Morphine Increases Susceptibility to Oral Salmonella typhimurium Infection

Amanda Shearer MacFarlane,1 Xiaohui Peng,1 Joseph J. Meissler, Jr.,1 Thomas J. Rogers,1 Ellen B. Geller,2 Martin W. Adler,2,3 and Toby K. Eisenstein1,3

This study examined the effect of morphine on oral infection with virulent Salmonella typhimurium. Animals were treated with a 75-mg slow-release morphine pellet followed by inoculation with salmonellae. Morphine markedly sensitized mice to oral infection, as assessed by survival, mean survival time, and colony culture. By 24 h after Salmonella inoculation, morphine-treated mice had a 105-fold difference in number of organisms in the Peyer’s patches, compared with controls. The opioid antagonist naltrexone significantly blocked Salmonella colonization in Peyer’s patches and reduced Salmonella burden in other organs, indicating that morphine acts at least in part via an opioid receptor–mediated pathway. The data show that morphine markedly potentiates Salmonella infection at the gastrointestinal portal of entry and enhances subsequent dissemination of Salmonella organisms. The results have implications for potentiating gastrointestinal opportunistic infections in intravenous drug abusers and in opioid-mediated postsurgical patients.

Since the 1930s opioid abusers have been known to have increased incidence of infectious diseases [1, 2]. More recent reports continue to substantiate an increased risk of infections in intravenous drug users (IDUs) who abuse heroin [3–5]. Right-side endocarditis caused by Serratia marcescens [6] and enterococci [7], which is relatively rare in the general population, is among the more frequently documented infections in IDUs. There is also a significant intersection of IDUs and human immunodeficiency virus (HIV) infection. Intravenous drug use is the second most frequently reported risk behavior for HIV infection [8]. It is well documented that HIV-infected persons have a greater risk of Salmonella infections than the general population [9–15]. However, despite the high incidence of IDUs among HIV-positive populations, there have been minimal attempts to determine if the opioid drugs, per se, are cofactors in Salmonella infection. One study documented that persons with AIDS who were IDUs had increased incidence of recurrent nontyphoidal Salmonella septicemia [11].

Previously, infections observed in IDUs were solely attributed to contaminated drugs, nonsterile needles, poor hygiene, promiscuity, or malnutrition. More recent literature shows that opioids are immunosuppressive, and impaired immunity has been proposed to be an additional cause of increased infections in IDUs [16–18]. Studies on drug abusers are confounded by differences in drug dosage, intervals between drug use, duration of drug use, and use of multiple drugs, making it difficult to correlate use of a particular drug with increased infection. Animal models permit isolation of the variables needed to examine these questions.

Our laboratory has reported that implantation of a 75-mg slow-release morphine pellet in mice results in sepsis due to the escape of normal enteric bacteria into the systemic compartment [19]. This suggests that morphine alters the capacity of the gastrointestinal (GI) tract to contain microbes. We also found that after oral inoculation with attenuated salmonellae, morphine-treated mice had increased salmonellae in their mesenteric lymph nodes (MLNs; M. E. Hilburger, T. K. Eisenstein, et al., unpublished data). As the major metabolite of heroin is morphine, these observations are relevant for use of opioids as analgesics and for consequences of heroin abuse. The present study was designed to examine whether morphine administration can sensitize to infection with an oral pathogen.

Materials and Methods

Animals. Pathogen-free, female, 6-week-old C3HeB/FeJ mice were purchased from Jackson Laboratories (Bar Harbor, ME). Mice were allowed to acclimate for 1 week before use. Rodent chow (Purina, St. Louis) and fresh water were available ad libitum. Bacteria. Salmonella typhimurium W118-2 was originally isolated from a patient with a fulminant case of human gastroenteritis (courtesy of S. Formal, Walter Reed Army Institute for Research, Washington, DC). The oral LD50 under 4-h fasting conditions (see

Received 18 October 1999; revised 5 January 2000; electronically published 13 April 2000.


