Published in final edited form as:
Cell Immunol. 2008 ; 252(1-2): 146–154. doi:10.1016/j.cellimm.2007.09.00.

Opioid and Nociceptin Receptors Regulate Cytokine and Cytokine Receptor Expression

M. J. Finley[1,4], **C. M. Happel**[1,4], **D. E. Kaminsky**[2,4], and **T. J. Rogers**[1,3,4]

1 *Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, Philadelphia, PA 19140*

2 *Departments of Microbiology and Immunology, Temple University School of Medicine, Philadelphia, PA 19140*

3 *Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140*

4 *Center for Substance Abuse Research. Temple University School of Medicine, Philadelphia, PA 19140*

Abstract

Opioids were originally discovered because of their ability to induce analgesia, but further investigation has shown that the opioids regulate the function of cells involved in the immune response. We suggest that the regulation of cytokine, chemokine, and cytokine receptor expression is a critical component of the immunomodulatory activity of the opioids. In this paper we review the literature dealing with the regulation of cytokine and cytokine receptor expression by agonists for the three major opioid receptor types (μ, κ, and δ), and nociceptin, the natural agonist for the orphanin FQ/nociceptin receptor. Although the opioid receptors share a high degree of sequence homology, opposing roles between the kappa opioid receptor (KOR) and the mu opioid receptor (MOR) have become apparent. We suggest that activation of the KOR induces an anti-inflammatory response through the down-regulation of cytokine, chemokine and chemokine receptor expression, while activation of the MOR favors a pro-inflammatory response. Investigation into the opioid receptor-like (ORL1)/nociceptin system also suggests a role for this receptor as a down-regulator of immune function. These effects suggest a broad role for opioids in the modulation of the function of the immune system, and suggest possible targets for the development of new therapeutics for inflammatory and infectious diseases.

Keywords

Opioid; Opioid receptors; Chemokine; Chemokine receptors; Cytokine; Nociceptin; GPCR; HIV; ORL1

1. Introduction

For many centuries, opium derived from secretions of *Papaver somniferum* seedpods have been utilized for analgesic purposes. Morphine, an opium alkaloid, was isolated in 1803 by

Address all correspondence to: Dr. Thomas J. Rogers, Fels Institute for Cancer Research and Molecular Biology, Temple University School of Medicine, 3307 N. Broad Street, Philadelphia, PA 19140. Email: rogerst@temple.edu, Phone: 215-707-3215; FAX: 215-707-4743.

Serturner and was later found to be primarily responsible for the analgesic properties of opium. However, other metabolites of heroin may also posses pharmacological activities considering that the heroin metabolites 6-monoacetylmorphine (6 MAM), morphine, morphine-6-β-D-glucuronide (M6G), and morphine-3-β-D-glucuronide (M3G) can induce discriminative stimulus effects in rhesus monkeys similar to heroin [1;2].

Three distinct opioid receptor classes have been identified and cloned, and are designated μ, κ, and δ [3–7]. All three opioid receptors are widely distributed in the central nervous system (CNS). The expression of opioid receptors on cells of the immune system was first implicated by the ability of opioids to alter immune function. Wybran et al. [8] provided the first experimental evidence that cells of the immune system express opioid receptors. These studies showed that morphine inhibited the ability of human T lymphocytes to rosette with sheep erythrocytes, and this effect could be blocked by the opioid antagonist naloxone. Based on this evidence, Wybran et al. hypothesized that naloxone-sensitive receptors were expressed on human T lymphocytes. Since these initial studies, pharmacological, molecular, and, more recently, immunologic evidence for the expression of opioid receptors on immune cells have been reported [9–11]. Furthermore, μ-, κ-, and δ-opioid receptors (MOR, KOR, and DOR) have been cloned from mRNA isolated in primary lymphocytes, primary macrophages, and several cell lines with sequences that are essentially identical to opioid receptors found in the CNS [12–16].

The receptor designated Opioid Receptor-Like 1 (ORL1) exhibits substantial sequence homology with the classic opioid receptors [17–20]. Similar to the classic opioid receptors, ORL1 is coupled to G proteins and expressed within the CNS as well as on cells of the immune system [21]. Moreover, human immune cells also express the precursor protein for nociceptin, prepronociceptin, suggesting the presence of an intact ORL1-nociceptin circuit entirely within the immune system [22]. The functional capacity of ORL1 was demonstrated by the ability of nociceptin to induce the chemotaxis of immune cells [23;24]. A recent study suggests that expression of ORL1 may actually be greater on cells of the immune system [25], but despite this observation, little attention has been given to potential regulation of the immune response by the ORL1-nociceptin system.

Treatment with various opioid compounds has been shown to modulate a number of aspects of the immune response, including antibody responses in vitro and in vivo [26–28], phagocytic cell function [29;30], Natural Killer Cell activity [31], the development and function of T cells in the thymus [32–34], and cytokine and cytokine receptor expression (reviewed below). As mentioned above, it is clear that cells of the immune system express opioid receptors. However, at the present time it is not possible to state with certainty how universal this expression is. It is clear that levels of expression of the opioid receptors on leukocytes are lower than for neurons. In contrast, the nociceptin receptor appears to be expressed by some cells of the immune system at relatively high levels, and at levels which are equivalent to expression in the brain [21]. Once a set of reliable monoclonal antibodies is available, which would permit direct analysis of the expression of the opioid and nociceptin receptors by sub-populations of leukocytes, we will be in a position to learn a great deal more about the overall role of opioid and nociceptin receptors in the immune system.

2. Effects of Opioids and Nociceptin on the Expression of Cytokines and Cytokine Receptors

Regulation of immune and inflammatory responses is dependent on the functions of cytokines, and we suggest that many of the immunomodulatory activities of the opioids and nociceptin are due, at least in part, to the modulation of cytokine expression. The experience of investigators with the opioids, particularly the relatively non-selective opioid agonist

morphine, is complicated by effects which are not sensitive to treatment with an opioid antagonist such as naloxone. This is also true for the opioid β-endorphin [35], a peptide which appears to interact with a binding site on some leukocytes that is not a classic opioid receptor (reviewed in [36]). These effects are assumed to be due to mechanisms which are not classical opioid receptor mediated. For example, Brown and Van Epps [37] showed that both β-endorphin and met-enkephalin elevated IFN-γ production by Concanavalin A (ConA)-stimulated human peripheral blood mononuclear cells (PBMCs), but the increase in IFN-γ production was not reversible by the opioid antagonist naloxone. Further, Mandler et al. [38] also found that β-endorphin increased IFN-γ production from phytohemagglutinin (PHA)-stimulated human lymphocytes, and this effect was partially reversed with naloxone. On the other hand, Peterson et al. [39] found that human PBMCs pretreated for 3 hours with either β-endorphin or morphine followed by ConA stimulation failed to produce control levels of IFN-γ, and this effect was reversed in the presence of naloxone.

