Morphine Reciprocally Regulates IL-10 and IL-12 Production by Monocyte-Derived Human Dendritic Cells and Enhances T Cell Activation

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We evaluated the effect of morphine on human dendritic cells (DCs). Interestingly, immature DCs were found to express all 3 (μ, κ, δ) opioid receptors on the cell surface. Chronic morphine treatment (10^{-8} to 10^{-12} M) during the development of DCs from monocytes augmented LPS-induced upregulation of HLA-DR, CD86, CD80, and CD83 and increased the T cell stimulatory capacity of DCs, which could be inhibited by naloxone, an opioid receptor antagonist. The change in surface phenotype was paralleled by a p38 MAPK-dependent decrease in IL-10 and increase in IL-12 secretion. Our data indicate that morphine exerts an immunostimulatory effect by modulating LPS-induced DC maturation.

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INTRODUCTION

The observation that individuals under severe stress or addicted to opioids are generally immunosuppressed (1-3) has led to the notion of a neuroendocrine-immune network. Neuroactive ligands exert a powerful effect on cells of the immune system. There is growing evidence that opioid receptors are expressed by cells of the immune system and that opioids modulate immune responses. In contrast to endogenous opiates that exert immunostimulatory effects (3,4), both the therapeutic and chronic use of morphine compromise the optimal function of the immune system (5). Opiate addicts are prone to infection. This effect of opiates has been attributed to a variety of its immunomodulatory effects (6). Chronic administration of morphine affects both innate and adaptive immunity (7). Morphine given in vivo suppresses a variety of immune responses, including rat T and natural killer (NK) cells (8), macrophages, rat and macaque polymorphonuclear leukocytes (PMNs) (9,10), and lymphocyte circulation in macaques (11). Morphine-receiving mice have splenic and thymic atrophy (12), morphine triggers T cell apoptosis in in vitro studies (13), and enhances macrophage apoptosis in murine macrophages (14). Several reports address the role of macrophages in morphine-induced modulation of immunity (15-20). These studies pertain to a variety of macrophage functions including phagocytosis, tumoricidal activity, generation of nitric oxide and reactive oxygen species, and cytokine synthesis. Undoubtedly, these studies have significantly advanced our understanding of the role of opiates in the modulation of immunity; nevertheless, the effect of morphine on immunity still remains a complex puzzle.

Dendritic cells (DCs) play a central role in the initiation and control of an adaptive immune response (21). Dendritic cells link the innate to the adaptive immune response by their ability to detect and capture foreign antigens and efficiency to present antigens to T cells. Following the uptake and processing of antigens in the periphery, immature DCs migrate to the T cell–rich areas of lymphoid organs and undergo a maturation process. Mature DCs are potent stimulators of primary T cell and memory responses; they also produce an array of cytokines and chemokines (21). Factors that modify the DC maturation process can influence the immune response against pathogens and or vaccines. In addition to pathogen components and inflammatory cytokines, DCs respond to a growing number of neuropeptides; for example, calcitonin gene-related peptide inhibits the antigen-presenting capacity of DCs (22,23), substance P enhances nuclear factor kappa B (NF-κB) activation

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(24), and the vasoactive intestinal peptide synergizes with TNF-α to increase IL-12 production and enhance DC maturation (25). Murine DCs have been shown to express functional κ-opioid (26) as well as δ- and μ-opioid (27) receptors. Stimulation with the κ agonist dynorphin suppressed the T cell stimulatory capacity of DCs without affecting antigen uptake or phenotypic maturation (26). Expression of δ-opioid receptor has also been detected on human DCs (27).

We sought to investigate the consequence of morphine on the differentiation process of human myeloid DCs from monocytes. Our data show that exposure of monocytes to morphine during the differentiation process into DCs enhances the T cell stimulatory capacity of lipopolysaccharide (LPS)-matured DCs, which is mediated through classic opioid receptors. This is paralleled by a p38 MAPK–dependent increase in IL-12 p70 secretion and decrease in IL-10 production. In contrast to studies in T cells and some reports on macrophages, morphine enhances the response of DCs to a stimulus and exerts an immunomodulatory function that is likely to amplify a Th1 immune response. Thus, human monocytes and DCs can be participants in the neuroimmune dialog.

MATERIALS AND METHODS

Reagents

The p38 MAPK–specific inhibitor, SB203580, a pyridinyl imidazole compound, the MEK inhibitor PD98059, and *N*-tosyl-L-phenylalanine chloromethyl ketone (TPCK) were purchased from Sigma (St. Louis, MO, USA). The inhibitors were used at a final concentration of 5 μM. These reagents were solubilized in DMSO; therefore, DMSO, in identical concentrations, was also used in controls. Dr. Singhal obtained morphine from the National Institute for Drug Abuse.