Animal studies were approved and carried out under protocols of the Temple University Institutional Animal Care and Use Committee.

Grant support: NIH (DA-11134 and DA-06650).

Reprints or correspondence: Dr. Toby K. Eisenstein, Dept. of Microbiology and Immunology, Temple University School of Medicine, Philadelphia, Pennsylvania.

The Journal of Infectious Diseases 2000;181:1350–8
© 2000 by the Infectious Diseases Society of America. All rights reserved.
0022-1899/200018110-0015$02.00

1350
overnight, and Salmonella blue agar plates (Difco, Detroit). Plates were incubated at 37°C for 48 h before inoculation. The mice were anesthetized with methoxyflurane (Metofane, Pitman-Moore, Mundelein, IL) and orally inoculated with a blunt-end feeding needle with W118-2.2. After inoculation, slow-release pellets were subcutaneously implanted dorsally [23]. Animals received either a 75-mg morphine pellet or multiple 8-mg morphine pellets. Controls received either a 30-mg naltrexone pellet, a placebo pellet, or a morphine plus a naltrexone pellet. Pellets were obtained from the National Institute on Drug Abuse (Rockville, MD).

In some experiments, mice were implanted with the pellets 24 h or 48 h before or after Salmonella inoculation. Mice were anesthetized for all oral Salmonella inoculations. Morphine and naltrexone slow-release pellets are preferred to experimentally deliver these drugs, because the continuous release of the opiate prevents cycles of withdrawal [24, 25]. Serum levels of morphine are initially ~2 µg/mL, which stabilizes at 0.6 µg/mL 48 h after implantation [26]. These levels are in the range of levels reached in patients given morphine for analgesia or anesthesia and are easily in the range achieved by IDUs.

Determination of bacteria burden. On the designated day after infection, 5 animals from each group were killed by breaking their necks. The Peyer’s patches (PPs), MLNs, spleen, and/or liver of each animal were aseptically removed. Three PPs from 1 animal were prepared with a Tekmar tissueizer, model SDT (Tekmar, Cincinnati). PPs, MLNs, and spleens were each suspended in 3 mL of sterile water. Liver samples were suspended in 5 mL of sterile water. A 0.1-mL sample of homogenate or of an appropriate dilution was plated on Levine’s eosin-methylene blue agar plates (Difco, Detroit). Plates were incubated at 37°C overnight, and Salmonella colonies were counted. Select individual colonies were taken to the Clinical Microbiology Laboratory at Temple University Hospital to verify their identity as salmonellae by use of a semiautomated microbial identification system (Bio-merieux, Hazelwood, MO). Data are expressed as colony-forming units per organ. In studies that used pW118-2 containing the pHSG422 plasmid, the homogenates were plated on both TSA and TSA-kanamycin. Plates were incubated overnight at 30°C. Salmonellae recovered from PPs that contained the plasmid grew on TSA-kanamycin plates. Their number indicates persistence of organisms from the original inoculum. Both salmonellae containing the plasmid and daughter cells that had lost the plasmid grew on TSA agar plates. The difference in colony counts from the PPs on the 2 types of media was used to determine the degree of Salmonella replication in vivo. pW118-2 does not spontaneously lose the plasmid over 96 h [22].

Survival. Groups of mice given morphine, naltrexone, or placebo and orally inoculated with salmonellae were observed daily, and mortality was recorded for 40 days. Percent survival and mean survival time (MST) were calculated.

