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INVITED REVIEW

Role of the mu-opioid receptor in opioid modulation of immune function

Jana Ninković · Sabita Roy

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Abstract Endogenous opioids are synthesized in vivo to modulate pain mechanisms and inflammatory pathways. Endogenous and exogenous opioids mediate analgesia in response to painful stimuli by binding to opioid receptors on neuronal cells. However, wide distribution of opioid receptors on tissues and organ systems outside the CNS, such as the cells of the immune system, indicate that opioids are capable of exerting additional effects in the periphery, such as immunomodulation. The increased prevalence of infections in opioid abuser-based epidemiological studies further highlights the immunosuppressive effects of opioids. In spite of their many debilitating side effects, prescription opioids remain a gold standard for treatment of chronic pain. Therefore, given the prevalence of opioid use and abuse, opioid-mediated immune suppression presents a serious concern in our society today. It is imperative to understand the mechanisms by which exogenous opioids modulate immune processes. In this review, we will discuss the role of opioid receptors and their ligands in mediating immune-suppressive functions. We will summarize recent studies on direct and indirect opioid modulation of the cells of the immune system, as well as the role of opioids in exacerbation of certain disease states.

J. Ninković
Department of Surgery, University of Minnesota,
420 Delaware St SE, MMC195, Minneapolis,
MN 55455, USA
e-mail: nink0003@umn.edu

S. Roy (✉)
Division of Infection, Inflammation and Vascular Biology,
Department of Surgery and Pharmacology, University
of Minnesota, MMC 195, 420 Delaware Street SE,
Minneapolis, MN 55455, USA
e-mail: royxx002@umn.edu

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Opioid receptors

Opioid receptors are activated both by endogenously produced opioid peptides and by exogenously administered opioids, such as morphine (Waldhoer et al. 2004). Classical opioid receptors are seven transmembrane G protein-coupled receptors (GPCRs) that have three major receptor subtypes: μ (mu for morphine), δ (delta for deferens because it was first identified in mouse vas deferens) and kappa κ (kappa for ketocyclazocine, an agonist that is a benzomorphan derivative) (Lord et al. 1977). As a class, GPCRs are of fundamental physiological importance, mediating the actions of the majority of known neurotransmitters and hormones.

Structure and function

Analgesia induced by opioids is predominately mediated through the μ-opioid receptor. Endogenous opioids have been implicated in activating all three receptor types. β-endorphins and enkephalins bind to μ and δ, while dynorphin binds predominately to the κ receptor. μ Opioid agonists (endogenous and exogenous) induce analgesic effects by regulating both the pre- and post-synaptic sensory neurons. At the pre-synaptic neurons, opioids bind to μ-opioid receptors (MOR), block voltage-gated calcium (Ca^{2+}) channels and, hence, block Ca^{2+} influx. Lower intracellular Ca^{2+} leads to an inhibition of excitatory neurotransmitter release from presynaptic vesicles. Activation of MOR on postsynaptic terminals promotes the efflux of potassium (K^+) via K^+ channels. The net effect of

MOR receptor activation results in hyperpolarization of the post-synapse causing inhibition of neuronal firing. Studies have shown that MOR affects the pre- and post-synaptic neuron synergistically, thereby decreasing the perception of pain (Yoshimura and North 1983; Glaum et al. 1994; Kohno et al. 1999).

Opioid receptors are GPCRs that are classified into two distinct classes: classical (μ, δ, κ) and non-classical opioid receptors (Goodsell 2005). Three decades of extensive pharmacological studies have uncovered a variety of opioid receptor types; however, only four have been cloned to date: μ, δ, κ and the n-opioid receptor (initially called LC132) (Bunzow et al. 1994), ORL-1 (Mollereau et al. 1994) or nociceptin/orphanin FQ receptor (Meunier et al. 1995). Although only four receptor genes have been discovered, there is substantial pharmacological evidence to suggest the existence of spliced variants of opioid receptor subtypes (Pan et al. 2005; Pan 2005; Choi et al. 2006; Mizoguchi et al. 2011; Xu et al. 2011).

The sigma receptor (σ = sigma for SKF10047) was initially classified as an opioid receptor (Martin et al. 1976). However, since the time it was cloned in 1996 (Hanner et al. 1996), it has become evident that the sigma receptor is a single transmembrane-spanning protein targeted by other drugs of abuse, for example phencyclidine and its analogs [for review see (Monassier and Bousquet 2002)]. The sigma receptor is no longer regarded as a member of the opioid receptor family. Moreover, a variety of other opioid receptors have been described on the basis of pharmacological profiles that do not match with any of the classical receptors. These include a ζ (zeta) receptor, which has recently been cloned and classified as an opioid growth factor receptor (OGFr) with no homology to the classical opioid receptors (Zagon et al. 1991, 2000). In addition, a λ (lambda) receptor and a β-endorphin-sensitive ε (epsilon) opioid-binding site have been described (Wuster et al. 1979). However, these receptors are poorly characterized, and proof of their existence by identifying their respective genes is still lacking (Waldhoer et al. 2004).