Isolation and Generation of DCs

DCs were generated from peripheral blood mononuclear cells (PBMCs). Blood of healthy volunteers was purchased from the Long Island Blood Services (Melville, NY, USA). PBMCs were isolated over a Ficoll-Hypaque (Amersham Biosciences, Uppsala, Sweden) density gradient. $CD14^+$ monocytes were isolated from PBMCs by positive selection using anti-CD14 beads (Miltenyi Biotech, Auburn, CA, USA) following the manufacturer's instructions. To generate DCs, $CD14^+$ cells were cultured in RPMI 1640 supplemented with 2 mM L-glutamine (Gibco-BRL Life Technologies, Grand Island, NY, USA), 50 μM 2-mercaptoethanol (Sigma), 10 mM HEPES (Gibco-BRL), penicillin (100 units/mL), streptomycin (100 μg/mL) (Gibco-BRL), and 5% human AB serum (Gemini Bio-Products, Woodland, CA, USA). Cultures were maintained for 7 days in 6-well trays (3×10^6 cells/well) supplemented with 1000 units/mL GM-CSF (Immunex, WA, USA) and 200 units/mL IL-4 (R&D Systems, Minneapolis, MN, USA) at days 0, 2, 4, and 6. In all experiments, morphine was added to the differentiating DCs at days 1, 3, and 5 at the indicated doses. Morphine was tested for the presence of endotoxin using the limulus assay (Biowhittaker), and the measured endotoxin levels in 10^{-2} M morphine were EU 0.001.

To analyze potential receptors used by morphine on DCs, the antagonist naloxone (10^{-6} M) (Sigma) was added to DCs on alternate days to morphine.

Stimulation of DCs

Monocytes (3×10^6) were cultured in 6-well plates for 7 days in the presence or absence of morphine and GM-CSF/IL-4 as described above. At day 7 of culture, immature DCs were either left untreated (immature [IM]) or stimulated with 100 ng/mL lipopolysaccharide (LPS) (*E. coli* serotype O26:B6; Sigma). DCs were left in the same wells, and LPS was added directly to the cultures. DCs were not washed before LPS addition; residual morphine could still be present in the cultures. Characteristics of DCs were analyzed after 48 h of stimulation.

Analysis of DC Phenotype

DCs (1×10^4) were labeled (by incubating in 100 μL PBS/5% FCS/0.1% sodium azide, staining buffer) with phycoerythrin (PE)-conjugated IgG specific for HLA-DR (Becton Dickinson Immunostaining Systems [BD], San Jose, CA, USA), CD83 (Immunotech-Beckman-Coulter, Marseille, France), or fluorescein isothiocyanate (FITC)-conjugated IgG mAb specific for CD86 and CD80 (BD) for at least 30 min at 4°C. Cells were then washed 4 times with staining buffer, fixed with 3.7% formaldehyde in PBS (pH 7.2-7.4), and examined by flow cytometry using FACScan (BD). In all experiments, isotype controls were included using an appropriate PE- or FITC-conjugated irrelevant mAb of the same Ig class. To detect the expression of opioid receptors on the cell surface, unlabeled primary antibodies against the opioid μ, κ, and δ receptors were used (Oncogene Research Products, San Diego, CA, USA) and detected using FITC-conjugated goat anti-mouse Ig (Biosource International, Camarillo, CA, USA).

Measurement of Cytokines and Chemokines

Cell culture supernatants were collected from day-9 DCs. Cells were left untouched after the initial seeding. The production of cytokines and chemokines in DC supernatants was measured by ELISA (Pierce Boston Technology Center, SearchLight Proteome Arrays Multiplex Sample Testing Services, Woburn, MA, US) 48 h after activation with LPS at day 7.

T Cell Isolation

T cells were isolated by negative selection using the RosetteSep antibody cocktail from Stem Cell Technologies (Vancouver, Canada) according to the manufacturer's instructions. The isolated T cells were routinely ~99% pure.

Mixed Leukocyte Reaction

To assess levels of cellular activation and proliferation, cells were plated at

10^5 cells/well in 96-well plates at a DC:T cell ratio of 1:100. After 5 days, the microcultures were pulsed with [^{3}H]thymidine (1 μCi/well) during the last 8 h of culture. At the end of the incubation period, the cells were harvested onto glass fiber filters with an automated multiple sample harvesters], and the amount of isotope incorporation was determined by liquid scintillation β-emission. Data is reported as mean cpm of thymidine incorporation per well; each experiment was carried out in triplicate.

RESULTS

Morphine Modifies LPS-Induced DC Maturation

The effects of opioids are mediated by at least 3 different opioid receptors termed μ, κ, and δ, which belong to the G-protein coupled membrane receptor family (28). The expression of morphine receptors on the cell surface of CD14$^+$ monocytes was investigated by flow cytometry. Monocytes were found to express all 3 opioid receptors (Figure 1A,B). Immature DCs and LPS-matured DCs expressed similar levels of all 3 opioid receptors, and the expression did not differ significantly from that of monocytes (Figure 1B). Chronic morphine stimulation was mimicked by adding morphine to the monocyte cultures at days 1, 3, and 5 during the differentiation process. The presence of morphine during the development of DCs from monocytes did not significantly alter the cell surface phenotype of immature DCs (data not shown). When immature DCs that had been cultured in the presence of morphine were stimulated with LPS, however, morphine-treated DCs showed increased expression levels of HLA-DR, CD86, and CD83 compared with DCs differentiated in the absence of morphine (Figure 2). No changes were observed in the expression levels of CD80, CD40, and CD54 (data not shown). The doses tested were physiological ($< 10^{-8}$) as well as pharmacological (10^{-6} to 10^{-8}). No difference in morphology was observed between morphine-exposed and nonexposed DCs, and the yield of DCs was not affected by morphine (data not shown).