Determination of mRNA levels of cytokines by reverse-transcriptase polymerase chain reaction (RT-PCR). Groups of 5 mice were implanted with a morphine pellet, a naltrexone pellet, a placebo pellet, or a morphine plus a naltrexone pellet. Within each group, 5 animals were orally inoculated with salmonellae, and 5 were not inoculated. Control groups that received no pellets and were salmonella infected or uninfected were also included. Mice were killed 48 h after implantation, and 5 PPs from each of 5 mice were pooled together. RNA was extracted using RNeasy B according to the manufacturer’s instructions. First-strand cDNA was synthesized from total RNA by use of Superscript II RT (Gibco BRL, Grand Island, NY) at 42°C for 50 min in a reaction containing 2.5 µg of total RNA, 2 µL of random hexamer primers (Promega, Madison, WI), 0.5 µL of RNaase inhibitor, 4 µL of 5× first-strand buffer, 1 µL of dNTP, 2 µL of 0.1 M DTT, and 1 µL of Superscript II RT. Sample cDNAs were first assayed for levels of the constitutively expressed gene hypoxanthine-guanine phosphoribosyl transferase (HPRT). Input cDNA volumes were then adjusted in order to standardize the HPRT levels among the groups. The HPRT-normalized cDNAs were then used to determine levels of inducible nitric oxide synthase (iNOS) and cytokines by means of specific primers. cDNA was amplified for 35 cycles (94°C for 40 s, 60°C for 20 s, 72°C for 40 s, and a final extension at 72°C for 10 min) by use of Taq polymerase (Boehringer Mannheim, Indianapolis). PCR products were analyzed by electrophoresis on 2% agarose gels and visualized by ethidium bromide staining.

Statistical analysis. We used Fisher’s exact test to test differences in survival, the Kaplan-Meier product limit estimate to determine significance for mean survival time, the Kruskal-Wallis test to examine differences in bacteria burden in various organs over time, and the Wilcoxon rank order test to determine differences in bacteria burdens between treatment groups at a single time point.

Results

Morphine potentiates oral Salmonella infection. To examine the effect of morphine on Salmonella infection, mice were orally inoculated with 2.5 × 10⁴ salmonellae and implanted with a 75-mg morphine pellet. Figure 1 shows that morphine greatly sensitized mice to Salmonella infection, resulting in 100% mortality with an MST of 3.2 days. To test whether the effects of morphine were mediated through an opioid receptor, Salmonella-
Figure 1. Morphine potentiates oral *Salmonella* infection. Mice were orally inoculated with salmonellae and implanted with indicated pellets. Survival % was scored, and mean survival time (MST) was calculated. For survival or for MST, $P < .009$ for morphine vs. naltrexone or placebo; $P < .05$ for morphine + naltrexone vs. naltrexone or placebo; $P$ not significant for morphine vs. morphine + naltrexone.

Infected mice were treated with a morphine plus a naltrexone pellet. Naltrexone alone had no effect on mice when compared with placebo controls. Both controls groups had survival rates of 46%, which is in keeping with a 1 LD$_{50}$ challenge dose and 30-day MSTs. Naltrexone only partially blocked the effects of morphine. In animals receiving morphine plus naltrexone, lethality remained high (92%), but the MST was significantly extended to 11.6 days.

**Effect of morphine dose on potentiation of oral *Salmonella* infection.** To examine the effect of morphine dose on *Salmonella* infection, mice were orally inoculated with $2.9 \times 10^6$ salmonellae and implanted with a 75-mg morphine pellet, various numbers of 8-mg morphine pellets, or a placebo pellet (figure 2). All doses of morphine resulted in 100% mortality of mice within 9 days, compared with a 50% survival rate in those receiving placebos. At the lowest morphine dose tested, 16 mg, there was a small difference in MST compared with mice receiving the 75-mg dose (5.3 days vs. 3.2 days, respectively), which was statistically significant ($P < .01$). However, placebo-treated mice had an MST of 28.3 days, which was significantly different from all morphine groups ($P < .001$).