It is also important to note that like other GPCRs, opioid receptors not only act as monomers but can form homomeric and heteromeric complexes with other opioid receptor subtypes as well as with other GPCRs, creating new receptors with novel pharmacological properties (van Rijn et al. 2010). The role of dimers in modulation of immune function has not been well explored and therefore will not be the focus of this review.

Opioid receptor expression in immune cell

Opioid receptors are expressed throughout the body, in various tissues and cell types. They are found in the periphery, at pre-synaptic and postsynaptic sites in the spinal cord dorsal horn, and in the brainstem, thalamus and cortex, in what constitutes the ascending pain transmission system. Receptors are also found in the structures that comprise the descending inhibitory system that modulates pain at the level of the spinal cord (Inturrisi 2002). Until recently, it was thought that opioid receptors were only expressed in the central nervous system. However, recent findings have proven that opioid receptors are also expressed by the cells of the immune system such as T cells, B cells and macrophages (for details, see Table 1) (Chuang et al. 1995).

The earliest examination of opioid receptor expression used pharmacological and ligand binding studies to

Table 1 Opioid receptor expression in immune cells

Cell type	μ	δ	κ	References
T cell	CEM cell line, human CD4+ T cells	MOLT cell line, human cell line, murine splenic T cells,	MOLT cell line, human immature thymic T cells, murine thymocytes and splenocytes	Chuang et al. (1995), Wick et al. (1996), Sharp et al. (1998), Ignatowski and Bidlack (1998)
B cell	CEM cell line, Raji line	Human cell line	CEM cell line	Chuang et al. (1995), Gaveriaux et al. (1995), Suzuki et al. (2001)
Dendritic cell	Human and murine primary DC	Human and murine primary DC	Murine DC	Makarenkova et al. (2001), Kirst et al. (2002)
Macrophage/ monocyte	Primary rat peritoneal mac., human and simian mono	Human cell line	Macrophage-like murine cell line P388D1	Chuang et al. (1995), Sedqi et al. (1995) Lopker et al. (1980), Carr et al. (1991)
Neutrophil	Human granulocytes	Human granulocytes	Murine bone marrow neutrophils	Lopker et al. (1980), Falke et al. (1985), Stefano et al. (1993), Kulkarni-Narla et al. (2001)
Microglia	Human fetal microglia murine microglia	Murine microglia	Human fetal microglia murine microglia	Chao et al. (1996), Stiene-Martin et al. (2001)

provide support for the existence of opioid receptors on the cells of the immune system. With the advent of genetic cloning and polymerase chain reaction (PCR) techniques, expression of receptors that are in low abundance on cells of the immune system was established. These techniques enabled an alternative way to demonstrate expression of all three opioid receptors on several immune cells, including CD4+ T-helper cells [reviewed in (Sharp et al. 1998)]. Specifically, using reverse transcriptase-polymerase chain reaction (RT-PCR) cDNA transcripts of the μ (Chuang et al. 1995), δ and κ (Wick et al. 1996) opioid receptors were amplified from several immune cells. Chuang et al. (1995) demonstrated the expression of the mu-opioid receptor gene in various cell types, including the human hybrid B- and T-cell CEM line, the Raji line (human B cells), human CD4+ cells, human monocytes and macrophages, and various others. In addition, studies have demonstrated the existence of delta and kappa opioid cDNA transcripts in MOLT-4 and CEM T cell lines as well as in human peripheral blood lymphocytes using similar techniques (Wick et al. 1996). Furthermore, existence of mu-opioid receptor transcripts has been demonstrated to be expressed in rat peritoneal macrophages, while the delta-opioid receptor has been found in inactivated mouse thymocytes (Sedqi et al. 1995, 1996). In all cases, the transcripts obtained from the immune cells were nearly identical to opioid receptor cDNAs isolated from neuronal cells. In addition to previously described studies examining message levels of opioid receptors, several groups have reported μ-, δ- and κ-opioid receptor protein presence in T cells, B cells, dendritic cells and mouse thymocytes using FACS or western blot analysis (Ignatowski and Bidlack 1998; Makarenkova et al. 2001; Suzuki et al. 2002; Liu et al. 2010).