Bessler et al. [40] showed that pretreatment with β-endorphin significantly enhanced IL-1-induced IL-2 production in two murine lymphoma cell lines, and that this effect was blocked by pretreatment with naloxone. However, pretreatment of rat splenocytes with β-endorphin followed by 24 hour stimulation with Con-A increased both IL-2 production and IL-2 receptor α-chain expression [41], and the latter effect was not reversed by naloxone, suggesting the participation of a non-classical opioid receptor. In contrast to these results with the relatively non-selective agonist β-endorphin, a dose-dependent inhibition of IL-2 and IL-4 was observed when murine splenocytes were incubated for 24 hours with relatively high concentrations of morphine and then stimulated with ConA [42]. Lysle et al. [43] observed that morphine injected subcutaneously into rats induced naltrexone-reversible, dose-dependent suppression of IL-2 and IFN-γ production by splenic lymphocytes. These results are consistent with more recent studies which show that lymph node T cells from mice treated with low doses of morphine failed to produce control levels of either IL-2 or IFN-γ in response to ConA [44], and these effects were absent in MOR-knockout mice.

The immunomodulatory properties of the opioids and nociceptin are not fully understood at the present time. However, it is possible that the altered expression of some of the cytokines is due to the opioid-induced production of regulatory mediators which, in turn, lead to altered levels of cytokines such as IL-2 and IFN-γ. A good candidate for this type of two-step regulation would involve Transforming Growth Factor (TGF)- β. Chao et al. [45] has shown that morphine induces a naloxone-reversible increase in TGF-β production following treatment of lipopolysaccharide (LPS) - or PHA-stimulated PBMCs. The inhibition of IL-2 and IFN-γ production following morphine administration might be explained by the well documented immunomodulatory activity of TGF-β. A role for TGF-β in the opioid-induced regulation of chemokine expression is described below.

An additional factor in the consideration of effects of opioids on the immune response is related to the nature of the opioid administration. For example, there is evidence that chronic morphine treatment results in reduced IL-2 and IFN-γ production, with an increase in IL-4 and IL-5 [46;47], and here again, these effects are lost in MOR-knockout mice. These effects have been attributed to a polarization of the T cells to the Th2 phenotype [47;48]. This is in contrast to studies which show that peritoneal macrophages from mice given relatively high doses of morphine in vivo, followed by stimulation ex vivo, exhibit elevated expression of IL-12 and TNFα [49]. The latter two sets of results [47;49] are difficult to rectify since an increase in the production of IL-12 would not be consistent with an overall Th2 shift in the T cell phenotype. However, the results do serve to demonstrate the disparate effects achieved when comparing acute and chronic morphine administration.

The expression of the pro-inflammatory monocyte/macrophage-derived cytokines, such as IL-1, IL-6, and TNFα is also regulated following morphine administration. Several laboratories have reported evidence which shows that endogenous opioid administration leads to the modulation of cytokine expression. Apte et al. [50;51] have shown that endogenous endorphins and enkephalins increase IL-1 production. Studies reported by Roy et al. [52] suggest that morphine can synergize with LPS to augment the production of both IL-6 and TNFα. In a recent study reported by Wang et al. [53] using a murine model of *Streptococcal pneumoniae* infection, chronic morphine administration impairs resistance to infection. Furthermore, in the morphine-treated animals, there was a significant decrease in the levels of TNFα, IL-1 and IL-6 in the bronchoalveolar lavage fluid. It appeared that the impaired cytokine levels, together with reduced levels of the chemokines CXCL2 and CXCL1, and impaired galectin-3 in the alveolar space, resulted in a reduction in the migration of protective neutrophils to the site of infection [53]. These results are particularly interesting when viewed in the context of earlier results showing that the reduced capacity of splenocytes from morphine-treated mice to produce antibody ex vivo could be reversed by the addition of exogenous IL-1, IL-6 or IFN-γ [54]. The latter results suggest impairment in the levels of IL-1 and/or IL-6, and would be entirely consistent with the results of Wang et al. [53] which suggest that morphine inhibits the production of some of the pro-inflammatory monocyte/macrophage-derived cytokines. However, studies carried out with human peripheral blood cells (described below) to determine the effects of morphine on pro-inflammatory chemokines appear to suggest that opioid administration does not induce a universal impairment in pro-inflammatory cytokine expression.

Studies on the KOR have led to the conclusion that activation of this opioid receptor has, in general, an immunosuppressive effect [55]. Studies carried out by Belkowski et al. (1995b) showed that the KOR agonist trans-3,4-dichloro-N-methyl-N[2-(1-pyrolidinyl)cyclohexyl] benzeneacetamide methanesulfonate (U50,488H) decreased the LPS-induced augmentation of IL-1 and TNFα produced by the macrophage-like cell line $P388D_1$. More recently, Alicea et al. [12] demonstrated, using non-elicited peritoneal macrophages activated with LPS, that treatment with U50,488H at concentrations as low as 1nM resulted in decreased production of IL-1, TNFα and IL-6. Additional studies showed that the inhibition in cytokine expression was apparent at the transcriptional level. The molecular basis for these effects remains uncertain.

Guan et al. [56] have determined that murine thymocytes stimulated with Staphylococcal enterotoxin B (SEB) in the presence of activated macrophages exhibit significantly reduced IL-2 production following administration of U50,488H. Indeed, expression of KOR by developing T cells in the thymus appears to be relatively high [57;58], suggesting that the KOR may play a role in regulating T cell differentiation. The expression of KOR by double-negative (DN; CD4/CD8-negative) thymocytes as well as the immature T-cell lines R1.1 and DPK has been established by both radioactive ligand binding assays and RT-PCR [10;13;57;59]. The R1.1 cell line represents a very immature double negative developing T cell with low surface expression of CD3 and CD25, and the DPK line is representative of the somewhat more mature CD3+CD4+CD8+ stage of thymocyte differentiation. Guan and coworkers [59] observed that following stimulation with superantigen in culture for 4 days, DPK cells progressed from the double-positive stage to a CD4+ CD8intermediate stage, with further differentiation of approximately 25% of these stimulated cells to the CD4+CD8- phenotype. These investigators found that U50,488H inhibited the superantigen-induced differentiation of DPK cells. Approximately 60% of the DPK cells exposed to U50,488H remained CD4+CD8+ following SEA stimulation. Cells that did undergo differentiation failed to progress beyond the CD4 +CD8intermediate stage.