**Effect of morphine on bacteria burden in *Salmonella*-inoculated mice.** To examine whether mortality in morphine-treated, *Salmonella*-infected mice correlated with increased bacteria burdens, mice were orally inoculated with $8.7 \times 10^7$ salmonellae and implanted with either a 75-mg morphine pellet or a naltrexone pellet. At various times after infection, the animals were killed, and bacteria burdens were determined (figure 3). Of 45 tissue samples from mice that received naltrexone pellets that were examined over the 3-day period, only 3 had culturable levels of salmonellae, and the bacteria burdens were $<10^3$ cfu in each. In contrast, 24 h after morphine treatment, all tissues sampled had detectable salmonellae, with a median burden of $1.2 \times 10^5$ cfu in PPs. By day 2, there was $>10^6$-fold difference in *Salmonella* burden in the PPs compared with animals given naltrexone. The median *Salmonella* burden in the MLNs continued to rise in mice given morphine pellets over the 3-day period.

**Effect of naltrexone on morphine-induced increases in *Salmonella* burden.** To test whether the morphine-induced increases in *Salmonella* burden were mediated through an opioid receptor, groups of mice were implanted with either a 75-mg morphine pellet, a naltrexone pellet, a placebo pellet, or both a naltrexone and a morphine pellet and inoculated orally with $3 \times 10^4$ salmonellae. Three days after infection, the mice were killed, and bacteria burdens were determined (figure 4). In the PPs, none of the placebo- or naltrexone-treated mice had detectable salmonellae. However, in the MLNs, 3 of 5 mice given naltrexone had culturable salmonellae. On the basis of median bacteria burdens, naltrexone partially blocked the effect of morphine in spleens, livers, and MLNs.

**Effect of time of morphine administration on susceptibility to...**

![Figure 2](image)
Salmonella infection. We did 2 separate experiments to determine the effect of time of morphine administration on susceptibility to salmonellae. In the first experiment (figure 5A), mice were given morphine at the same time as the Salmonella inoculation (T-0 as in the previous experiments) or at 48 h (T-48) or 24 h (T-24) before infection. The inoculating dose in this experiment was very low, only 231 cfu (0.01 LD₅₀). At this inoculum, all placebo-treated mice survived, but in mice given morphine at T-0, there was 100% mortality with an MST of 4.3 days. Morphine given 24 or 48 h before inoculation with salmonellae significantly increased the MSTs to 17.3 and 19.9 days, respectively, although the number of survivors was not significantly altered from that of animals receiving morphine at T-0. In the second experiment (figure 5B), mice were given morphine 24 (T-124) or 48 (T-148) h after infection. The inoculating dose in this experiment was also low, 592 cfu (0.02 LD₅₀). When the time of morphine administration was changed to after Salmonella inoculation, mortality was not significantly altered (90% in all morphine-treated groups). However, when morphine was given 24 or 48 h after inoculation, the MST rose from 4.6 days (T-0) to 12.8 days (T-24) and 10.4 days (T-48).

Effect of morphine on bacterial uptake and proliferation in PPs. To test whether the higher Salmonella levels in PPs of morphine-treated mice are associated with bacterial proliferation, an experiment was designed using W118-2 containing a single copy of the temperature-sensitive plasmid, pHSG422. (The plasmid has no effect on virulence compared with wild-type salmonellae [22].) The plasmid exhibits defective replication at body temperature, so with each Salmonella division it is passed to 1 daughter cell, and the other has no plasmid. Enumeration of plasmid-bearing versus plasmid-devoid bacteria in the original inoculum and the organisms retrieved from PPs after inoculation provides a measure of the proportion of burden in PPs that is derived from bacterial replication. The results in figure 6 show that the increase in bacteria burden observed in the PPs of infected mice 18 h after morphine inoculation results mainly from replication of the original inoculum, as 97.6% of the bacterial cultures from the 17 mice that were tested were free of the plasmid. In 12 of 17 mice, salmonellae harboring the plasmid were also cultured, although they generally comprised <5% of the residual organisms (mean, 2.4%). In contrast, 70% of the original inoculum contained the plasmid. Of note, in mice given placebo, no bacteria were recovered from the PPs at days 1–3 (compare figures 3 and 4 with figure 6), so the degree of bacterial replication in control and morphine-treated animals could not be compared.

