Differential morphine tolerance development in the modulation of macrophage cytokine production in mice

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Abstract: Morphine has been shown to affect cell-mediated and humoral immune parameters. In this study, we investigated the capacity of in vivo acute and chronic morphine treatment to modulate interleukin (IL)-10 and IL-12 production by LPS and interferon-γ-stimulated resident and thioglycollate-elicited murine peritoneal macrophages and the development of tolerance to these effects. One hour after the acute administration of 5, 10, and 20 mg/Kg morphine, a dose-related decrease of IL-10 and IL-12 levels was present. The pretreatment with naltrexone at doses up to 20 mg/Kg did not prevent the decrease of IL-10 and IL-12 induced by morphine. When the drug was administered chronically, a differential development of tolerance to the immune effects was observed. After 3 days of treatment, the effect of the acute challenge with 20 mg/Kg morphine on IL-12 was lost. In contrast, morphine-induced inhibition of IL-10 disappeared between 10 and 12 days of treatment, in parallel with tolerance to the antinociceptive effect. These results suggest that morphine treatment affects macrophage cytokine production and that tolerance affects this modulation differently. *J. Leukoc. Biol.* 72: 43–48; 2002.

Key Words: *IL-10 · IL-12 · antinociception · naltrexone · TNF-α*

INTRODUCTION

It is well known that the opiate drug morphine affects natural and adaptive immunity in the experimental animal as well as in the human [1]. Acute and chronic morphine administration has been shown to decrease most T lymphocyte functions, such as proliferation and cytokine production [2–5], natural killer (NK) cell activity [2, 6, 7], and macrophage functions including phagocytosis, tumoricidal activity, and superoxide formation [3, 8, 9].

Macrophages play a central role in innate and adaptive immunity. Interleukin (IL)-1, IL-6, and tumor necrosis factor α (TNF-α) produced by macrophages are prominent inflammatory cytokines [10, 11]. Moreover, monocytes/macrophages and professional antigen presenting cells are the main producers of IL-12 [12], the critical factor driving the development of T helper (Th)-1 cells [13], linked to cellular immune responses and tissue injury.

Conversely, Th2 cells, responsible for humoral responses and allergy [13], are stimulated by the T lymphocyte cytokine IL-4 and by IL-10 [13, 14], produced by T lymphocytes as well as by monocytes/macrophages. Therefore, when considering the importance of macrophage cytokines in orchestrating the immune response, we evaluated the effect of morphine in vivo administration on IL-12 and IL-10 production by murine peritoneal macrophages.

Although it is well known that tolerance develops to many of the effects of morphine, the timing of the development of tolerance to the immune effects of morphine has been scarcely taken into consideration. Therefore, we paid particular attention to the development of tolerance to the effect of morphine on cytokines and its relation with the antinociceptive effect of the drug.

MATERIALS AND METHODS

Animals

BALB/cJ male mice, 18–20 g body weight (Charles River, Calco, Italy), were used in the study. Animals were kept on a 12-h light-dark cycle with water and food ad libitum and were housed six mice to a cage. Each experimental group consisted of eight animals. Experiments were repeated three times.

Treatment protocols

Mice were inoculated intraperitoneally with 2 ml 3% Brewer's thioglycollate medium (Difco, Detroit, MI) for macrophage elicitation. In the acute experiments, morphine hydrochloride (S.A.L.A.R.S., Como, Italy) was injected subcutaneously (s.c.) 4 days after the macrophage elicitation at the doses of 5, 10, or 20 mg/kg. Control animals were treated with the same volume of saline.

To evaluate the effects of morphine on resident-nonelicited peritoneal macrophages, some groups of mice were treated acutely only with morphine at the doses of 10 and 20 mg/Kg or with the same volume of saline. Naltrexone (S.A.L.A.R.S.) was injected s.c. at the dose of 10 and 20 mg/Kg, alone or 15 min before 20 mg/Kg morphine. In the chronic treatment experiments, 48 animals were used. The same animals were used for antinociceptive and immune evaluation. Animals underwent the last hot-plate test the day before being killed for the immune studies. Starting from the thioglycollate injection day, 24 animals were treated s.c. with morphine twice daily at the doses of 10 mg/Kg for 2 days and 20 mg/Kg for the following days. Control groups (24 animals) received the same volume of saline.

On the day of the hot-plate test, animals received 5 mg/Kg morphine challenge, supplemented, after the hot-plate test, with a second injection of 15 mg/Kg morphine in order to achieve the maintenance dose of 20 mg/Kg. At the 3rd, 7th, and 12th day of treatment, 16 animals (8 morphine+8 saline) were

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killed for evaluation of cytokine production. To evaluate the effects of morphine on resident-nonelicited peritoneal macrophages, two groups of mice, for a total of 16 animals, were treated chronically with saline or morphine for 7 days according to the same dose schedule described above. In all experiments, animals received the last morphine injection 1 h before being killed for macrophage collection.