In an effort to further examine the role of the KOR in the function of developing T cells in the thymus, we have tested the impact of U50,488H administration on the expression of cytokines

and cytokine receptors in superantigen-stimulated thymocytes by RNase-protection analysis (RPA) [60]. We have measured detectable levels of the cytokines IL-2, IL-4, IL-5, IL-13 and IFNγ, and the chemokines lymphotactin and RANTES in stimulated thymocyte cultures; however, addition of U50,488H did not alter the expression of these cytokines. Examination of cytokine receptor expression by these thymocytes revealed a significant inhibition in the expression of the transcript for the IL-7 receptor α-chain (IL-7Rα), and these results were confirmed at the protein level by flow cytometric analysis of the IL-7 receptor. These results are particularly interesting given the critical role of IL-7 in the maturation of both T and B cells. The IL-7 receptor is essential for the normal maturation of T cells in the thymus based on the reduced numbers of mature T cell subsets in mice with deletions of the IL-7 or IL-7 receptor genes [61;62], and overexpression of IL-7 results in a restoration of T cell populations in nude mice [63]. It is also apparent that IL-7 acts to support the survival of immature T cells in part by providing an anti-apoptotic signal to these cells [64]. Very little is known about the regulation of IL-7 receptor expression. Surprisingly, the expression of several other cytokine receptor chains, including the common γ-chain, IL-2Rβ, IL-2Rα, IL-4Rα, and IL-15Rα chains were not altered by U50,488H treatment.

Analyses of DOR function in the regulation of cytokine expression have primarily centered on IL-2 expression. A study reported by Shahabi and Sharp [65] showed that murine splenic $CD4^+$ T-cells treated with the DOR agonist deltorphin and then stimulated with plate-bound anti-CD3 exhibited an increase in IL-2 production when deltorphin was given at very low doses (10^{-11}M). In contrast, a decrease in IL-2 production was observed following deltorphin administration at a higher dose (10^{-7}M) revealing an unexpected biphasic modulation of lymphokine production. House et al. [66] demonstrated that the DOR agonists [D-Pen^2, D-Pen^5]enkephalin (DPDPE) and deltorphin administration elevated anti-CD3-induced IL-2, IL-4 and IL-6 production. Studies carried out with Jurkat T cells transfected to express the DOR have confirmed that DOR agonist administration elevates the production of IL-2 [67]. Studies with these transfected cells suggest that the enhanced IL-2 production occurs through an opioid-induced increase in the activity of the Nuclear Factor of Activated T cells (NF-AT)/ Activator Protein-1 (AP-1) element of the IL-2 gene promoter.

Very little is known about the influence of ORL1 on the expression of cytokines by cells of the immune system. Zhao and colleagues [68] used a rat model to induce inflammation and analyze the effects of nociceptin on proinflammatory cytokine production. Following the induction of trauma in these animals, they found typical, robust increases in both TNFα and IL-1β transcripts in peritoneal macrophages. However, when nociceptin was administered by intracerebroventricular injection, both TNFα and IL-1β expression was attenuated in these cells. While these results might suggest an anti-inflammatory influence by nociceptin, the results from Goldfarb et al., [69] would argue that a simple pro- versus anti-inflammatory designation for nociceptin might not be entirely accurate. In this study, the effect of nociceptin administration on staphylococcal enterotoxin A (SEA)-induced expression of the proinflammatory cytokines TNFα, IL-1β, IL-2, and INFγ was examined. They determined that nociceptin injection into mice prior to SEA challenge produced an enhancement of TNFα and INFγ mRNA in the spleen, as well as increased plasma levels of TNFα. It is apparent that more research will be needed to further define how nociceptin is involved in regulating inflammation.

3. Effects of Opioids and Nociceptin on Chemokine Expression

Chemokines are a group of small proteins that were discovered because of their association with the inflammatory response and for their ability to regulate cell trafficking of leukocytes. Chemokines are now known to be involved in mediating chemotaxis, as well as integrin activation and leukocyte-endothelial cell interactions, leukocyte degranulation, and release of inflammatory mediators and angiogenesis [70;71]. Chemokines are classified by the location

of conserved cysteine residues and divided into 4 main families: CC, CXC, C and CX_3C. Increased expression of chemokines has been associated with a wide range of inflammatory diseases and pathologies and chemokine antagonists are being hailed as the new generation of inflammatory therapeutics with the hope of more specific inhibition and fewer adverse reactions.

The μ-selective agonist, [$_D$-Ala2,N-Me-Phe4,Gly-ol5]enkephalin (DAMGO) has theability to increase the expression of several pro-inflammatory chemokines, including CCL2, CCL5 and CXCL10, in non-activated and PHA-stimulated human PBMCs [72]. This increase in chemokine expression was seen at both the RNA and protein levels and pretreatment with D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH_2 (CTAP), a selective MOR antagonist, abolished the DAMGO-induced increase of CCL2 and CCL5 [72]. Recent studies have also shown that activation of the MOR by either morphine or DAMGO induces a significant increase in CCL2 expression in primary human neurons [73].

Studies investigating the mechanism of opioid receptor regulation of chemokine expression could be particularly significant in understanding the inflammatory response. Preliminary results from our laboratory have implicated a role for TGF-β in MOR regulation of CCL5. Although generally considered anti-inflammatory, TGF-β has been shown have a pivotal function in the immune system [74]. TGF-β is a pleiotropic cytokine affecting a number of cellular processes, including cell growth and differentiation [75]. The cellular response to TGF-β is diverse depending on the cell context and the physiological environment resulting in differential regulation of TGF-β. There is some evidence that TGF-β acts as a chemoattractant for human peripheral blood monocytes and in this way may play an additional role in the regulation of monocyte recruitment to the site of injury or inflammation [76]. Moreover, TGF-β can induce transcription of pro-inflammatory regulators such as IL-1 and IL-6 in human monocytes [77;78].

Regulation of TGF-β by opioids has not been fully investigated. Chao et al. [45] reported that PBMCs exposed to morphine for 24 hours resulted in an increased amount of TGF-β. Preliminary results from our laboratory show that DAMGO also increases TGF-β expression in PBMCs, an effect which is blocked by addition of CTAP. Although the biochemical basis for this effect remains uncertain, however, there are published results which suggest a possible molecular mechanism. An examination of the MOR signal transduction pathway shows that MOR activation results in an increase in the activity and DNA-binding activity of the transcription factors Nuclear Factor-κB (NF-κB) and AP-1 in primary neurons [79]. Bilecki et al. [80] reported that stimulation of MOR resulted in the increased phosphorylation and activity of cAMP Response Element-Binding (CREB) transcription factor, as well as the increased activity and DNA-binding activity of AP-1 in the Neuro2aMOR neuronal cell line. The promoters for CCL2 and CCL5 have been shown to possess consensus sequences for these transcription factors [81;82], and activation of the TGF-β receptor may serve to induce the expression of these chemokines by this mechanism. In addition, TGF-β is known to induce its own mRNA expression, and results show that this is mediated by the binding of AP-1 (Jun-Fos) to a binding site within the TGF-β promoter [83]. Taken together these results suggest a novel role for TGF-β in the immune system as an inducer of pro-inflammatory chemokine expression.