Effect of morphine on iNOS and cytokine mRNA expression in PPs of Salmonella-infected mice. The effect of morphine and naltrexone on mRNA levels for iNOS and cytokines was examined in the PPs of uninfected and Salmonella-infected mice (T-0). As shown in figure 7A and 7B, uninfected control mice (no pellets) expressed a visible mRNA band for tumor necrosis factor (TNF)-α and a barely detectable band for iNOS. Pellet implantation intensified the band for iNOS in mice receiving morphine, placebo, or naltrexone pellets but did not affect the TNF-α level and did not induce other cytokine mRNAs. Salmonella inoculation of the control mice markedly increased iNOS mRNA. In infected mice, morphine significantly enhanced iNOS and TNF-α message levels, compared with those in control and placebo groups. Furthermore, interleukin (IL)-12 p40 and interferon (IFN)-γ mRNA were induced. In contrast, IL-2 and type 2 cytokines, IL-4 and IL-10 mRNA, were not detected in morphine-treated mice. The enhancement
Figure 4. Effect of naltrexone on *Salmonella* colonization in morphine-treated mice 3 days after oral inoculation. Dashes are median values for each group. A, Peyer’s patches: $P < .05$ for morphine vs. morphine + naltrexone, placebo, or naltrexone; $P < .01$ for morphine + naltrexone vs. placebo or naltrexone and not significant (NS) for placebo vs. naltrexone. B, Mesenteric lymph nodes: $P < .05$ for morphine vs. morphine + naltrexone, placebo, or naltrexone; $P < .05$ for morphine + naltrexone vs. placebo and NS for morphine + naltrexone vs. naltrexone and for placebo vs. naltrexone. C, Spleen: $P < .05$ for morphine vs. morphine + naltrexone, placebo, or naltrexone; $P < .01$ for morphine + naltrexone vs. placebo; $P < .05$ for placebo vs. naltrexone and NS for morphine + naltrexone vs. naltrexone. D, Liver: $P < .05$ for morphine vs. morphine + naltrexone, naltrexone, or placebo; $P < .05$ for morphine + naltrexone vs. placebo or naltrexone; $P < .05$ for placebo vs. naltrexone. ND, none detected.

of iNOS and TNF-α and the induction of IL-12 and IFN-γ mRNA cytokines were antagonized by implantation of naltrexone pellets with the morphine pellets, showing that the effects were mediated through an opioid receptor.

**Discussion**

Morphine administered by implantation of a 75-mg slow-release pellet dramatically sensitized mice to oral *Salmonella* inoculation. Morphine-treated mice given 1 LD$_{50}$ of *S. typhimurium* rapidly died of the infection (MST, 3.2 days), while 46% of mice given placebo pellets survived with a 10-fold greater MST of 30.2 days. Modulation of the doses of morphine or of salmonellae (in morphine-treated mice) had little effect on survival outcome. Pellets containing 16 mg of morphine also sensitized mice to salmonellae: there was 100% mortality, and the MST was only slightly extended compared with that of mice given the 75-mg pellet. Inoculation of mice treated with morphine and given *Salmonella* doses as low as 0.01 LD$_{50}$ also resulted in 100% mortality, while all placebo-pelleted mice survived.