Harvest of elicited peritoneal macrophages

Peritoneal exudate cells (PEC) were harvested in cold RPMI-1640 medium (Sigma Chemical Co., St. Louis, MO) plus 10% of fetal calf serum (FCS; Gibco-BRL, Life Technology, Italy). Viability of cells was checked by the trypan blue exclusion test. Then PEC from each mouse were resuspended in RPMI plus 10% FCS at 1×10^6/ml, and 1 ml aliquots were dispensed into wells of a 24-well culture plate. Isolation and purification of macrophages were carried out by adherence to culture plates. After a period of 2 h, nonadherent cells were removed with the medium, and adherent cells were washed twice with warm RPMI plus 10% FCS. A differential staining with Diff-Quick (Dade, Biomap, Italy) and nonspecific esterase staining with α-naphthyl acetate (Sigma Chemical Co.) were used to assess the percentage of macrophages in the PEC.

Harvest of resident peritoneal macrophages

Peritoneal cells were harvested in cold RPMI plus 10% FCS and were pooled from four mice per replication. Cells were resuspended in RPMI plus 10% FCS at 2×10^6/ml, and 1 ml aliquots were dispensed into wells of a 24-well culture plate. Nonadherent cells were removed with the medium after an incubation of 2 h, and adherent cells were washed twice with warm RPMI plus 10% FCS.

Cytokine production

Elicited macrophages were primed with 1 μg/ml lipopolysaccharides (LPS; Sigma Chemical Co.) for IL-10 production or with 1 μg/ml LPS and 50 U/ml interferon-γ (IFN-γ) (Pharmingen, San Diego, CA) for IL-12 stimulation. Nonelicited macrophages were stimulated with 1 μg/ml LPS for IL-10 production or with 10 μg/ml LPS and 100 U/ml IFN-γ for IL-12 evaluation. The different stimuli were added to the macrophage cultures in a final volume of 1 ml/well RPMI plus 10% FCS, 1% glutamine (Sigma Chemical Co.), 2% penicillin/streptomycin solution (Sigma Chemical Co.), and 0.1% 2-mercaptoethanol (Sigma Chemical Co.; complete medium). The plates were incubated at 37°C and 5% CO_2, and supernatants were collected after 24 h in culture and stored frozen at −80°C for cytokine analysis.

Cytokine enzyme-linked immunosorbent assay (ELISA)

The levels of IL-12 p70 protein were determined by ELISA protocol as standardized by Pharmingen. The anti-IL-12 (p35/p70)-capture monoclonal antibody (mAb; 9 μg/ml) was absorbed on a polystyrene 96-well plate, and the IL-12 present in the sample was bound to the antibody-coated wells. The biotinylated anti-IL-12 (p40/p70)-detecting mAb (0.25 μg/ml) was added to bind the IL-12 captured by the first antibody. After washing, avidin-peroxidase (Sigma Chemical Co.) was added to the wells to detect the biotinylated-detecting antibody, and finally, 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (Sigma Chemical Co.) substrate was added. A colored product was formed in proportion to the amount of IL-12 present in the sample that was measured at an optical density of 405 nm. The amount of cytokine in each supernatant was extrapolated from the standard curve. The standards were recombinant cytokine curves generated in doubling dilutions from 30 to 4000 pg/ml.

IL-10 production was measured with the same ELISA protocol except for the use of anti-IL-10-capture mAb at 2 μg/ml, biotinylated anti-IL-10-detecting mAb at 0.5 μg/ml, and a standard curve ranging from 15 to 2000 pg/ml. (All mAb and recombinant cytokines were from Pharmingen.)

Evaluation of tolerance

The hot-plate test was used to assess nociceptive thresholds. The apparatus (Basile, Comerio, Italy) was set at a temperature of 54 ± 0.5°C; the cutoff time was 30 s, chosen to avoid tissue damage to footpads [15]. Time latency was recorded between the placement of the animal on the hot plate and the moment at which the mouse licked both the fore paws. To prevent tissue damage and adaptation, only one hot-plate response was measured per time point. To evaluate the development of tolerance to the analgesic effect of morphine, the antinociceptive response was recorded 60 min after challenge with 5 mg/Kg morphine every other day of chronic treatment. The same animals were used for antinociceptive and immune evaluation. Animals underwent the last hot-plate test the day before being killed for the immune studies. The results are expressed as percentage of the maximal possible effect (% MPE): $MPE = [(TL-BL)/(ML-BL)] \times 100$, where BL is the mean basal latency, TL is the test latency measured after treatments, and ML is the maximal latency accepted (30 s).

Statistical analysis

Significant differences between groups were assessed by one-way analysis of variance (ANOVA), followed by Bonferroni's *t*-test for multiple comparisons. % MPE was evaluated by the Kruskall Wallis ANOVA for nonparametric data.

RESULTS

Acute experiments

One hour after the acute administration of 5, 10, and 20 mg/kg morphine, elicited or resident peritoneal macrophages were incubated for 24 h in the presence of stimuli for the production of cytokines. Unstimulated macrophages did not produce detectable levels of IL-12 or IL-10. Activation of cells with LPS and IFN-γ induced the production of IL-12 and with LPS of IL-10.