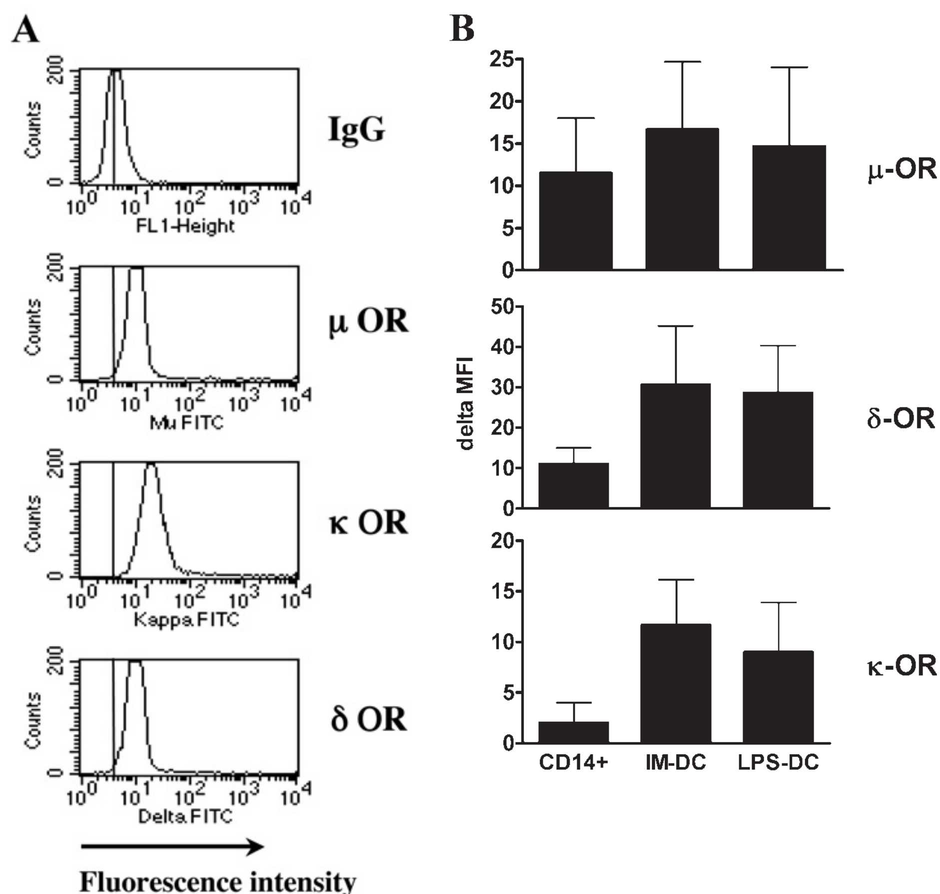

Figure 1. Expression of opioid receptors on monocytes and DCs. (A) Monocytes were analyzed for expression of the μ, κ, and δ opioid receptors by surface membrane immunofluorescence techniques using FITC-conjugated mAbs. The result shown is one representative of five independent experiments using cells from different donors. (B) Monocytes, immature DCs (IM-DC), and LPS-matured DCs (LPS-DC) were analyzed for expression of the μ, κ, and δ opioid receptors by surface membrane immunofluorescence techniques using FITC-conjugated mAbs. Results shown are mean ± SEM of five (monocytes) and three (IM-DC, LPS-DC) experiments using cells from different donors.

Another characteristic of mature DCs is the secretion of cytokines. To analyze whether morphine treatment altered the cytokine profile of LPS-matured DCs, cell culture supernatants were analyzed for IL-10 and IL-12. Morphine consistently reduced LPS-induced IL-10 secretion almost completely (to the levels present in immature DCs) in all 6 donors tested and increased LPS-induced IL-12 (p70) production in 4 of 6 donors tested (Figure 3). Furthermore, morphine did not induce changes in the cytokine profile in immature DCs (data not shown).

Morphine Mediates Changes in IL-12 Secretion through p38 MAPK

To investigate the involvement of MEK, p38 MAPK, PI3K, and NF-κB pathways in morphine-mediated changes of IL-12 and IL-10 production, we used their specific inhibitors, PD98059, SB203580, LY294002, and TPCK, respectively (Figure 4A). The LPS-induced secretion of IL-10 was completely reduced by the p38 MAPK inhibitor SB203580 (Figure 4A,B). Morphine-mediated reduction of IL-10