In contrast to the increase in chemokine expression seen following stimulation of MOR in leukocytes, activation of MOR in neuronal cells results in a decrease in chemokine expression. Mahajan et al. [84;85] reported that normal human astrocytes show diminished expression of the chemokines, CCL4, CCL2 and CXCL8. These findings are consistent with results showing that morphine inhibits the production of, and migration towards, CCL5 by human microglia [86]. The disparity between the results obtained with PBMCs, reviewed above, and neuronal

cells, is unclear. However, it is possible that other factors, such as TGF-β, may signal in the neuronal and immune cells in distinct ways.

Despite the high degree of homology among the opioid receptors, MOR and KOR seem to have opposing effects on cells of the immune system. The exogenous KOR-selective alkaloid agonist U50,488H has been reported to down-regulate CCL2 production in primary human astrocytes stimulated by the HIV-1 nuclear protein, Tat [87]. Activation of KOR following stimulation with U50,488 H has also been shown to decrease IL-1β-induced CXCL8 secretion [88]. Additionally, treatment with the U50,488H has been shown to suppress the production of pro-inflammatory cytokines IL-1, IL-6, and TNF-α in primary macrophages and macrophage/monocyte cell lines [12]. These results are interesting given that MOR activation in the same cell populations (reviewed above) has the opposing effect on chemokine expression. The biochemical basis for the disparate effects of MOR and KOR on chemokine expression is the subject of ongoing research in our laboratory.

Finally, studies on the role of ORL1 in the regulation of chemokine expression have led to very interesting results. Preliminary results from our laboratory suggest that administration of nociceptin to human monocytes results in suppression of both CCL2 and CCL5 protein levels (unpublished results). However, nociceptin does not appear to regulate the expression of these chemokines at the level of transcription. Inhibition of pro-inflammatory cytokines and chemokines would suggest nociceptin may serve as an anti-inflammatory signal by limiting proliferation and differentiation of immune cells as well as reducing the migration of peripheral responder cells.

4. Effects of Opioids on Chemokine Receptor Expression

Both the chemokine and opioid receptors are members of the G protein-coupled receptor (GPCR) family. These receptors form seven transmembrane α-helixes that span the lipid bilayer to create three extracellular and three intracellular loops. The extracellular amino-terminal domain is generally post-translationally modified by glycosylation. On the intracellular or carboxyl-terminal domain end, several signaling events are initiated. Coupling of the G protein and threonine and serine phosphorylation sites are found on the C-terminal domain and mediate downstream signaling cascades. Distinctions between the signaling capacities of these receptors are likely to be determined by structural elements at the C-terminal domain.

Angiotensin receptors are likely the closest neighbor to the chemokine receptors as are the opioid, somatostatin, and melanin concentrating hormone receptors [89]. Chemokine receptors have been found to be involved in disease processes such as inflammation and cancer. This creates an avenue for the exploration of novel therapeutic targets and strategies for the treatment of disease. For example, the chemokine receptors CCR5 and CXCR4 are the two major co-receptors for HIV-1 infection. CXCR4 participates in T cell-tropic HIV-1 infection [90], while CCR5 participates in monocyte/macrophage-tropic HIV-1 infection [91]. Further understanding of the function and expression of these co-receptors is of interest because of their role in inflammatory diseases, as well as their participation in infection by HIV-1. Moreover, other chemokine receptors are known to participate in the progression of disease following HIV-1 infection. Most notably, CCR2 is a critical chemokine receptor involved in the traffic of HIV-infected monocytes across the blood-brain barrier during the development of HIV encephalitis (reviewed in [92]. Finally, it is clear that a number of neuronal factors can influence the development of this inflammatory disease process, including substance P, dopamine, and neuropeptide Y, as well as the endogenous opioid peptides [92].

The influence of opioids on the expression of chemokine receptors has been studied in both leukocytes and neuronal cell populations. Recent results show that administration of morphine

to the human astrocytoma cell line U87 resulted in the elevation of CXCR2 transcript levels [84]. These studies also showed that CCR5 and CCR3 transcript levels are increased with morphine administration, as determined by RT-PCR, in normal human astrocytes. A more recent study using a human brain derived astrocytoma/glioblastoma cell line termed U373 showed marked increases in CCR3 and CCR5 expression following morphine treatment [93]. Recent results also show that CCR5 protein levels were elevated when non-human primate PBMCs were treated with morphine for 24 hours [94]. The ability of MOR activation to induce CCR5 expression suggests MOR agonists, such as morphine, may promote susceptibility to HIV-1 infection and disease progression associated with this infection.

Studies carried out with human PBMCs showed that both morphine and DAMGO induce the expression of both CCR5 and CXCR4 [95]. These studies showed that the induction of chemokine receptor expression was apparent at the mRNA level as well. In addition, analysis of protein expression using flow cytometry showed that both CCR5 and CXCR4 was induced following MOR activation in both CD14-positive monocytes as well as in CD3-positive blast cells.

Studies with the KOR have been somewhat contradictory. We have reported results from studies in mice which showed that treatment with U50,488H results in an increase in CCR2 expression by developing T cells in the thymus [60]. This is in contrast to our preliminary experiments which show that activation of the KOR in human PBMCs suppresses the expression of both CCR5 and CXCR4. Here again, the effect is apparent for both monocytes and T cell blasts. The biochemical basis for the opposing effects of MOR and KOR on chemokine receptor expression remains unclear at this time. However, our studies suggest that TGF-β is involved in the increased CXCR4 levels following MOR activation by DAMGO, based on the ability of MOR agonists to induce TGF-β expression, and the ability of this cytokine to induce CXCR4 levels in PBMCs. The mechanism of CCR5 transcript elevation by DAMGO treatment remains in question, but the presence of AP-1 and NF-κB transcription factor binding sites in addition to several other possible regulatory elements may be responsible for these effects. The biochemical basis for the effect of KOR activation on chemokine receptor expression is an important issue, and an area of ongoing research in our laboratory. However, our results show that the inhibition of CCR5 and CXCR4 expression is apparent at the mRNA level. The distinct functions of these opioid receptors are very interesting, and suggest a complex signaling mechanism in which different opioids are able to elicit very different physiological responses.

In a study using differential display RT-PCR, morphine treatment of human lymphocytic cells for a period of 6 hours showed that several genetic sequences are regulated by activation of the opioid receptors. Using a comparison between cDNA sequences and those sequences found to be differentially regulated, one sequence which is substantially up-regulated is the Kruppel-like factor 7 (KLF7), an essential zinc finger transcriptional factor normally expressed during development of the nervous system [96]. A close relative to KLF7 is the Specificity Protein-1 (SP-1) family of transcriptional regulators. Both SP-1 and KLF7 bind GT-boxes or CACCC elements found in the promoters of several gene families [97]. Interestingly, the promoter of CXCR4 contains several SP-1 binding sites, but at this time it is not clear if KLF7 has any involvement in the regulation of CXCR4 or any chemokine receptor.