As demonstrated in **Figure 1**, acute morphine administration decreased the production of IL-12 (upper panel) and IL-10 (lower panel) by thioglycollate-elicited macrophages. The effect on IL-12 started to be present at 10 mg/Kg morphine, but reached statistical significance only with 20 mg/Kg opiate drug. Also, IL-10 was significantly reduced only by the highest dose of morphine.

Although the thioglycollate-elicited macrophages are commonly used for cytokine evaluation studies, the possibility exists that a thioglycollate-mediated activation could interfere with the results obtained. To rule out this case, we evaluated the ability of acute morphine administration to alter cytokine production also by resident nonelicited macrophages.

As shown in **Table 1**, the levels of IL-12 produced by resident macrophages after in vitro stimulation with LPS (10 μg) plus IFN-γ (100 units) were extremely low. However, the acute administration of 20 mg/kg morphine was able to decrease the release of lL-12 significantly. It has to be pointed out, however, that in macrophage cultures derived by morphine-treated mice, the levels of IL-12 were reduced to hardly detectable levels in our ELISA assays. Basal levels of IL-10 were comparable in thioglycollate-elicited and resident macrophages (Table 1). Also in this case, acute morphine treatment significantly decreased IL-10 production.

To rule out a toxic effect of morphine on macrophages, 1 h after morphine administration, the viability of PEC was checked by the test of trypan blue exclusion. The number of viable PEC was not affected by morphine:saline: $6.13 \pm 1.8 \times 10^6$ (mean±SD) PEC/mouse; morphine, 20 mg/Kg: $6.83 \pm 1.7 \times 10^6$ PEC/mouse. Moreover, we evaluated by morphological criteria (Diff-Quick staining) and by nonspecific esterase

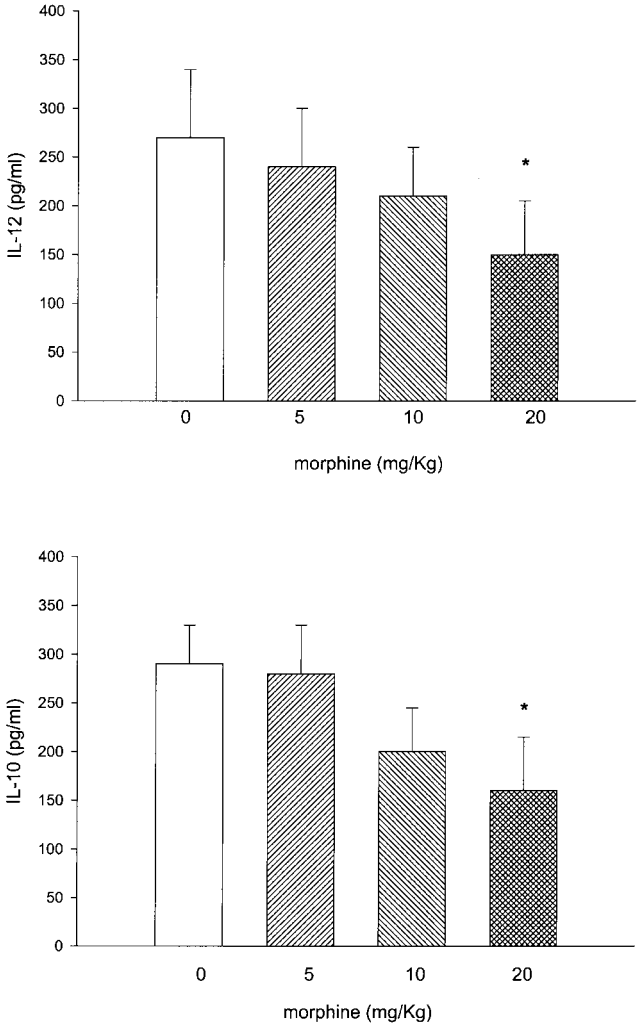

Fig. 1. Effect of the acute administration of three doses of morphine on IL-12 (upper panel) and IL-10 (lower panel) production by thioglycollate-elicited murine peritoneal macrophages. One hour after morphine administration, macrophages were stimulated in vitro for 24 h in the presence of LPS (IL-10) or LPS + IFN-γ (IL-12). Values are means ± SD of eight animals (one experimental session). The experiments were repeated three times with similar results. *, $P < 0.01$ versus saline-treated animals (0).

staining that the percentage of adherent macrophages was not altered in the different treatment groups (unpublished results).

Effect of naltrexone pretreatment

Figure 2 shows the effects of the acute administration of 10 and 20 mg/Kg naltrexone alone or in combination with 20 mg

TABLE 1. IL-12 and IL-10 Production by Resident Macrophages Obtained from Saline- and Morphine Acutely Treated Mice

	IL-12 (pg/mL) (10 μg LPS+100 U IFN-γ)	IL-10 (pg/mL) (1 μg LPS)
Saline	30.2 ± 8[a]	299 ± 15
Morphine 10 mg/Kg	21.8 ± 6	266 ± 15
Morphine 20 mg/Kg	15.0 ± 6.2*	216 ± 25*

[a] Values are mean ± SD of eight replications. Each replication consisted of a pool of four mice. * $P < 0.05$ versus saline-treated animals.