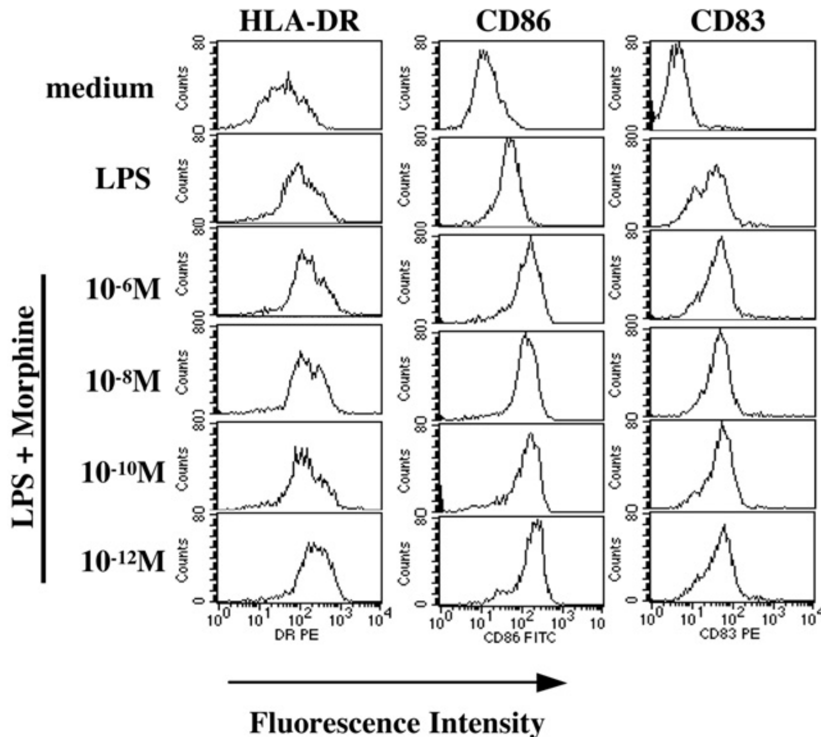

Figure 2. Morphine enhances expression of surface molecules in LPS-matured DCs. Immature DCs were differentiated in the absence or presence of different doses of morphine (10^{-6} to 10^{-12} M) for 7 days. DCs were exposed to medium only (IM) or LPS (100 ng/mL) for 48 h. DCs were analyzed for expression of the indicated markers by flow cytometry using PE- or FITC-conjugated mAbs. One representative experiment of 3 is shown on nongated DCs.

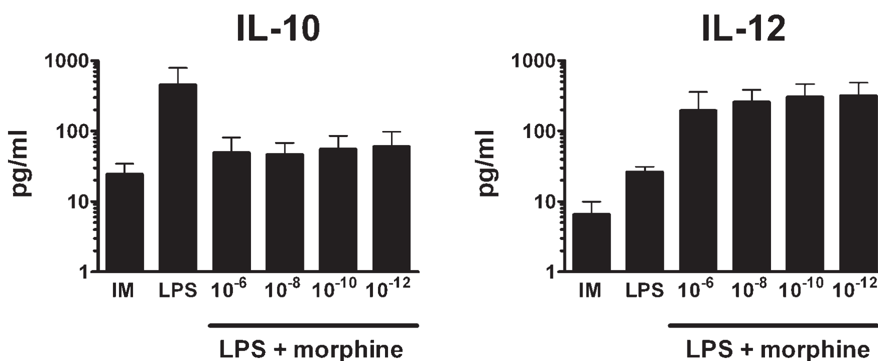

Figure 3. Effect of morphine on IL-10 and IL-12 production in LPS-matured DCs. Immature DCs were differentiated in the absence or presence of different doses of morphine (10^{-6} to 10^{-12} M) for 7 days. DCs were exposed to medium only (IM) or LPS (100 ng/mL) for 48 h. LPS, immature DCs exposed to LPS; LPS + morphine, morphine-treated immature DCs exposed to LPS. Cell culture supernatants were analyzed for the presence of IL-10 and IL-12 (p70) by ELISA. The results shown are mean ± SEM of 4 independent experiments.

secretion in LPS-matured DCs could not be reversed by any of the other inhibitors tested (Figure 4A). The LPS-induced IL 12 secretion and the morphine-mediated increase in IL-12 production were both reduced using the p38 MAPK inhibitor (Figure 4A,B). None of the other inhibitors reduced the morphine-augmented IL-12 levels (Figure 4A).

Morphine Enhances the T Cell Stimulatory Capacity of LPS-Matured DCs

Mature, cytokine-producing DCs induce T cell activation and proliferation, leading to the development of adaptive immunity (21). Because we found that morphine reduces IL-10 production in LPS-matured DCs, and because IL-10 is known to reduce T cell proliferation (29), we tested whether morphine-treated mature DCs would show an increased T cell stimulatory capacity. Immature DCs were generated in the presence or absence of morphine for 7 days and matured by exposure to LPS for 48 h. DCs were harvested and cocultured with allogeneic T cells for 5 days. Morphine-treated LPS-matured DCs showed increased activation of T cells (Figure 5A). To further evaluate whether morphine exerts its effect on DCs though opioid receptors, immature DCs were generated in the presence or absence of morphine as described above. The morphine-treated group was divided into two groups: one received only morphine every other day of culture and the other was exposed to the opioid antagonist naloxone on alternating days of morphine treatment. We found that the increased T cell stimulatory capacity of morphine-treated mature DCs could be blocked by naloxone (Figure 5B).