These observations suggest that opiate drugs may be directly mediating their diverse array of effects by binding to receptors expressed on cells of the immune system. It is also important to note that the cDNA clone, AT7-5EU, was isolated after a screen of an activated human lymphocyte cDNA library in search of homology to brain opioid receptors. This clone was demonstrated to encode for the opioid 'orphan' receptor, and the protein coding region shared complete homology with a reported opioid 'orphan' receptor cloned from human brain. There have also been reports that implicate the existence of novel, non-classical opioid receptors and binding sites on immune cells that are selective for morphine (Sharp et al. 1998). The importance and relevance of all of these findings (summarized in Table 1) are centered on the idea that opioids, both endogenous and exogenous, may be exerting their myriad effects on the immune system in a direct and indirect manner.

Opioid receptor functions

Tissue injury and inflammation increase the excitability of peripheral pain signaling sensory neurons called nociceptors. Excitation of these receptors leads to generation of currents, which when sufficiently large are able to lead to perception of pain. In response to chemical stimuli, high-threshold transducer ion channels at the peripheral terminals of nociceptors depolarize the peripheral terminal activating voltage-dependent sodium channels. Transmission occurs in response to calcium influx at the central terminal, releasing glutamate as well as multiple synaptic modulators and signaling molecules, and yielding an increase in synaptic action potentials (Woolf and Ma 2007). It is the net effect of increased rate of action potentials that leads to conscious awareness of pain. The sensation of pain leads to activation of analgesic pathways resulting in activation of endogenous opioid signaling through opioid receptors.

Opioid receptors belong to the class A (Rhodopsin) family of Gi/Go protein-coupled receptors with an extracellular N-terminal domain, seven transmembrane helical domains connected by three extracellular and three intracellular domains, as well as an intracellular C-terminal tail. Seven transmembrane helices of opioid receptors are arranged sequentially in a counterclockwise fashion, to form a tight helical bundle. Together with the extracellular domains of the receptor, this provides a dynamic interface for the binding of various opioid ligands. The opioid receptors are about 60% identical to each other with the greatest homology in the transmembrane helices, and the greatest diversity in their N and C termini as well as their extracellular loops.

Opioids initiate a signal through a G-protein cascade. When morphine binds to an opiate receptor, the receptor changes shape and interacts with a G protein inside the cell. The activated receptor causes the G protein to replace its GDP molecule with a GTP, causing the G protein to break into Gα and G$\beta\gamma$ subunits. The half with the GTP molecule then diffuses along the membrane until it finds its target. Opioid receptor activation leads to upregulation of the cAMP pathway, which is believed to be a mechanism for opiate tolerance and dependence [reviewed by (Nestler 2001)]. Opiates acutely inhibit the functional activity of the cAMP pathway (indicated by cellular levels of cAMP and cAMP-dependent protein phosphorylation). With continued opiate exposure, functional activity of the cAMP pathway gradually recovers, and increases far above the control levels following removal of the opiate (precipitated withdrawal, e.g., by administration of the opioid receptor antagonist naloxone). These changes in the functional state of the cAMP pathway are mediated via the induction of adenylyl cyclase and protein kinase A (PKA) in response to

chronic administration of opiates. Induction of these enzymes accounts for the gradual recovery in the functional activity of the cAMP pathway that occurs during chronic opiate exposure (tolerance and dependence) and activation of the cAMP pathway observed on opiate withdrawal (Nestler 2001).

Structure and site of opioid peptide production

Endogenous opioids

During the decade spanning the mid-1970s to the late 1980s, more than 20 different endogenous opioid peptides were identified and shown to possess differential affinity for the different types of opioid receptors (Evans 2004). The endogenous opioids are derived from three opioid protein precursors by a process of selective proteolytic cleavages. Although there is a wide variety of endogenous peptides, they consist of three major classes: enkephalins, endorphins and dynorphins. All endogenous opioids have an N-terminal enkephalin sequence (Tyr–Gly–Gly–Phe–Met/Leu), with many peptides containing a C-terminal extension, which modulates receptor selectivity and susceptibility to degradation by extracellular proteases [reviewed by (Weber et al. 1983)].