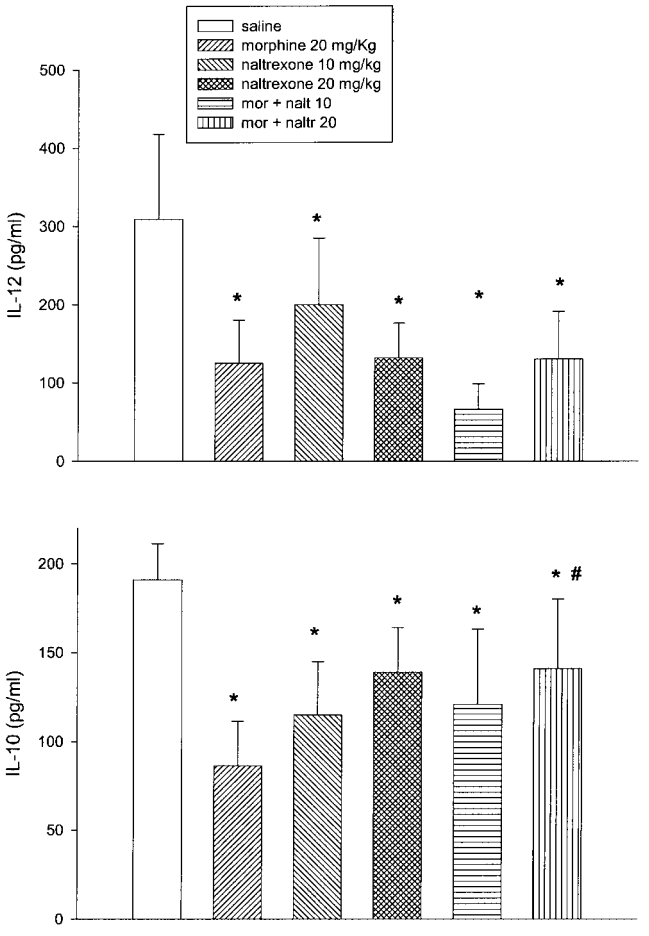

Fig. 2. Effect of the pretreatment with naltrexone (10 and 20 mg/Kg) on morphine (20 mg/Kg) induced decrease of IL-12 and IL-10 production by elicited macrophages. One hour after morphine administration, macrophages were stimulated in vitro for 24 h in the presence of LPS (IL-10) or LPS + IFN-γ (IL-12). Values are means ± SD of eight animals. mor + nalt, Morphine + naltrexone. *, $P < 0.01$ versus saline-treated animals. #, $P < 0.05$ versus morphine-treated animals.

mg/Kg morphine on IL-12 (upper panel) and IL-10 (lower panel) production. Both doses of the opiate antagonist by themselves were able to induce a slight although significant decrease of IL-12 and IL-10 production by elicited peritoneal macrophages. When the antagonist doses were administered 15 min before 20 mg/Kg morphine, IL-12 remained significantly lower than the one in saline-treated animals. As for IL-10, at the high dose of 20 mg/Kg, naltrexone seems to partially prevent the morphine-induced IL-10 reduction.

Chronic experiments

To study the development of tolerance to the antinociceptive and immunosuppressive effects of morphine, animals were treated for 12 days with morphine. The animals treated chronically did not seem to suffer from morphine treatment, as shown by the observation that no body weight difference was observed between saline- and morphine-treated animals at any of the treatment days (body weight at the 12th day of treatment: saline, 23.8±0.6 grams; morphine, 24.7±1 grams; mean±SD). Moreover, when we counted the number of PEC at the last day of morphine treatment (12th day), no significant difference was

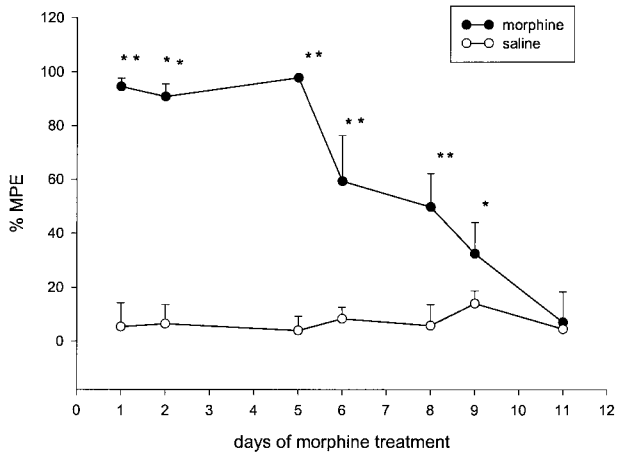

Fig. 3. Analgesic responses to acute administration of morphine (5 mg/Kg) in mice treated chronically with morphine (see Materials and Methods). Nociceptive thresholds were measured using the hot-plate test immediately before and 60 min after acute morphine treatment. As controls, animals chronically treated with saline were used. Values are means ± SD of eight animals. Each point represents the mean response of eight mice, which were killed the following day for immune evaluation. *, $P < 0.05$ versus saline-treated animals. **, $P < 0.001$ versus saline-treated animals.

observed in comparison with saline-treated animals: saline, $3.3 \pm 1.9 \times 10^6$ (mean±SD) PEC/mouse; chronic morphine, $3.5 \pm 2.3 \times 10^6$ PEC/mouse.