5. Conclusions

It is clear that MOR activation by opioids results in altered transcriptional and protein regulation of several cytokines, chemokines, and chemokine receptors. Evidence reviewed above clearly links opioid use to altered immune function. Furthermore, the observation that CCR5 and CXCR4 are elevated by MOR activation would be predicted to have a profound

influence on susceptibility to infection by HIV-1. Not surprisingly, activation of MOR by morphine or DAMGO leads to an increase in susceptibility to infection by both R5- and X4-strains of HIV-1, and this is associated with the induction of CCR5 and CXCR4 in these cells [95].

In contrast with MOR, with few exceptions, KOR activation exhibits a broad inhibitory influence on cytokine, chemokine, and chemokine receptor expression. A brief summary of the contrasting effects of MOR and KOR activation on the expression of chemokine receptors is shown in Table 1. We have previously suggested that KOR activation induces a generalized anti-inflammatory influence on cells of the immune system [55]. This is particularly apparent for the expression of the chemokine receptor CCR5, and suggests important regulatory signaling pathways are distinct for these two opioid receptors. Clarification of the molecular basis for these signaling pathways may reveal very useful information about the participation of these receptors in inflammatory disease states. This information may also yield useful targets for the development of therapeutic strategies for the treatment of inflammatory and infectious diseases.

Acknowledgements

The authors wish to thank the National Institutes of Health for the generous grant support: DA14230, DA16544, P30DA13429, T32DA07237, and DA06650.

Reference List

1. Platt DM, Grech DM, Rowlett JK, Spealman RD. Discriminative stimulus effects of morphine in squirrel monkeys: stimulants, opioids, and stimulant-opioid combinations. J Pharmacol Exp Therapeut 1999;290:1092–1100.
2. Platt DM, Rowlett JK, Spealman RD, Platt DM, Rowlett JK, Spealman RD. Discriminative stimulus effects of intravenous heroin and its metabolites in rhesus monkeys: opioid and dopaminergic mechanisms. J Pharmacol Exp Therapeut 2001;299:760–767.
3. Evans CJ, Keith D Jr, Morrison H, Magendzo K, Edwards RH. Cloning of a delta opioid receptor by functional expression [see comments]. Science 1992;258:1952–1955. [PubMed: 1335167]
4. Kieffer BL, Befort K, Gaveriaux-Ruff C, Hirth CG. The delta-opioid receptor: isolation of a cDNA by expression cloning and pharmacological characterization [published erratum appears in Proc Natl Acad Sci U S A 1994 Feb 1;91(3):1193]. Proc Natl Acad Sci(USA) 1992;89:12048–12052. [PubMed: 1334555]
5. Chen Y, Mestek A, Liu J, Yu L. Molecular cloning of a rat kappa opioid receptor reveals sequence similarities to the mu and delta opioid receptors. Biochem J 1993;295:625–628. [PubMed: 8240267]
6. Li S, Zhu J, Chen C, Chen YW, Deriel JK, Ashby B, Liu-Chen LY. Molecular cloning and expression of a rat kappa opioid receptor. Biochem J 1993;295:629–633. [PubMed: 8240268]
7. Meng F, Xie GX, Thompson RC, Mansour A, Goldstein A, Watson SJ, Akil H. Cloning and pharmacological characterization of a rat kappa opioid receptor. Proc Natl Acad Sci(USA) 1993;90:9954–9958. [PubMed: 8234341]
8. Wybran J, Appelboom T, Famaey JP, Govaerts A. Suggestive evidence for receptors for morphine and methionine-enkephalin on normal human blood T lymphocytes. J Immunol 1979;123:1068–1070. [PubMed: 224107]
9. Bidlack JM, Khimich M, Parkhill AL, Sumagin S, Sun B, Tipton CM. Opioid receptors and signaling on cells from the immune system. Journal of Neuroimmune Pharmacology 2006;1(3):260–269. [PubMed: 18040803]Ref Type: Generic
10. Bidlack JM, Saripalli LD, Lawrence DM. kappa-Opioid binding sites on a murine lymphoma cell line. Eur J Pharmacol 1992;227:257–265. [PubMed: 1335414]
11. Carr DJ, DeCosta BR, Kim CH, Jacobson AE, Guarcello V, Rice KC, Blalock JE. Opioid receptors on cells of the immune system: evidence for delta- and kappa-classes. J Endocrinol 1989;122:161–168. [PubMed: 2549147]

12. Alicea C, Belkowski S, Eisenstein TK, Adler MW, Rogers TJ. Inhibition of primary murine macrophage cytokine production in vitro following treatment with the kappa-opioid agonist U50,488H. J Neuroimmunol 1996;64:83–90. [PubMed: 8598393]
13. Belkowski SM, Zhu J, Liu-Chen LY, Eisenstein TK, Adler MW, Rogers TJ. Sequence of kappa-opioid receptor cDNA in the R1.1 thymoma cell line. J Neuroimmunol 1995;62:113–117. [PubMed: 7499487]
14. Chuang TK, Killam KF Jr, Chuang LF, Kung HF, Sheng WS, Chao CC, Yu L, Chuang RY. Mu opioid receptor gene expression in immune cells. Biochem Biophys Res Comm 1995;216:922–930. [PubMed: 7488213]
15. Chuang LF, Chuang TK, Killam KF Jr, Chuang AJ, Kung HF, Yu L, Chuang RY. Delta opioid receptor gene expression in lymphocytes. Biochem Biophys Res Comm 1994;202:1291–1299. [PubMed: 8060306]
16. Sharp BM, Shahabi N, McKean D, Li MD, McAllen K. Detection of basal levels and induction of delta opioid receptor mRNA in murine splenocytes. J Neuroimmunol 1997;78:198–202. [PubMed: 9307245]
17. Fukuda K, Kato S, Mori K, Nishi M, Takeshima H, Iwabe N, Miyata T, Houtani T, Sugimoto T. cDNA cloning and regional distribution of a novel member of the opioid receptor family. FEBS Lett 1994;343:42–46. [PubMed: 8163014]
18. Mollereau C, Parmentier M, Mailleux P, Butour JL, Moisand C, Chalon P, Caput D, Vassart G, Meunier JC. ORL1, a novel member of the opioid receptor family. Cloning, functional expression and localization. FEBS Lett 1994;341:33–38. [PubMed: 8137918]
19. Wang JB, Johnson PS, Imai Y, Persico AM, Ozenberger BA, Eppler CM, Uhl GR. cDNA cloning of an orphan opiate receptor gene family member and its splice variant. FEBS Lett 1994;348:75–79. [PubMed: 8026588]
20. Bunzow JR, Saez C, Mortrud M, Bouvier C, Williams JT, Low M, Grandy DK. Molecular cloning and tissue distribution of a putative member of the rat opioid receptor gene family that is not a mu, delta or kappa opioid receptor type. FEBS Lett 1994;347:284–288. [PubMed: 8034019]
21. Peluso J, LaForge KS, Matthes HW, Kreek MJ, Kieffer BL, Gaveriaux-Ruff C. Distribution of nociceptin/orphanin FQ receptor transcript in human central nervous system and immune cells. J Neuroimmunol 1998;81:184–192. [PubMed: 9521621]
22. Fiset ME, Gilbert C, Poubelle PE, Pouliot M. Human neutrophils as a source of nociceptin: a novel link between pain and inflammation. Biochem 2003;42:10498–10505. [PubMed: 12950177]
23. Serhan CN, Fierro IM, Chiang N, Pouliot M. Cutting edge: nociceptin stimulates neutrophil chemotaxis and recruitment: inhibition by aspirin-triggered-15-epi-lipoxin A4. J Immunol 2001;166:3650–3654. [PubMed: 11238602]
24. Trombella S, Vergura R, Falzarano S, Guerrini R, Calo G, Spisani S. Nociceptin/orphanin FQ stimulates human monocyte chemotaxis via NOP receptor activation. Peptides 2005;26:1497–1502. [PubMed: 15922491]
25. Pampusch MS, Serie JR, Osinski MA, Seybold VS, Murtaugh MP, Brown DR. Expression of nociceptin/OFQ receptor and prepro-nociceptin/OFQ in lymphoid tissues. Peptides 2000;21:1865–1870. [PubMed: 11150647]
26. Taub DD, Eisenstein TK, Geller EB, Adler MW, Rogers TJ. Immunomodulatory activity of mu- and kappa-selective opioid agonists. Proc Natl Acad Sci(USA) 1991;88:360–364. [PubMed: 1846441]
27. Eisenstein TK, Meissler JJ Jr, Rogers TJ, Geller EB, Adler MW. Mouse strain differences in immunosuppression by opioids in vitro. J Pharmacol Exp Therapeut 1995;275:1484–1489.
28. Guan L, Townsend R, Eisenstein TK, Adler MW, Rogers TJ. Both T cells and macrophages are targets of kappa-opioid-induced immunosuppression. Brain Behavior Immun 1994;8:229–240.
29. Szabo I, Rojavin M, Bussiere JL, Eisenstein TK, Adler MW, Rogers TJ. Suppression of peritoneal macrophage phagocytosis of Candida albicans by opioids. J Pharmacol Exp Therapeut 1993;267:703–706.
30. Rojavin M, Szabo I, Bussiere JL, Rogers TJ, Adler MW, Eisenstein TK. Morphine treatment in vitro or in vivo decreases phagocytic functions of murine macrophages. Life Sci 1993;53:997–1006. [PubMed: 8361330]

