); apoptosis in control rats, 2.2% ± 0.7% apotosed cells/field, vs. morphine-treated rats, 29.4% ± 2.1% apotosed cells/field; necrosis in control rats, 1.3% ± 0.5% necrosed cells/field, vs. morphine-treated rats, 29.8% ± 2.0% necrosed cells/field). Peritoneal macrophages isolated from rats receiving 11 doses of morphine also showed greater macrophage injury than those from untreated rats (\( P < .001 \); apoptosis in control rats, 4.3% ± 0.6% apotosed cells/field, vs. morphine-treated rats, 22.1% ± 1.7% apotosed cells/field; necrosis in control rats, 2.5% ± 1.0% necrosed cells/field, vs. morphine-treated rats, 6.5% ± 1.9% necrosed cells/field).

Naloxone treatment of rats attenuated the effect of morphine on peritoneal macrophage injury (\( P < .001 \); control rats, 4.3% ± 0.6% peritoneal macrophages/field; morphine-treated rats, 22.1% ± 1.7% peritoneal macrophages/field; rats treated with naloxone and morphine, 2.5% ± 1.5% peritoneal macrophages/field). As shown in table 2, treatment of mice with nalox-
one also attenuated morphine-induced peritoneal macrophage injury. To determine the effect of bacterial leakage in the peritoneal cavity in nondrugged mice, aliquots (0.1 mL) of either normal saline or normal saline containing $10^7$ bacteria/mL (E. coli) were administered to mice (in groups of 4). Twenty-four hours later, peritoneal macrophages were harvested and studied for macrophage injury. Administration of bacteria in the peritoneal cavity in smaller concentrations triggered neither blood monocyte injury nor peritoneal macrophage injury (table 3). However, when large numbers of bacteria were administered in the peritoneal cavity in smaller concentrations triggered neither blood monocyte injury nor peritoneal macrophage injury (table 3). However, when large numbers of bacteria were administered in the peritoneal cavity, the mice showed both blood monocyte and peritoneal macrophage injury (table 3).

To determine the effect of morphine on bone marrow macrophage injury, we examined the occurrence of apoptosis of bone marrow macrophages in control mice and mice treated with morphine (3 and 11 doses). Mice receiving 11 doses of morphine showed greater injury of bone marrow macrophages than did control mice and mice receiving 3 doses of morphine ($P < .001$; table 2).

### Table 2. Effect of morphine on macrophage injury.

<table>
<thead>
<tr>
<th>Macrophage source</th>
<th>Control</th>
<th>Morphine, 3 doses</th>
<th>Morphine, 11 doses</th>
<th>Naloxone and morphine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Apoptosis</td>
<td>Necrosis</td>
<td>Apoptosis</td>
<td>Necrosis</td>
</tr>
<tr>
<td>Peritoneal</td>
<td>2.3 ± 0.6</td>
<td>2.0 ± 0.4</td>
<td>6.2 ± 0.4</td>
<td>3.2 ± 0.5</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>2.2 ± 0.4</td>
<td>1.7 ± 0.3</td>
<td>4.3 ± 0.3</td>
<td>2.8 ± 0.3</td>
</tr>
</tbody>
</table>

NOTE. Data are mean percentage ± SEM of total macrophages isolated. Mice (in groups of 4) received 11 doses of normal saline, morphine, or naloxone and morphine, administered at 8-h intervals. Subsequently, peritoneal macrophages were harvested and stained with Hoechst 33342 (H-33342) and propidium iodide. Five mice received three 0.5-mL doses of either normal saline (control) or normal saline containing morphine sulfate, administered at 8-h intervals. Another 5 mice received 11 doses of normal saline (control) or normal saline containing morphine sulfate, administered at 8-h intervals. Subsequently, bone marrow macrophages were harvested and stained with H-33342 and propidium iodide. To compare values between multiple groups, analysis of variance was applied, and a Bonferroni multiple comparison test was used to calculate $P$ values. ND, not done.