DISCUSSION

Morphine is widely used in the clinic. When given in vivo, morphine suppresses a variety of immune responses, including rat T and NK cells (8), macrophages, rat and macaque PMNs (9,10), and lymphocyte circulation in macaques (11). Although some studies

have recently been performed in mouse DCs, the function of opioid receptors on human DCs has not been investigated.

We show that human monocytes express all 3 opioid receptors on the cell surface and that chronic exposure of monocytes to morphine during the DC differentiation process enhances the T cell stimulatory capacity of LPS-matured DCs. It has been shown that mouse DCs that were cultured with the κ-opioid–specific antagonist dynorphin overnight showed suppressed T cell activation, but their phenotypic maturation was not affected (26). The mouse spleen contains several subtypes (plasmacytoid and myeloid) of DCs that are $CD11c^{+}$, each of which could respond in a different way to dynorphin. Therefore, mouse splenic $CD11c^{+}$ cells cannot be directly compared with our human myeloid DCs. Also, in our system, we are chronically exposing monocytes to morphine during the differentiation processes into DCs. Our experiments were designed to mimic constant exposure of differentiating DCs to morphine, which would be the case in people chronically exposed to morphine. Morphine was added to the cultures until day 5, and potential leftover morphine was not removed when the DCs were exposed to LPS. We do not know how a one-time exposure of immature DCs to morphine would affect their response to LPS. Morphine stability and turnover of opioid receptors bound to morphine on DCs remains to be tested. Also, it still needs to be evaluated whether morphine acts specifically on stimuli that go through toll-like receptors, such as LPS, and whether the same effects will be observed with other DC maturation stimuli such as CD40L.

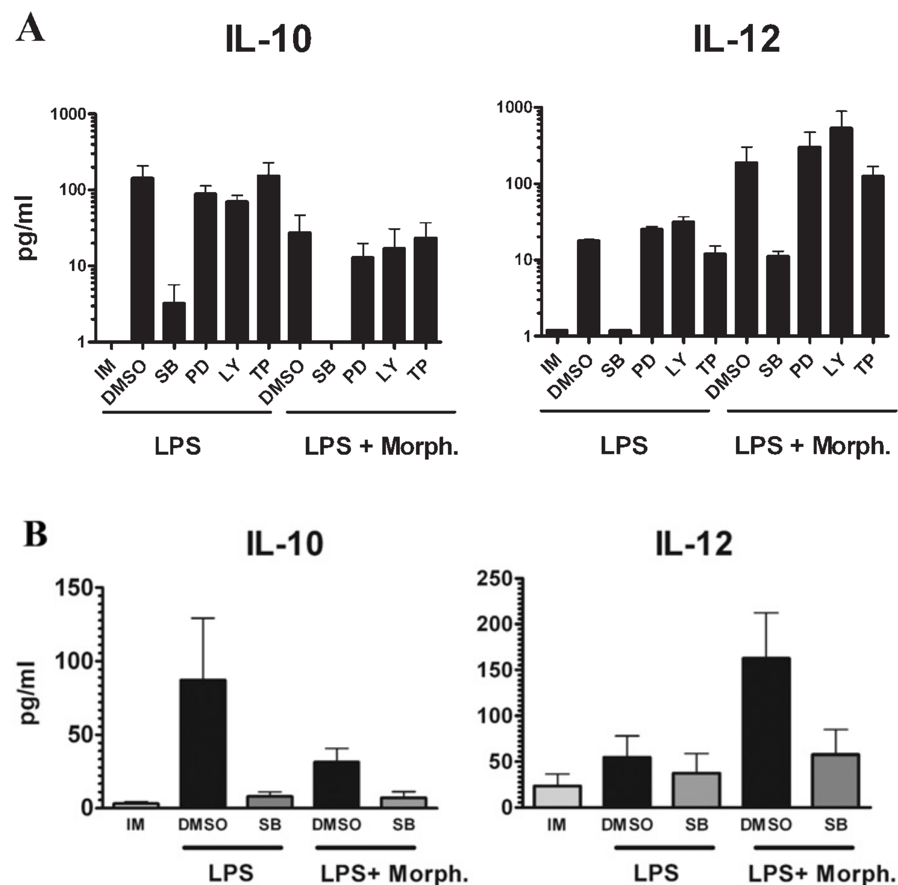

Figure 4. Morphine modulates IL-12 production through p38 MAPK. Immature DCs were differentiated in the absence or presence of morphine (10^{-8} M) for 7 days. DCs were exposed to medium only (IM) or LPS (100 ng/mL). LPS, immature DCs exposed to LPS; LPS + morphine, morphine-treated immature DCs exposed to LPS. (A) One hour before LPS exposure, immature d 7 DCs were incubated with DMSO or 5 μM SB203580 (SB), PD98059 (PD), LY294002 (LY), or TPCK (TP). Forty-eight hours after addition of LPS, cell culture supernatants were analyzed for the presence of IL-10 and IL-12 (p70) by ELISA. The results shown are mean ± SEM of 2 independent experiments. (B) One hour before LPS exposure, immature d 7 DCs were incubated with DMSO or 5 μM SB203580 (SB). Forty-eight hours after addition of LPS, cell culture supernatants were analyzed for the presence of IL-10 and IL-12 (p70) by ELISA. The results shown are mean ± SEM of 4 independent experiments.