31. Weber RJ, Pert A. The periaqueductal gray matter mediates opiate-induced immunosuppression. Science 1989;245:188–190. [PubMed: 2749256]
32. Linner KM, Quist HE, Sharp BM. Expression and function of proenkephalin A messenger ribonucleic acid in murine fetal thymocytes. Endocrinol 1996;137:857–863.
33. McCarthy L, Szabo I, Nitsche JF, Pintar JE, Rogers TJ. Expression of functional mu-opioid receptors during T cell development. J Neuroimmunol 2001;114:173–180. [PubMed: 11240029]
34. McCarthy LE, Rogers TJ. Alteration of early T cell development by opioid and superantigen stimulation. Adv Exp Med Biol 2001;493:163–167. [PubMed: 11727762]
35. Shahabi NA, Peterson PK, Sharp B. Beta-endorphin binding to naloxone-insensitive sites on a human mononuclear cell line (U937): effects of cations and guanosine triphosphate. Endocrinol 1990;126:3006–3015.
36. McCarthy L, Wetzel M, Sliker JK, Eisenstein TK, Rogers TJ. Opioids, opioid receptors, and the immune response. [Review] [128 refs]. Drug & Alcohol Dependence 2001;62:111–123. [PubMed: 11245967]
37. Brown SL, Van Epps DE. Opioid peptides modulate production of interferon gamma by human mononuclear cells. Cell Immunol 1986;103:19–26. [PubMed: 3026654]
38. Mandler RN, Biddison WE, Mandler R, Serrate SA, Mandler RN, Biddison WE, Mandler R, Serrate SA. beta-Endorphin augments the cytolytic activity and interferon production of natural killer cells. J Immunol 1986;136:934–939. [PubMed: 2934481]
39. Peterson PK, Sharp B, Gekker G, Brummitt Keane WF. Opioid-mediated suppression of interferon-gamma production by cultured peripheral blood mononuclear cells. J Clin Invest 1987;80:824–831. [PubMed: 3040807]
40. Bessler H, Sztein MB, Serrate SA, Bessler H, Sztein MB, Serrate SA. Beta-endorphin modulation of IL-1-induced IL-2 production. Immunopharmacol 1990;19:5–14.
41. van den Bergh P, Rozing J, Nagelkerken L. Two opposing modes of action of beta-endorphin on lymphocyte function. Immunol 1991;72:537–543.
42. Jessop JJ, Taplits MS, Jessop JJ, Taplits MS. Effect of high doses of morphine on Con-A induced lymphokine production in vitro. Immunopharmacol 1991;22:175–184.
43. Lysle DT, Coussons ME, Watts VJ, Bennett EH, Dykstra LA. Morphine-induced alterations of immune status: dose dependency, compartment specificity and antagonism by naltrexone. J Pharmacol Exp Therapeut 1993;265:1071–1078.
44. Wang J, Charboneau R, Balasubramanian S, Barke RA, Loh HH, Roy S. Morphine modulates lymph node-derived T lymphocyte function: role of caspase-3, -8, and nitric oxide. J Leuk Biol 2001;70:527–536.
45. Chao CC, Hu S, Molitor TW, Zhou Y, Murtaugh MP, Tsang M, Peterson PK. Morphine potentiates transforming growth factor-beta release from human peripheral blood mononuclear cell cultures. J Pharmacol Exp Therapeut 1992;262:19–24.
46. Roy S, Balasubramanian S, Sumandeep S, Charboneau R, Wang J, Melnyk D, Beilman GJ, Vatassery R, Barke RA, Roy S, Balasubramanian S, Sumandeep S, Charboneau R, Wang J, Melnyk D, Beilman GJ, Vatassery R, Barke RA. Morphine directs T cells toward T(H2) differentiation. Surgery 2001;130:304–309. [PubMed: 11490364]
47. Roy S, Wang J, Gupta S, Charboneau R, Loh HH, Barke RA. Chronic morphine treatment differentiates T helper cells to Th2 effector cells by modulating transcription factors GATA 3 and T-bet. J Neuroimmunol 2004;147:78–81. [PubMed: 14741432]
48. Roy S, Wang J, Kelschenbach J, Koodie L, Martin J. Modulation of Immune Function by Morphine: Implications for Susceptibility to Infection. Journal of Neuroimmune Pharmacology Mar 1;2006 1 (1):77–89. [PubMed: 18040793]Ref Type: Generic
49. Peng X, Mosser DM, Adler MW, Rogers TJ, Meissler JJ Jr, Eisenstein TK. Morphine enhances interleukin-12 and the production of other pro-inflammatory cytokines in mouse peritoneal macrophages. J Leuk Biol 2000;68:723–728.
50. Apte RN, Oppenheim JJ, Durum SK. Beta-endorphin regulates interleukin 1 production and release by murine bone marrow macrophages. Int Immunol 1989;1:465–470. [PubMed: 2535139]
51. Apte RN, Durum SK, Oppenheim JJ. Opioids modulate interleukin-1 production and secretion by bone-marrow macrophages. Immunol Lett 1990;24:141–148. [PubMed: 2162329]