* $P < .01$, vs. respective control mice (apoptosis).
* $P < .001$, vs. respective control mice and mice receiving morphine (apoptosis).
* $P < .05$, vs. respective mice receiving morphine (necrosis).
* $P < .001$, vs. respective control mice and mice receiving naloxone and morphine (apoptosis).
* $P < .05$, vs. respective control mice.
* $P < .001$, vs. respective control mice and mice receiving morphine, 3 doses.
* $P < .05$, vs. respective control mice.

### Table 3. Effect of bacterial peritoneal leak on macrophage injury.

<table>
<thead>
<tr>
<th>Peritoneal macrophages</th>
<th>Blood monocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leak size</td>
<td>Peritoneal</td>
</tr>
<tr>
<td></td>
<td>Apoptosis</td>
</tr>
<tr>
<td>Small</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>Large</td>
<td>1.5 ± 0.2</td>
</tr>
</tbody>
</table>

NOTE. Data are mean percentage ± SEM of total macrophages isolated. Aliquots (0.1 mL) of either normal saline or normal saline containing a small number of Escherichia coli ($10^7$ cells/mL) were administered intraperitoneally (ip) to mice in groups of 4 (small-leak group). In parallel experiments, aliquots (0.1 mL) of either normal saline or normal saline containing $10^7$ cells/mL (E. coli) were administered ip to mice in groups of 4 (large-leak group). Peritoneal macrophages were harvested 24 h later and studied for macrophage injury (apoptosis and necrosis). To compare values between multiple groups, analysis of variance was applied, and a Bonferroni multiple comparison test was used to calculate $P$ values. * $P < .001$, vs. respective control mice.

**Effect of morphine on survival of phagocytosed bacteria.** Morphine-treated MCBP were assessed for survival at 1, 2, 3, and 4 h (figure 3) and showed a higher number of surviving bacteria than seen with control MCBP. These results indicate that morphine-treated MCBP had decreased killing power, which allowed bacteria to survive.

**Effect of morphine on the escape of phagocytosed bacteria.** Morphine-treated MCBP, not control MCBP, showed escape of bacteria. Bacterial counts in supernatants of morphine-treated and control MCBP are shown in figure 4. These results suggest that morphine not only affected the killing of bacteria by macrophages but also, by compromising the integrity of the macrophage cell wall, allowed bacteria to escape.

To determine whether morphine may have directly modulated the replication of bacteria, an aliquot of E. coli ($2 \times 10^8$ bacterial/mL) was added to 5 mL of Lennom Luria broth base containing either vehicle (control) or variable concentrations of morphine ($10^{-10}$ to $10^{-4}$ M) and incubated for 24 h. Subsequently, bacteria were counted by measuring absorbance at 540 nm by spectrophotometer (Spectronic 20; Spectronic Analytical
In the present study, mice that received 3 doses of morphine showed no peritoneal leak but did sustain macrophage injury. It appears that either macrophage injury preceded peritoneal leak or a small number of leaked bacteria had already been phagocytosed by peritoneal macrophages and were not available in the PF. It is also plausible that, in mice that received 3 doses of morphine, macrophage injury may have been the result of a morphine-induced small peritoneal leak, rather than the direct effect of morphine. However, in the present study, we did not observe macrophage injury after introduction of a small number of bacteria into the peritoneal cavity. Although a direct link between macrophage injury and bacterial escape cannot be drawn from the present study, the data favor a causal relationship.

Hilburger et al. [6] showed that entry of bacteria into the peritoneal cavity is associated with colonization of bacteria in the abdominal viscera in morphine-treated mice. However, it is possible that bacterial dissemination into the abdominal viscera may have occurred through the peritoneal cavity. Because these investigators did not study the spread of bacteria into the blood or into organs outside the peritoneal cavity, it is difficult to infer the role played by blood in the systemic dissemination of bacteria after morphine administration. Nevertheless, morphine directly affects intestinal motility and permeability, and we cannot rule out the possibility that morphine treatment results in a direct leak of bacteria from the intestines into the peritoneal cavity and then to the abdominal viscera. In the present study, bacteremia after morphine administration in rats and mice was also associated with dissemination of bacteria into the organs both inside (i.e., liver, kidney, and spleen) and outside (i.e., lungs and heart) the abdominal cavity. Spread of bacteria outside the abdominal cavity suggests that the morphine-induced dissemination of bacteria may also occur via the blood.