Figure 3 illustrates the development of tolerance to the antinociceptive of morphine. Each point represents the mean response of eight mice that were killed the following day for immune evaluation. Tolerance started by day 6 of treatment and was complete at the 11th day of chronic morphine administration. In fact, at this time the acute challenge with 5 mg/kg morphine did not elicit any significant antinociceptive effect in comparison to saline-treated animals. The effects of chronic morphine on cytokine production are shown in **Figure 4**. As reported in the upper panel, tolerance developed very rapidly to the suppressive effect of morphine on IL-12, as it appeared between the 1st and 3rd day of treatment; indeed, after 3 days of treatment, no reduction of this cytokine was present after 20 mg/kg morphine. In contrast, the effect of the opiate on IL-10 decrease persisted longer, as it disappeared between the 7th and the 12th day, in parallel with the development of tolerance to the antinociceptive effect. Similar results were obtained also when resident-nonelicited macrophages were used (**Table 2**). After 7 days of chronic morphine treatment, tolerance already developed to the effect of the drug on IL-12 production, and morphine was still able to decrease IL-10 release.

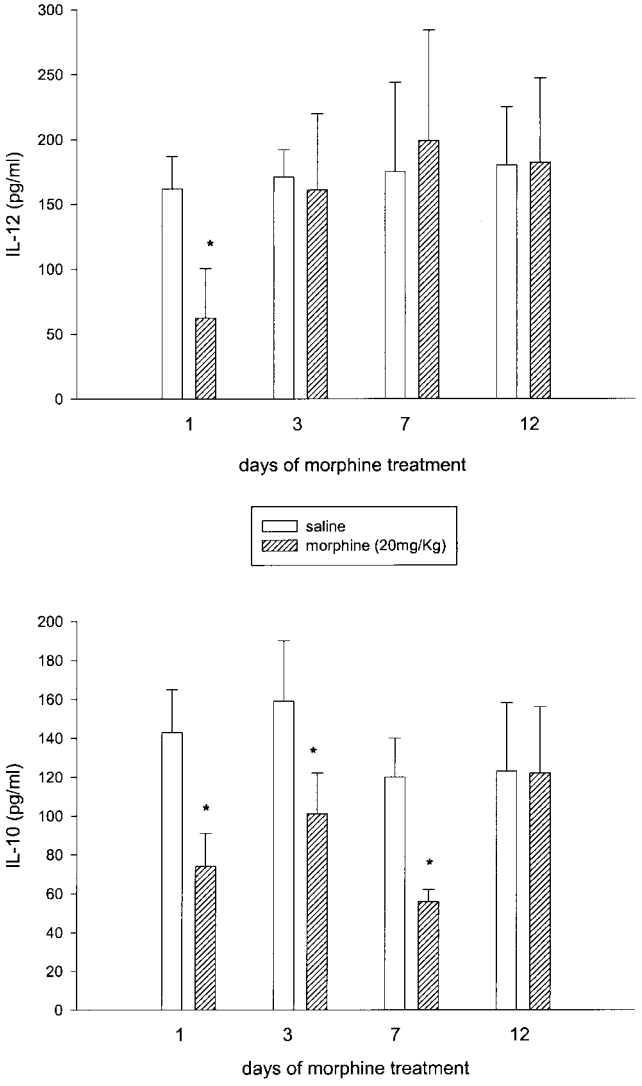

Fig. 4. Development of tolerance to morphine induced decrease of IL-10 and IL-12 production by elicited peritoneal macrophages. Mice treated chronically with morphine for the time indicated were challenged with 20 mg/Kg morphine. One hour after morphine administration, macrophages were stimulated in vitro for 24 h in the presence of LPS (IL-10) or LPS + IFN-γ (IL-12). Day 1 represents the data obtained from a single, acute injection of 20 mg/Kg morphine. Values are means ± SD of eight animals (one experimental session). The experiments were repeated three times with similar results. *, $P < 0.01$ versus saline-treated animals.

DISCUSSION

In this study, we demonstrate that the acute administration of morphine decreased IL-12 and IL-10 production significantly by LPS and IFN-γ-stimulated murine peritoneal macrophages. IL-12, a heterodimeric cytokine produced mainly by monocytes/macrophages, is a central inducer of cell-mediated immunity that promotes the development, proliferation, and function of Th1 cells [12, 13]. Th1 cells promote the activation and function of NK cells and of T cell cytotoxicity and produce IL-2 and IFN-γ [13]. In contrast, IL-10, produced as a result of activation of Th2 cells, B lymphocytes, and macrophages, is an endogenous inhibitor of IL-12 and Th1 responses and stimulates Th2 cell population and humoral immunity [13, 14].