52. Roy S, Cain KJ, Chapin RB, Charboneau RG, Barke RA. Morphine modulates2 NF kappa B activation in macrophages. Biochem Biophys Res Comm 1998;245:392–396. [PubMed: 9571161]
53. Wang J, Barke RA, Charboneau R, Roy S, Wang J, Barke RA, Charboneau R, Roy S. Morphine impairs host innate immune response and increases susceptibility to Streptococcus pneumoniae lung infection. J Immunol 2005;174:426–434. [PubMed: 15611267]
54. Bussiere JL, Adler MW, Rogers TJ, Eisenstein TK. Cytokine reversal of morphine-induced suppression of the antibody response. J Pharmacol Exp Therapeut 1993;264:591–597.
55. Rogers TJ, Peterson PK. Opioid G protein-coupled receptors: signals at the crossroads of inflammation. [Review] [43 refs]. Trends in Immunology 2003;24:116–121. [PubMed: 12615205]
56. Guan L, Eisenstein TK, Adler MW, Rogers TJ. Inhibition of T cell superantigen responses following treatment with the kappa-opioid agonist U50,488H. J Neuroimmunol 1997;75:163–168. [PubMed: 9143250]
57. Belkowski SM, Zhu J, Liu-Chen LY, Eisenstein TK, Adler MW, Rogers TJ. Detection of kappa-opioid receptor mRNA in immature T cells. Adv Exp Med Biol 1995;373:11–16. [PubMed: 7545346]
58. Alicea C, Belkowski SM, Sliker JK, Zhu J, Liu-Chen LY, Eisenstein TK, Adler MW, Rogers TJ. Characterization of kappa-opioid receptor transcripts expressed by T cells and macrophages. J Neuroimmunol 1998;91:55–62. [PubMed: 9846819]
59. Guan L, Eisenstein TK, Adler MW, Rogers TJ. Modulation of DPK cell function by the kappa opioid agonist U50,488H. Adv Exp Med Biol 1998;437:125–136. [PubMed: 9666264]
60. Zhang L, Rogers TJ. Kappa-opioid regulation of thymocyte IL-7 receptor and C-C chemokine receptor 2 expression. J Immunol 2000;164:5088–5093. [PubMed: 10799865]
61. Peschon JJ, Morrissey PJ, Grabstein KH, Ramsdell FJ, Maraskovsky E, Gliniak BC, Park LS, Ziegler SF, Williams DE, Ware CB. Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. J Exp Med 1994;180:1955–1960. [PubMed: 7964471]
62. Freeden-Jeffry U, Vieira P, Lucian LA, McNeil T, Burdach SE, Murray R. Lymphopenia in interleukin (IL)-7 gene-deleted mice identifies IL-7 as a nonredundant cytokine. J Exp Med 1995;181:1519–1526. [PubMed: 7699333]
63. Rich BE, Leder P. Transgenic expression of interleukin 7 restores T cell populations in nude mice. J Exp Med 1995;181:1223–1228. [PubMed: 7869039]
64. Kim K, Lee CK, Sayers TJ, Muegge K, Durum SK. The trophic action of IL-7 on pro-T cells: inhibition of apoptosis of pro-T1, -T2, and -T3 cells correlates with Bcl-2 and Bax levels and is independent of Fas and p53 pathways. J Immunol 1998;160:5735–5741. [PubMed: 9637482]
65. Shahabi NA, Sharp BM. Delta opioid agonists inhibit proliferation of highlypurified murine CD4+ and CD8+ T-cells. Adv Exp Med Biol 1995;373:29–36. [PubMed: 7668157]
66. House RV, Thomas PT, Bhargava HN. A comparative study of immunomodulation produced by in vitro exposure to delta opioid receptor agonist peptides. Peptides 1996;17:75–81. [PubMed: 8822513]
67. Hedin KE, Bell MP, Kalli KR, Huntoon CJ, Sharp BM, McKean DJ. Delta-opioid receptors expressed by Jurkat T cells enhance IL-2 secretion by increasing AP-1 complexes and activity of the NF-AT/AP-1-binding promoter element. J Immunol 1997;159:5431–5440. [PubMed: 9548483]
68. Zhao H, Wu GC, Cao XD. Immunomodulatory activity of orphanin FQ/nociceptin on traumatic rats. Acta Pharmacologica Sinica 2002;23:343–348. [PubMed: 11931711]
69. Goldfarb Y, Reinscheid RK, Kusnecov AW. Orphanin FQ/nociceptin interactions with the immune system in vivo: gene expression changes in lymphoid organs and regulation of the cytokine response to staphylococcal enterotoxin A. J Neuroimmunol 2006;176:76–85. [PubMed: 16762423]
70. Howard OM, Oppenheim JJ, Wang JM. Chemokines as molecular targets for therapeutic intervention. [Review] [98 refs]. J Clin Immunol 1999;19:280–292. [PubMed: 10535604]
71. Taub DD, Oppenheim JJ. Chemokines, inflammation and the immune system. [Review] [190 refs]. Therapeutic Immunology 1994;1:229–246. [PubMed: 7584498]
72. Wetzel MA, Steele AD, Eisenstein TK, Adler MW, Henderson EE, Rogers TJ. Mu-opioid induction of monocyte chemoattractant protein-1, RANTES, and IFN-gamma-inducible protein-10 expression in human peripheral blood mononuclear cells. J Immunol 2000;165:6519–6524. [PubMed: 11086093]