Because mice receiving only 3 doses of morphine did not develop either bacteremia or visceral bacterial colonization, it
appears that a sustained opiate milieu is needed before a breach will develop in the host defense barrier. To clarify this issue, we investigated whether the suppression of macrophage growth in the reservoir (bone marrow) plays a role in the morphine-induced degradation of the host defense barrier. Interestingly, morphine-treated mice showed significant suppression of macrophage colony formation in response to M-CSF. These findings are consistent with the earlier findings of Roy et al. [12]. We propose that administration of a few doses of morphine has the potential to induce minimal macrophage injury but that a new crop of macrophages from the bone marrow rapidly replaces the injured macrophages. On the other hand, during prolonged morphine treatment, bone marrow macrophages are significantly injured, and their growth is suppressed; this may not only compromise the replacement of injured macrophages in the reservoir but also deplete the pool of macrophages available for migration to the site of bacterial entry. However, morphine-induced bacteremia may also have impaired the response of macrophages to M-CSF. These studies require further, future work.

Morphine modulates immune function by multiple means [12–21] and inhibits phagocytosis, chemotaxis, and antibody and cytokine production by macrophages. In addition, it may modulate macrophage function by altering the function of T cells [11]. These effects of morphine, individually and in combination, may compromise the role of macrophages in the host defense system. Because injured macrophages are functionally compromised, many of the reported effects of morphine may also contribute indirectly to morphine-induced macrophage apoptosis and necrosis.

The gastrointestinal tract is a staging area for bacteria. Usually, submucosal macrophages phagocytose intestinal bacteria and deliver them to mesenteric lymph nodes [22–24]. During the transit period, macrophages kill the phagocytosed bacteria and thus deliver dead bacteria instead of live bacteria to the mesenteric lymph nodes [22, 23]. The present in vitro study suggests that, when macrophages are under the influence of morphine, not only is the survival of phagocytosed bacteria allowed but their escape from macrophages is facilitated. Hypothetically, in patients who are receiving opiates, submucosal macrophages may deliver live bacteria to mesenteric lymph nodes. Bacteria that are able to invade through enterocytes are usually phagocytosed by peritoneal macrophages [24]; morphine may compromise the replacement of injured peritoneal macrophages not only through the suppression of growth of bone marrow macrophages but also by decreasing the chemoattraction of such macrophages [25].

The degradation of the host defense barrier does not seem to be species specific. Morphine-induced bacteremia and visceral colonization occurred in rats and mice. Similarly, we
found that morphine promoted the apoptosis and necrosis of peritoneal macrophages in rats and mice. These findings further support our notion that morphine-induced macrophage apoptosis and degradation of the host defense barrier may be linked.

As shown in our study, a small leak of bacteria into the peritoneal cavity is unlikely to trigger peritoneal macrophage or blood monocyte injury. However, a larger dose of bacteria induced macrophage injury. Because morphine can trigger macrophage injury, a small leak of bacteria in patients receiving opiates may also contribute to ongoing injury. Is it possible that the morphine-induced bacterial leak may have triggered both monocyte and macrophage injury? We consider this unlikely, because 3 doses of morphine treatment could produce both blood monocyte and peritoneal macrophage injury without any evidence of a bacterial leak. Further studies are needed to clarify this issue.

We conclude that morphine induces both bacteremia and monocyte injury in mice. Morphine administration in mice not only induced peritoneal macrophage injury but also allowed entry of bacteria into the peritoneal cavity. Moreover, naloxone, an opiate receptor antagonist, not only inhibited peritoneal macrophage injury but also prevented the entry of bacteria into the peritoneal cavity after morphine administration. Because morphine promotes the survival and the escape of phagocytosed bacteria, it appears that degradation of the host defense barrier and monocyte injury may be linked.

References