TABLE 2. IL-12 and IL-10 Production by Resident Macrophages Obtained from Mice Treated Chronically (7 days) with Saline or Morphine

	IL-12 (pg/mL) (10 μg LPS+100 U IFN-γ)	IL-10 (pg/mL) (1 μg LPS)
Saline	44.3 ± 1.9[a]	273 ± 19
Morphine	38.7 ± 13	179 ± 11*

[a] Values are mean ± SD of four replications. Each replication consisted of a pool of four mice. * $P < 0.05$ versus saline-treated animals.

It has been reported that morphine administration can alter the distribution of cells in immune organs: both a decrease and an increase of peritoneal macrophage numbers have been observed after administration of morphine [16–18]. However, the cytokine alterations that we observed do not seem to be because of this condition, as, with our protocol treatment, the number and viability of macrophages in the peritoneal fluid were not affected by morphine treatments. Moreover, in our experimental model, we measured the peritoneal macrophages production of TNF-α, which was never modified by morphine treatment (unpublished results). We can therefore suggest that morphine seems to affect specifically the level of IL-10 and IL-12 rather than alter the composition of peritoneal cells.

Only a few other studies in the literature demonstrate the effects of morphine administered in vivo on IL-12 and IL-10 production. Peng et al. [19] observed that 48 h after the implantation of a 75 mg slow-release pellet, IL-12 production by murine macrophage was indeed increased, and IL-10 was reduced [18, 19]. The discrepancy with our results showing IL-12 decrease can be explained easily, however, on the basis of the different treatment schedules. The peak morphine plasma concentration reached after the acute injection of 20 mg/kg morphine cannot be compared with the concentrations reached by the slow release of morphine from the pellet. Moreover, the authors suggest that the IL-12 increase they observe could be a result of occult sepsis caused by the 48 h morphine treatment [20, 21]. Our model is indeed very acute, as animals are killed only 1 h after morphine administration; therefore, the possibility of the presence of an underlying sepsis at this time was unlikely. Moreover, in chronically treated animals, we did not observe any bacterial growth (unpublished results). Different morphine dosage and route of administration can be responsible for these different results.

The question of the development of tolerance to the immunological effects of morphine is still an open one. A certain degree of tolerance has been shown to develop to some, though not all, immune effects of morphine. West et al. [22, 23], using a model with morphine delivered in drinking water, showed that tolerance develops to the suppression of NK activity in parallel to tolerance to antinociception, whereas the lymphoproliferation suppression does not seem to go into tolerance. Similar results were reported also in the Rhesus monkey [24]. In contrast, other authors [25, 26] with a twice daily injection protocol showed that the suppression of concanavalin A lymphoproliferation induced by morphine underwent tolerance in 4–8 days, in parallel to antinociceptive tolerance. Also in a recent paper [27], development of tolerance to morphine inhibition of lymphoproliferation was shown, while, in this case, no tolerance to suppression of NK activity was observed up to the 8th day of chronic morphine treatment. Moreover, a differential development of tolerance to antinociception and to T and B lymphoproliferation suppression was described also in a chronic pellet implantation model [28]. On the whole, it seems clear that the development of tolerance to morphine effects can depend on many factors such as the immune parameter evaluated and the way of drug administration. Our data on the effects of chronic morphine on macrophage cytokines seem to confirm this observation. In fact, when the effects of morphine and the production of IL-12 disappeared completely after 3 days of morphine treatment, the reduction of IL-10 was still present. Therefore, it has to be considered that the final status of the immune responses can vary with different morphine regimens.

It is difficult to hypothesize how the modifications of macrophage cytokine production induced by acute or chronic morphine can impact on Th1/Th2 balance, especially considering that a complex regulatory loop exists between Th1 and Th2 cytokines [12–14]. The results of the present study indicate that in vivo morphine seems to be a potent inhibitor of macrophage activity, suppressing IL-12 and IL-10 secretion. In theory, these modifications should diminish Th1 and Th2 responses or, in any case, should not affect one of the T subsets particularly. Consistently, the data present in the literature show that morphine affects cellular and humoral immune responses [3, 29] as well as the production of Th1 cytokines (IL-2 and IFN-γ) [2, 30] and Th2 cytokines such as IL-4 [31].

In previous work, we demonstrated that the endogenous opioid system can find a role in skewing the Th1/Th2 balance toward a Th2 predominant response [5]. Conversely, it is not surprising that endogenous opioids could exert a fine modulation on Th1/Th2 responses that is different from the one achieved by injection of high pharmacological doses of morphine. Recently, it was reported that morphine administration in vitro can direct T cells toward Th2 differentiation [32]. This effect was probably mediated by a direct effect of morphine on T cells.

The differential development of tolerance to immune effects of morphine can be relevant when considering the chronic morphine treatment of patients. The final effect of morphine on immune function can, in fact, vary over time, depending on the balance of the effects still present at different moments. The effects induced by the administration of the opioid antagonist naltrexone on IL-12 and IL-10 production are surprising. Indeed, the antagonist seems to possess some agonistic activity, as it slightly decreases the cytokine levels, similar to the effects exerted by morphine. Moreover, the pretreatment with the antagonist does not block the morphine-induced decrease of IL-12 and only at the high dose of 20 mg/Kg seems to block IL-10 reduction slightly. However, we are aware that this dose is a very high one, and any specificity at the μ opioid receptor should be lost. Two possible scenarios can explain these results.