Here we show that morphine exerts stimulatory effects on human myeloid DCs in vitro. The different results obtained in comparison with the mouse DCs could be because morphine can signal through all 3 opioid receptors, whereas dynorphin is κ-receptor specific. Furthermore, it is likely that a mixture of myeloid and plasmacytoid DCs responds differently than a culture of only myeloid DCs, as in our experiments. Another study showed that mouse bone marrow–derived DCs show increased T cell activation when incubated with δ- or μ-specific agonist (30). These bone marrow–derived DCs were generated in the presence of GM-CSF, which yields only myeloid DCs (31). Therefore, this system resembles our human in vitro culture system, and the results obtained with regard to T cell activation are in concordance. A DC-stimulatory property of another neuroactive ligand has recently been identified. Basu and Srivastava (32) showed that DCs express the vanilloid receptor 1, which binds capsaicin and acts on neurons to convey the sensation of pain. Engagement of the vanilloid receptor with capsaicin caused maturation of DCs. Thus, it appears that the signals for pain and immunomodulation are intricately linked.

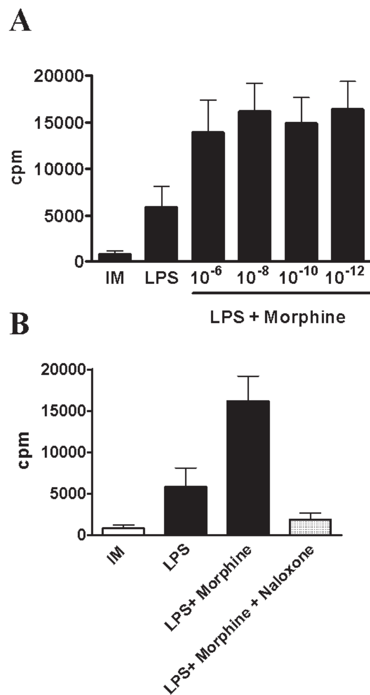

Figure 5. Morphine treatment increases the T cell stimulatory capacity of LPS-matured DCs. (A) Immature DCs were differentiated in the absence or presence of different doses of morphine (10^{-6} to 10^{-12} M) for 7 days. DCs were exposed to medium only (IM) or LPS (100 ng/mL) for 48 h. LPS, immature DCs exposed to LPS; LPS + morphine, morphine-treated immature DCs exposed to LPS. Two days after exposure to LPS, DCs were cocultured with 10^5 allogeneic T cells at a DC:T cell ratio of 1:100. T cell proliferation was assessed by measuring the amount of (^{3}H)thymidine incorporated during the last 8 h of a 5-day culture period. The results shown are mean ± SEM of 3 independent experiments using DCs generated from different donors. (B) Immature DCs were differentiated in the absence or presence of morphine (10^{-8} M) for 7 days. The morphine-treated group was divided into two groups: one received only morphine and the other morphine and naloxone (10^{-6} M) on alternating days until day 7. On day 7, DCs were exposed to medium only (IM) or LPS (100 ng/mL) for 48 h. On day 9, DCs were harvested, washed, and cocultured with 10^5 allogeneic T cells at a DC:T cell ratio of 1:100. T cell proliferation was assessed by measuring the amount of (^{3}H)thymidine incorporated during the last 8 h of a 5-day culture period. The results shown are mean ± SEM of 3 independent experiments.

Our results show that prolonged exposure of DCs to morphine during their differentiation from monocytes leads to a MAPK-dependent increase in IL-12 production when stimulated with LPS. This was paralleled by an increased T cell stimulatory capacity that could be blocked using the opioid antagonist naloxone, suggesting involvement of a classic opioid receptor.

IL-10 can inhibit LPS-induced DC maturation (33,34). It also functions as an endogenous inhibitor of IL-12 in DCs (35) and Th1 responses but stimulates Th2 cell population and humoral immunity, whereas IL-12 is an inducer of cell-mediated immunity that promotes the development, proliferation, and function of Th1 cells. IL-12 promotes the activation and function of NK cells, T cell cytotoxicity, and production of IL-2 and IFN-γ (36). Because morphine-treated DCs showed enhanced IL-12 production, this effect of morphine may be the result of the morphine-induced inhibition of IL-10 generation. In murine macrophages, morphine increased IL-12 production but did not alter IL-10 production (37). This could be due to species differences or different signaling pathways utilized in macrophages and DCs.

Only the p38 MAPK inhibitor SB203580, and none of the other inhibitors tested, reduced the morphine-augmented IL-12 levels, suggesting that morphine mediates increased IL-12 production via a p38 MAPK–dependent pathway. It is likely that morphine increased the amount of phosphorylated p38 in DCs. However, we cannot exclude that morphine also alters other signaling pathways that were not investigated in this study.