Mortality in morphine-treated *Salmonella*-infected mice correlated with increased bacteria burdens rather than simply with toxic death. Salmonellae invade the M cells of the PPs in the small intestine, seed through the MLNs into the blood, and finally colonize the spleen and liver [27, 28]. When bacteria
Figure 5. Effect of time of morphine administration on survival. Results are scored as % survival and mean survival time (MST). A, Morphine given before oral inoculation of salmonellae (231 cfu). Mice were implanted with placebo or a 75-mg morphine pellet at the same time (T-0) as infection or at 48 (T-48) or 24 (T-24) h before infection. Each group had 15 mice, except the placebo group ( ). For survival: for placebo vs. T-0, T-24, or T-48 and not significant (NS) for T-0 vs. T-24 or T-48 and for T-48 vs. T-24. For MST: \( P < .003 \) for placebo vs. T-0, T-24, or T-48 and not significant (NS) for T-0 vs. T-24 or T-48; \( P < .001 \) for placebo vs. T-0, T-24, or T-48 and NS for T-24 vs. T-48. B, Morphine administered after mice were orally inoculated with salmonellae (592 cfu) and implanted with a placebo or morphine pellet (75 mg; T-0), or pellets were implanted 24 (T-124) or 48 (T-148) h after infection. Each group comprised 11 mice. For survival: \( P < .006 \) for placebo vs. T-0, T-24, or T-48 and NS for T-0 vs. T-24 or T-48 and for T-48 vs. T-24 or T-124. For MST: \( P < .01 \) for T-0 vs. T-24 or T-48; \( P < .001 \) for placebo vs. T-0, T-24, or T-48 and NS for T-24 vs. T-48.

The time of morphine administration appears to be important in sensitization to infection. Morphine given at the same time as *Salmonella* inoculation resulted in the greatest mortality. Morphine administered before or after salmonellae still markedly enhanced mortality; however, the MSTs were significantly higher than those reached when morphine was given at T-0. The importance of drug scheduling in association with morphine’s modulation of infection has been studied, and Chao et al. [29] showed that repeated morphine injections, as well as a single morphine injection, sensitized animals to *Toxoplasma gondii* infection. However, mortality was prevented in mice given a series of low morphine doses prior to infection, which presumably induced tolerance. Starec et al. [30] showed that repeated injections of morphine before and during infection with murine Friend virus did not increase mortality; however, a single nonlethal morphine dose markedly increased mortality in infected mice. Donahoe et al. [31] also showed that opiates did not potentiate simian immunodeficiency virus infection in tolerant rhesus monkeys. However, if opiates were withdrawn, there was acute exacerbation of disease.

The mechanism by which morphine causes such a marked potentiation of oral *Salmonella* infection is of interest. Opioid receptors are present in the circular muscle, submucosal plexus, and myenteric plexus of the small intestine of animals and humans [32–35]. A common symptom of opioid administration is constipation due to decreased peristalsis and increased muscle tone and spasms [32]. Morphine also slows transit time of GI contents in rats [36, 37] and in humans [38]. In the present studies, the effect of morphine on sensitization to salmonellae was at least partially mediated through classical opioid receptors: Naltrexone significantly extended the MST and also significantly blocked colonization of salmonellae in morphine-treated mice. Why naltrexone did not block the morphine-induced potentiation of mortality is not clear. In previous

Figure 6. Effect of morphine on bacterial uptake and growth in Peyer’s patches (PPs). Left indicators, total salmonella (TS) inoculated and portion of TS that contained pHSYG422 plasmid (PS). Each bar represents colony-forming units (cfu) from 3 pooled PPs of individual mice. Within bars, hatched areas are total salmonellae in PPs; solid areas are total salmonellae with pHSYG422 plasmid. Values are % of plasmid-containing bacteria recovered from the original inoculum. The data are pooled results of 2 experiments. Median is of 17 mice.
Figure 7. Morphine enhances inducible nitric oxide synthase (iNOS) and cytokine gene expression in Peyer’s patches (PPs) in Salmonella-infected mice. Total RNA was isolated and reverse transcribed to cDNA. The data are representative of 3 separate experiments. A. cDNA was used as the template for reverse-transcription polymerase chain reaction (PCR) using primers specific for iNOS, interleukin (IL)-12 p40, interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and IL-2, -4, and -10. PCR products were analyzed on 2% agarose gels and visualized by ethidium bromide staining. M + N, morphine + naltrexone. B. Relative density of ethidium bromide bands was determined by NIH Image software.

studies, 30-mg naltrexone pellets completely blocked morphine-induced immunosuppression in mice [23] and the analgesic effects of the opioid [39]. In our studies, the 75-mg morphine pellets increased GI transit time, as measured by feeding mice a charcoal meal (placebo, 18.4 cm; morphine, 11.3 cm), and a 30-mg naltrexone pellet blocked the effect of morphine (19.0 cm; M. E. Hilburger, T. K. Eisenstein, et al., unpublished data). Therefore, it is unlikely that the gut is less sensitive to the effects of naltrexone and that a higher dose is required.