The most obvious is that the effects induced by morphine are not mediated by a classical opioid receptor. In the literature, most, but not all, of the immune effects of morphine have been shown to be blocked by the antagonists and therefore mediated by the μ opioid receptors [3, 33]. However, several papers have described the existence of nonclassical opioid binding sites on different cell populations that can mediate the non-naloxone-reversible effects of morphine [3, 33, 34]. Another explanation could be that under our experimental conditions, naltrexone could behave as an agonist. Conditions where the opioid antagonists naloxone and naltrexone behave as agonists have been described already for the main opioid effect, i.e., centrally and peripherally mediated analgesia [35–39]. Further studies are needed to better understand the effects of naltrexone.

CONCLUSION

In conclusion, we show that morphine treatment impairs production of cytokines by macrophage and that a different pattern

of modifications can be observed after acute or chronic treatments. Considering the importance of macrophage cytokines on modulating immune responses, it can be suggested that some of the effects of morphine on T- and B-cell function can be ascribed, at least in part, to the effect of the drug on macrophages.

REFERENCES

1. Lysle, D. T., Coussons, M. E., Watts, V. J., Bennet, E. H., Dykstra, L. A. (1993) Morphine-induced alterations of immune status: dose dependency, compartment specificity and antagonism by naltrexone. J. Pharmacol. Exp. Ther. 265, 1071–1078.
2. Sacerdote, P., Manfredi, B., Mantegazza, P., Panerai, A. E. (1997) Antinociceptive and immunosuppressive effects of opiate drugs: a structure-related activity study. Br. J. Pharmacol. 121, 834–840.
3. Eisenstein, T. K., Hillburger, M. E. (1998) Opioid modulation of immune responses: effects on phagocyte and lymphoyd cell populations. J. Neuroimmunol. 83, 36–44.
4. Pacifici, R., Di Carlo, S., Bacosi, A., Pichini, S., Zuccaro, P. (2000) Pharmacokinetics and cytokine production in heroin and morphine-treated mice. Int. J. Immunopharmacol. 22, 603–614.
5. Sacerdote, P., Manfredi, B., Gaspani, L., Panerai, A. E. (2000) The opioid antagonist naloxone induces a shift from type 1 cytokine pattern to type 2 cytokine pattern in Balb/cJ mice. Blood 95, 2031–2036.
6. Bryant, H. U., Roudebush, R. E. (1990) Suppressive effects of morphine pellet implants on in vivo parameters of immune function. J. Pharmacol. Exp. Ther. 255, 410–414.
7. Carr, D. J., France, C. P. (1993) Immune alterations in morphine-treated Rhesus monkeys. J. Pharmacol. Exp. Ther. 267, 9–15.
8. Tubaro, E., Santiangeli, C., Belogi, L., Borelli, G., Cavallo, G., Croce, C., Avico, U. (1987) Methadone vs morphine: comparison of their effect on phagocytic functions. Int. J. lmmunopharmacol. 9, 79–88.
9. Rojavin, M., Szabo, I., Bussiere, J. L., Rogers, T. J., Adler, M. W., Eisenstein, T. K. (1993) Morphine treatment in vitro or in vivo decreases phagocytic functions of murine macrophages. Life Sci. 53, 997–1006.
10. Miller, J. A., Hopkins, S. J., Luheshi, G. N. (1997) Sites of action of IL-1 in the development of fever and cytokine responses to tissue inflammation in the rat. Br. J. Pharmacol. 120, 1274–1279.
11. Beutler, B., Cerami, A. (1989) The biology of cachectin/TNF—a primary mediator of the host response. Annu. Rev. Immunol. 7, 625–655.
12. Trincheri, G. (1995) Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen specific adaptive immunity. Annu. Rev. Immunol. 13, 251–276.
13. Mosman, T. R., Sad, S. (1996) The expanding universe of T-cell subsets; Th1, Th2 and more. Immunol. Today 17, 138–146.
14. Moore, K. W., O'Garra, A., De Waal Malefyt, R., Vieira P., Mosmann, T. R. (1993) Interleukin-10. Annu. Rev. Immunol. 11, 165–190.
15. Bianchi, M., Maggi, R., Pimpinelli, F., Rubino, T., Parolaro, D., Poli, V., Ciliberto, G., Panerai, A. E., Sacerdote, P. (1999) Presence of a reduced opioid response in interleukin-6 knock out mice. Eur. J. Neurosci. 11, 1501–1507.
16. Hilburger, M. E., Adler, M. W., Rogers, T. J., Eisenstein, T. K. (1997) Morphine alters macrophage and lymphocyte populations in the spleen and peritoneal cavity. J. Neuroimmunol. 80, 106–114.