Endogenous opiates exert immunostimulatory effects (3,4). Helper T cells produce endogenous opioids, and memory T cells within inflammatory tissue are capable of synthesizing and releasing β-endorphins (38-40). Because DCs can respond to exogenous opioids, endogenous opioids might play a role in DC-T cell communication. When released from helper T cells, opioids could act on DCs to foster increased IL-12 production, which would shift the helper T cells to a Th1 phenotype.

Even though morphine has been shown to exert inhibitory effects on T cells and in certain conditions macrophages (6,14), our data show that it exerts a stimulatory effect on DCs in vitro. However, in vivo an increased Th1 response due to high levels of IL-12, and increased T cell proliferation in the context of an infection, might not be desirable and have negative effects on the host's immune response. As it has been shown that exaggeration of the IL-12 response may result in endotoxic shock (41,42) or allograft rejection (41-44) and can block antiparasitic immunity (45), the stimulatory effect of morphine on DCs might in fact have a negative impact on the development of an immune response in vivo. On the other hand, ex vivo–generated DCs that are used for immunotherapy of cancer patients, if generated in the presence of morphine, might induce a stronger immune response to tumor because of elevated IL-12 levels.

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REFERENCES

1. Rouveix B. (1992) Opiates and immune function: consequences on infectious diseases with special reference to AIDS. *Therapie* 47:503-12.
2. Sibinga NE, Goldstein A. (1988) Opioid peptides and opioid receptors in cells of the immune system. *Annu. Rev. Immunol.* 6:219-49.
3. Weigent DA, Blalock JE. (1987) Interactions between the neuroendocrine and immune systems: common hormones and receptors. *Immunol. Rev.* 100:79-108.
4. Vallejo R, de Leon-Casasola O, Benyamin R. (2004) Opioid therapy and immunosuppression: a review. *Am. J. Ther.* 11:354-65.
5. Roy S, Loh HH. (1996) Effects of opioids on the immune system. *Neurochem. Res.* 21:1375-86.
6. Eisenstein TK, Hilburger ME. (1998) Opioid modulation of immune responses: effects on phagocyte and lymphoid cell populations. *J. Neuroimmunol.* 83:36-44.