It is of interest that Chao et al. [29] found that naltrexone only partially blocked morphine-induced hypersusceptibility to T. gondii infection in mice. Pretreatment with naloxone, a related opioid antagonist, was also unable to block morphine’s potentiation of parasitemia during Plasmodium berghei infection in mice [40]. Since naltrexone blockade is competitively reversible, it is possible that a larger dose of naltrexone would produce a greater or even a complete blockade. In addition, it is possible that the infection itself causes release of endogenous opioids that are in competition with naltrexone for occupancy of the opioid receptors. Finally, it is possible that the infection causes up-regulation of opioid receptors, particularly in immune cells, such that the amount of naltrexone is insufficient to block the increased number of receptors.

Other studies support the conclusion that morphine increases the permeability of the gut, allowing organisms of the endogenous flora to penetrate intestinal barriers. For example, our laboratory has shown that morphine pellet implantation in mice results in sepsis, with colonization of enteric organisms in the peritoneal cavity, liver, and spleen [19]. Other investigators have reported that morphine or morphine plus TNF-α increases translocation of enteric bacteria across the intestinal barrier in rats [41, 42]. The mechanism by which opioids permit leakage of bacteria from the GI tract has not been determined, although some years ago Takeuchi [43] observed that opium potentiated Salmonella penetration of the epithelial cells in the guinea pig ileum.

In the present studies, we attempted to gain greater insight into the mechanism by which morphine enhances Salmonella colonization of PPs. Use of a Salmonella strain with a temperature-sensitive plasmid showed that some of the organisms carrying the plasmid from the original inoculum could penetrate the PPs but that most bacteria in the PPs of morphine-treated mice were plasmidless progeny, presumed to result from replication of the inoculated organisms. The experiment does not distinguish whether Salmonella replication occurred in the intestinal lumen or in the PPs, and future experiments will be needed to sort out these possibilities. The possibility that morphine might potentiate oral infection via suppression of innate immune function is supported by numerous studies showing that morphine is immunosuppressive [16, 44, 45]. Morphine depresses polymorphonuclear neutrophil and macrophage function [46–48], including chemotaxis [49–51], phagocytosis [48, 52], and generation of microbicidal oxygen intermediates [53]. In vivo, morphine potentiates Klebsiella pneumoniae and Candida albicans in mice while suppressing phagocytosis, killing, and superoxide production by polymorphonuclear leukocytes and macrophages [54]. Morphine also sensitizes mice to several other infections [29, 40, 55]. Thus, mice treated with morphine may be unable to mount an adequate inflammatory response to salmonellae in the PPs, either because of failure to attract inflammatory cells in sufficient quantities or because the cells that do respond have decreased phagocytic and microbicidal activity. Our cytokine studies indicate, however, that cells in the PPs of morphine-treated mice have elevated mRNA for...
iNOS, IL-12, IFN-γ, and TNF-α, so the proinflammatory response to infection is not totally suppressed. Further experiments will be required to examine whether other aspects of the immune response are down-regulated. These studies confirm previous observations that virulent *Salmonella* strains induce a type 1 inflammatory response in the gut [56, 57].

Our results have potentially important implications for patients treated with morphine as an analgesic and for heroin users. Both groups may be at increased risk for *Salmonella* infection and possibly for infection with other enteric bacterial pathogens.

Acknowledgments

We thank Alan Cowan for help with experiments that used the charcoal meal, Alan Truant for verifying the identity of *Salmonella* colonies, and John P. Gaughan for statistical analysis.

References