17. Singhal, P. C., Sharma, P., Kapasi, A. A., Reddy, K., Franki, N., Gibbons, N. (1998) Morphine enhances macrophage apoptosis. J. Immunol. 160, 1886–1893.
18. Tubaro, E., Borelli, G., Croce, C., Cavallo, G., Santiangeli, C. (1983) Effect of morphine on resistance to infection. J. Infect. Dis. 148, 656–666.
19. Peng, X. P., Mosser, D. M., Adler, M. A., Rogers, T. J., Meissler, J. J., Eisenstein, T. B. (2000) Morphine enhances interleukin-12 and the production of proinflammatory cytokines. J. Leukoc. Biol. 68, 723–728.
20. Hilburger, M. E., Adler, M. W., Truant, A. L., Meissler, J. J, Satishchandran, V., Rogers, T. J., Eisenstein, T. K. (1997) Morphine induces sepsis in mice. J. Infect. Dis. 176, 183–188.
21. Roy, S., Charboneau, R. G., Barke, R. A. (1999) Morphine synergizes with lipopolysaccharide in a chronic endotoxemia model. J. Neuroimmunol. 95, 107–114.
22. West, J. P., Dykstra, L. A., Lysle, D. T. (1997) Tolerance development to morphine-induced alterations of immune status administration. Drug Alcohol Depend. 46, 147–157.
23. West, J. P., Dykstra, L. A., Lysle, D. T. (1998) Differential tolerance to morphine's immunomodulatory effects following continuous administration. Drug Alcohol Depend. 53, 31–38.
24. Chuang, L. F., Killam, K. F., Chuang, R. Y. (1993) Opioid dependency and T-helper cell functions in Rhesus monkey. In Vivo 7, 159–166.
25. Bayer, B. M., Hernandez, M. C., Ding, X. Z. (1996) Tolerance and crosstolerance to the suppressive effects of cocaine and morphine on lymphocyte proliferation. Pharmacol. Biochem. Behav. 53, 227–234.
26. Bayer, B. M., Brechio, R. M., Ding, X. Z., Hernandez, M. C. (1994) Enhanced susceptibility of the immune system to stress in morphine-tolerant rats. Brain Behav. Immun. 8, 173–184.
27. Massi, P., Vaccani, A., Romorini, S., Parolaro, D. (2001) Comparative characterization in the rat of the interaction between cannabinoids and opiates for their immunosuppressive and analgesic effects. J. Neuroimmunol. 117, 116–124.
28. Bryant, H. U., Bernton, E. W., Holaday, J. W. (1988) Morphine pellet-induced imunomodulation in mice: temporal relationships. J. Pharmacol. Exp. Ther. 246, 913–920.
29. Bussiere, L. J., Adler, M. W., Rogers, T. J., Eisenstein, T. K. (1993) Cytokine reversal of morphine-induced suppression of the antibody response. J. Pharmacol. Exp. Ther. 264, 591–597.
30. Roy, S., Chapin, R. B., Cain, K. J., Charboneau, R. G., Ramakrishnan, S., Barke, R. A. (1997) Morphine inhibits transcriptional activation of IL-2 in mouse thymocytes. Cell. Immunol. 179, 1–9.
31. Bhargava, H. N., Thomas, P. T., Thorat, S., House, R. V. (1994) Effect of morphine tolerance and abstinence on cellular immune responses. Brain Res. 642, 1–10.
32. Roy, S., Balasubramanian, S., Sumandeep, S., Charnoneau, R., Wang, J., Melnyk, D., Vatassery, G. J., Barke, R. A. (2001) Morphine directs cells towards Th2 differentiation. Surgery 130, 304–309.
33. Madden, J. J., Whaley, W. L., Ketelsen, D., Donahoe, R. M. (2001) The morphine-binding site on human activated T-cells is not related to the mu opioid receptor. Drug Alcohol Depend. 62, 131–139.
34. McCarthy, L., Wetzel, M., Sliker, J. K., Eisenstein, T. K., Rogers, T. J. (2001) Opioids, opioid receptors and the immune response. Drug Alcohol Dep. 62, 111–123.
35. Sacerdote, P., Mantegazza, P., Panerai, A. E. (1985) Analgesic effects of mu antagonists after naloxone non-reversible stress induced analgesia. Brain Res. 359, 34–38.
36. Bianchi, M., Sacerdote, P., Locatelli, L., Mantegazza, P., Panerai, A. E. (1991) Corticotropin releasing hormone, interleukin 1-alpha, and tumor necrosis factor alpha share characteristics of stress mediators. Brain Res. 546, 139–142.
37. Bianchi, M., Panerai, A. E. (1993) Naloxone-induced analgesia; involvement of k-opiate receptors. Pharmacol. Biochem. Behav. 46, 145–148.
38. Levine, J. D., Gordon, N. C., Fields, H. L. (1979) Naloxone dose dependently produces analgesia and hyperalgesia in post-operative pain. Nature 278, 740–741.
39. Ferreira, S. H. (1980) Peripheral analgesia: mechanism of the analgesic action of aspirin-like drugs and opiate antagonists. Br. J. Clin. Pharmacol. 2, 237–245.