7. Bryant HU, Bernton EW, Holaday JW. (1987) Immunosuppressive effects of chronic morphine treatment in mice. *Life Sci.* 41:1731-8.
8. Liang-Suo J, Gomez-Flores R, Weber RJ. (2002) Immunosuppression induced by central action of morphine is not blocked by mifepristone (RU 486). *Life Sci.* 71:2595-602.
9. Luza J. (1992) Effect of morphine on phagocytic activity of the polymorphonuclears and monocytes. *Acta Univ. Palacki Olomuc. Fac. Med.* 134:47-50.
10. Liu Y, Blackbourn DJ, Chuang LF, Killam KF Jr, Chuang RY. (1992) Effects of in vivo and in vitro administration of morphine sulfate upon rhesus macaque polymorphonuclear cell phagocytosis and chemotaxis. *J. Pharmacol. Exp. Ther.* 263:533-9.
11. Donahoe RM, et al. (2001) Effects of morphine on T-cell recirculation in rhesus monkeys. *Adv. Exp. Med. Biol.* 493:89-101.
12. Bryant HU, Bernton EW, Holaday JW. (1988) Morphine pellet-induced immunomodulation in mice: temporal relationships. *J. Pharmacol. Exp. Ther.* 245:913-20.
13. Singhal P, Kapasi A, Reddy K, Franki N. (2001) Opiates promote T cell apoptosis through JNK and caspase pathway. *Adv. Exp. Med. Biol.* 493:127-35.
14. Bhat RS, Bhaskaran M, Mongia A, Hitosugi N, Singhal PC. (2004) Morphine-induced macrophage apoptosis: oxidative stress and strategies for modulation. *J. Leukoc. Biol.* 75:1131-8.
15. Malik AA, Radhakrishnan N, Reddy K, Smith AD, Singhal PC. (2002) Morphine-induced macrophage apoptosis modulates migration of macrophages: use of in vitro model of urinary tract infection. *J. Endourol.* 16:605-10.
16. Bhaskaran M, et al. (2001) Morphine-induced degradation of the host defense barrier: role of macrophage injury. *J. Infect. Dis.* 184:1524-31.
17. Singhal PC, Sharma P, Kapasi AA, Reddy K, Franki N, Gibbons N. (1998) Morphine enhances macrophage apoptosis. *J. Immunol.* 160:1886-93.
18. Gomez-Flores R, Suo JL, Weber RJ. (1999) Suppression of splenic macrophage functions following acute morphine action in the rat mesencephalon periaqueductal gray. *Brain Behav. Immun.* 13:212-24.
19. Roy S, Charboneau RG, Barke RA. (1999) Morphine synergizes with lipopolysaccharide in a chronic endotoxemia model. *J. Neuroimmunol.* 95:107-14.
20. Eisenstein TK, Rogers TJ, Meissler JJ Jr, Adler MW, Hilburger ME. (1998) Morphine depresses macrophage numbers and function in mouse spleens. *Adv. Exp. Med. Biol.* 437:33-41.
21. Banchereau J, Steinman RM. (1998) Dendritic cells and the control of immunity. *Nature* 392:245-52.
22. Asahina A, Hosoi J, Grabbe S, Granstein RD. (1995) Modulation of Langerhans cell function by epidermal nerves. *J. Allergy Clin. Immunol.* 96:1178-82.
23. Asahina A, et al. (1995) Specific induction of cAMP in Langerhans cells by calcitonin gene-related peptide: relevance to functional effects. *Proc. Natl. Acad. Sci. U. S. A.* 92:8323-7.
24. Marriott I, Mason MJ, Elhofy A, Bost KL. (2000) Substance P activates NF-kappaB independent of elevations in intracellular calcium in murine macrophages and dendritic cells. *J. Neuroimmunol.* 102:163-71.
25. Delneste Y, et al. (1999) Vasoactive intestinal peptide synergizes with TNF-alpha in inducing human dendritic cell maturation. *J. Immunol.* 163:3071-5.
26. Kirst A, Wack C, Lutz WK, Eggert A, Kampgen E, Fischer WH. (2002) Expression of functional kappa-opioid receptors on murine dendritic cells. *Immunol. Lett.* 84:41-8.
27. Makarenkova VP, et al. (2001) Identification of delta- and mu-type opioid receptors on human and murine dendritic cells. *J. Neuroimmunol.* 117:68-77.
28. Reisine T, Brownstein MJ. (1994) Opioid and cannabinoid receptors. *Curr. Opin. Neurobiol.* 4:406-12.
29. Taga K, Tosato G. (1992) IL-10 inhibits human T cell proliferation and IL-2 production. *J. Immunol.* 148:1143-8.
30. Esche C, et al. (1999) Murine dendritic cells express functional delta-type opioid receptors. *Ann. N. Y. Acad. Sci.* 885:387-90.
31. Gilliet M, et al. (2002) The development of murine plasmacytoid dendritic cell precursors is differentially regulated by FLT3-ligand and granulocyte/macrophage colony-stimulating factor. *J. Exp. Med.* 195:953-8.
32. Basu S, Srivastava P. (2005) Immunological role of neuronal receptor vanilloid receptor 1 expressed on dendritic cells. *Proc. Natl. Acad. Sci. U. S. A.* 102:5120-5.
33. McBride JM, Jung T, de Vries JE, Aversa G. (2002) IL-10 alters DC function via modulation of cell surface molecules resulting in impaired T-cell responses. *Cell Immunol.* 215:162-72.
34. Corinti S, Albanesi C, la Sala A, Pastore S, Girolomoni G. (2001) Regulatory activity of autocrine IL-10 on dendritic cell functions. *J. Immunol.* 166:4312-8.
35. Xia CQ, Kao KJ. (2003) Suppression of interleukin-12 production through endogenously secreted interleukin-10 in activated dendritic cells: involvement of activation of extracellular signal-regulated protein kinase. *Scand. J. Immunol.* 58, 23-32.
36. Trinchieri G. (1995) Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. *Annu. Rev. Immunol.* 13:251-76.
37. Peng X, Mosser DM, Adler MW, Rogers TJ, Meissler JJ Jr, Eisenstein TK. (2000) Morphine enhances interleukin-12 and the production of other pro-inflammatory cytokines in mouse peritoneal macrophages. *J. Leukoc. Biol.* 68:723-8.
38. Zurawski G, Benedik M, Kamb BJ, Abrams JS, Zurawski SM, Lee FD. (1986) Activation of mouse T-helper cells induces abundant preproenkephalin mRNA synthesis. *Science* 232:772-5.
39. Linner KM, Quist HE, Sharp BM. (1995) Met-enkephalin-containing peptides encoded by proenkephalin A mRNA expressed in activated murine thymocytes inhibit thymocyte proliferation. *J. Immunol.* 154:5049-60.
40. Cabot PJ, Carter L, Schafer M, Stein C. (2001) Methionine-enkephalin-and Dynorphin A-release from immune cells and control of inflammatory pain. *Pain* 93:207-12.
41. Gately MK, et al. (1998) The interleukin-12/interleukin-12-receptor system: role in normal and pathologic immune responses. *Annu. Rev. Immunol.* 16:495-521.
42. Ulevitch RJ, Tobias PS. (1995) Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. *Annu. Rev. Immunol.* 13:437-57.
43. Falcone M, Sarvetnick N. (1999) Cytokines that regulate autoimmune responses. *Curr. Opin. Immunol.* 11:670-6.
44. Li W, et al. (2001) Il-12 antagonism enhances apoptotic death of T cells within hepatic allografts from Flt3 ligand-treated donors and promotes graft acceptance. *J. Immunol.* 166:5619-28.
45. Finkelman FD, et al. (1997) Cytokine regulation of host defense against parasitic gastrointestinal nematodes: lessons from studies with rodent models. *Annu. Rev. Immunol.* 15:505-33.