73. Rock RB, Hu S, Sheng WS, Peterson PK. Morphine stimulates CCL2 production by human neurons. J Neuroinflammation 2006;3:32. [PubMed: 17156455]
74. Li MO, Wan YY, Sanjabi S, Robertson AK, Flavell RA. Transforming growth factor-beta regulation of immune responses. Annu Rev Immunol 2006;24:99–146. [PubMed: 16551245]
75. Roberts AB. TGF-beta signaling from receptors to the nucleus. [Review] [64 refs]. Microbes & Infection 1999;1:1265–1273. [PubMed: 10611754]
76. Wahl SM, Hunt DA, Wakefield LM, Cartney-Francis N, Wahl LM, Roberts AB, Sporn MB. Transforming growth factor type beta induces monocyte chemotaxis and growth factor production. Proc Natl Acad Sci USA 1987;84:5788–5792. [PubMed: 2886992]
77. Turner M, Chantry D, Feldmann M. Transforming growth factor beta induces the production of interleukin 6 by human peripheral blood mononuclear cells. Cytokine 1990;2:211–216. [PubMed: 2104224]
78. Wahl SM, Hunt DA, Wakefield LM, Cartney-Francis N, Wahl LM, Roberts AB, Sporn MB. Transforming growth factor type beta induces monocyte chemotaxis and growth factor production. Proc Natl Acad Sci USA 1987;84:5788–5792. [PubMed: 2886992]
79. Hou YN, Vlaskovska M, Cebers G, Kasakov L, Liljequist S, Terenius L. A mu-receptor opioid agonist induces AP-1 and NF-kappa B transcription factor activity in primary cultures of rat cortical neurons. Neurosci Lett 1996;212:159–162. [PubMed: 8843097]
80. Bilecki W, Wawrzczak-Bargiela A, Przewlocki R. Activation of AP-1 and CRE-dependent gene expression via mu-opioid receptor. J Neurochem 2004;90:874–882. [PubMed: 15287893]
81. Nelson PJ, Kim HT, Manning WC, Goralski TJ, Krensky AM. Genomic organization and transcriptional regulation of the RANTES chemokine gene. J Immunol 1993;151:2601–2612. [PubMed: 7689610]
82. Ueda A, Okuda K, Ohno S, Shirai A, Igarashi T, Matsunaga K, Fukushima J, Kawamoto S, Ishigatsubo Y, Okubo T, Ueda A, Okuda K, Ohno S, Shirai A, Igarashi T, Matsunaga K, Fukushima J, Kawamoto S, Ishigatsubo Y, Okubo T. NF-kappa B and Sp1 regulate transcription of the human monocyte chemoattractant protein-1 gene. J Immunol 1994;153:2052–2063. [PubMed: 8051410]
83. Kim SJ, Angel P, Lafyatis R, Hattori K, Kim KY, Sporn MB, Karin M, Roberts AB. Autoinduction of transforming growth factor beta 1 is mediated by the AP-1 complex. Mol Cell Biol 1990;10:1492–1497. [PubMed: 2108318]
84. Mahajan SD, Schwartz SA, Shanahan TC, Chawda RP, Nair MP. Morphine regulates gene expression of alpha- and beta-chemokines and their receptors on astroglial cells via the opioid mu receptor. J Immunol 2002;169:3589–3599. [PubMed: 12244149]
85. Mahajan SD, Schwartz SA, Aalinkeel R, Chawda RP, Sykes DE, Nair MP. Morphine modulates chemokine gene regulation in normal human astrocytes. Clin Immunol 2005;115:323–332. [PubMed: 15893700]
86. Hu S, Chao CC, Hegg CC, Thayer S, Peterson PK. Morphine inhibits human microglial cell production of, and migration towards, RANTES. Journal of Psychopharmacology 2000;14:238–243. [PubMed: 11106302]
87. Sheng WS, Hu S, Lokensgard JR, Peterson PK. U50,488 inhibits HIV-1 Tat-induced monocyte chemoattractant protein-1 (CCL2) production by human astrocytes. Biochem Pharmacol 2003;65:9–14. [PubMed: 12473373]
88. Neudeck BL, Loeb J, Buck J. Activation of the kappa-opioid receptor in Caco-2 cells decreases interleukin-8 secretion. Eur J Pharmacol 2003;467:81–84. [PubMed: 12706459]
89. Lio P, Vannucci M. Investigating the evolution and structure of chemokine receptors. Gene 2003;317:29–37. [PubMed: 14604789]
90. Doranz BJ, Rucker J, Yi Y, Smyth RJ, Samson M, Peiper SC, Parmentier M, Collman RG, Doms RW. A dual-tropic primary HIV-1 isolate that uses fusin and the beta-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. Cell 1996;85:1149–1158. [PubMed: 8674120]
91. Dragic T, Litwin V, Allaway GP, Martin SR, Huang Y, Nagashima KA, Cayanan C, Maddon PJ, Koup RA, Moore JP, Paxton WA. HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5 [see comments]. Nature 1996;381:667–673. [PubMed: 8649512]
92. Buckner CM, Luers AJ, Calderon TM, Eugenin EA, Berman JW. Neuroimmunity and the blood–brain barrier: molecular regulation of leukocyte transmigration and viral entry into the nervous system

with a focus on neuroAIDS. Journal of Neuroimmune Pharmacology 2006;1(2):160–181. [PubMed: 18040782]Ref Type: Generic

93. Mahajan SD, Aalinkeel R, Reynolds JL, Nair BB, Fernandez SF, Schwartz SA, Nair MP. Morphine exacerbates HIV-1 viral protein gp120 induced modulation of chemokine gene expression in U373 astrocytoma cells. Current HIV Research 2005;3:277–288. [PubMed: 16022659]
94. Suzuki S, Chuang AJ, Chuang LF, Doi RH, Chuang RY. Morphine promotes simian acquired immunodeficiency syndrome virus replication in monkey peripheral mononuclear cells: induction of CC chemokine receptor 5 expression for virus entry. J Infect Dis 2002;185:1826–1829. [PubMed: 12085334]
95. Steele AD, Henderson EE, Rogers TJ. Mu-opioid modulation of HIV-1 coreceptor expression and HIV-1 replication. Virology 2003;309:99–107. [PubMed: 12726730]
96. Suzuki S, Chuang LF, Doi RH, Chuang RY. Identification of opioid-regulated genes in human lymphocytic cells by differential display: upregulation of Kruppel-like factor 7 by morphine. Experimental Cell Research 2003;291:340–351. [PubMed: 14644156]
97. Laub F, Lei L, Sumiyoshi H, Kajimura D, Dragomir C, Smaldone S, Puche AC, Petros TJ, Mason C, Parada LF, Ramirez F. Transcription factor KLF7 is important for neuronal morphogenesis in selected regions of the nervous system. Mol Cell Biol 2005;25:5699–5711. [PubMed: 15964824]

Table 1

Effects of opioids on chemokine receptor transcriptional regulation

Opioid	Mu Opioid Receptor (MOR) Effect	Chemokine Receptor
Morphine	↑	CCR3[93]
	↑	CCR5 [93;95]
	↑	CXCR2[84]
	↑	CXCR4[95]
DAMGO	↑	CCR5[95]
	↑	CXCR4[95]
	Kappa Opioid Receptor (KOR)	
Opioid	Effect	Chemokine Receptor
U50,488H	↑	CCR2[60]
	↓	CCR5*
	↓	CXCR4*
	N/OFQ Receptor-Like (ORL)	
Opioid	Effect	Chemokine Receptor
Nociceptin	nc	CCR2*
	nc	CCR5*
	nc	CXCR4*

* Unpublished data. nc indicates no change in expression.