Pro-enkephalin contains multiple repeats of the enkephalin sequence, seven in the human pro-enkephalin precursor. The enkephalins are small, five amino acid peptides that exist in two forms, leucine enkephalin and methionine enkephalin. Pro-opiomelanocortin (POMC) contains beta-endorphin, 31 amino acid peptides that contain the met-enkephalin sequence, and shares the precursor protein with adrenocorticoptrophic hormone (ACTH), a critical pituitary hormone for coordination of stress responses. The endorphins and enkephalins act primarily on mu- and delta opioid receptors. Finally, pro-dynorphin contains three leu-enkephalin core opioid sequences analogous to pro-enkephalin, and differential processing of the core sequences leads to generation of multiple opioid peptides. The dynorphins exist in several forms that range from 10 to 17 amino acids in length, and they exert their effects primarily on kappa receptors. The biological significance of the multiplicity of endogenous opioids is still unclear.

Early studies in the 1970s and 1980s concluded that production of endogenous opioids was limited to the cells of the nervous system. However, each opioid peptide precursor has a unique pattern of expression, with POMC transcripts restricted to the pituitary, the arcuate nucleus of the hypothalamus and cells in the nucleus of the solitary tract, whereas both pro-enkephalin and pro-dynorphin have a considerably more expansive distribution (Akil et al. 1984). β-Endorphin production is localized mostly to the pituitary and hypothalamus, while enkephalins are more widely distributed through the neuraxis found mainly in the cortex and spinal cord as well as the adrenal medulla, while dynorphin production is limited to the hypothalamus and the brainstem. Table 2 summarizes the sites which have been known to generate endogenous opioids. Recent findings have shown that all opioid peptides are also found in leukocytes. Endorphins processing from POMC have been studied most extensively by (Rittner et al. 2005). POMC processing occurs in the endoplasmic reticulum and the trans-Golgi network. The enzymatic machinery required for this process includes carboxypeptidase E, the pro-hormone convertases 1 (PC1) and PC2, and the binding protein 7B2 (Mousa et al. 2004). β-Endorphin, POMC and all processing enzymes have been located in leukocytes in the blood and within inflamed tissue in rats (Mousa et al. 2004). Thus, leukocytes can process POMC into functionally active β-endorphin. Furthermore, met-enkephalin, dynorphin and endorphins are also detectable in leukocytes of inflamed tissues. Some opioid-containing immune cells identified to date are T and B lymphocytes, granulocytes and monocytes/macrophages (Przewlocki et al. 1992; Cabot et al. 1997; Mousa et al. 2001a; Rittner et al. 2001). Thus, opioid peptides are processed and present in the circulation and in the immune cells infiltrating injured tissue.

In macrophages, monocytes, granulocytes and lymphocytes, β-endorphin is present in secretory granules arranged

Table 2 Site of production of endogenous opioids

Opioid	Site of production	References
β-endorphin	Pituitary[a] Hypothalamus[a] Nucleus of the solitary tract T cell, B cell, Monocyte, Macrophage	Bloom et al. (1977), Moon et al. (1973), Pelletier et al. (1977, 1980), Bloch et al. (1978), Jacobowitz and O'Donohue (1978) Krieger et al. (1977), Pelletier et al. (1980), Stein et al. (1995), Rittner et al. (2005)
Enkephalin	Cortex Spinal cord Adrenal medulla Gi tract T cell, B cell, monocyte, macrophage	Elde et al. (1976), Hokfelt et al. (1977), Khachaturian et al. (1982a), Uhl et al. (1978), Watson et al. (1981), Rittner et al. (2005)
Dynorphin	Hypothalamus[a] Brainstem T cell, B cell, monocyte, macrophage	Khachaturian et al. (1982b), Watson et al. (1981), Weber et al. (1982), Rittner et al. 2005)

[a] Classical loci for production of peptides derived from POMC

at the cell periphery, ready for exocytosis. During the early inflammation, as the leukocytes migrate to the site of infection, they (along with the resident cells) secrete various chemokines such as CxCL8, CXCL1, IL-6, IL-1, etc., which lead to hyperalgesia. In late inflammation, macrophages and lymphocytes secrete IL-4, IL-10 and IL-13, inhibiting the hyperalgesic pathways at several different stages and leading to analgesia (see Fig. 1 for details). In addition, leukocytes can also release opioid peptides following stimulation from corticotropin-releasing factor (CRF) and IL-1β. The effects are mediated by CRF and IL-1β receptors (coexpressed by opioid-containing leukocytes) via a calcium-dependent mechanism and are mimicked by elevated extracellular concentrations of potassium (Cabot et al. 1997). This is consistent with a regulated pathway of neuronal or endocrine release from secretory vesicles. Furthermore, noradrenalin (NA) can release β-endorphin from leukocytes in vitro following activation of adrenergic receptors (Schafer et al. 1996). Adrenergic α1, β2 and, to a lesser degree, α2 receptors are expressed on β-endorphin-containing inflammatory cells located in close proximity to sympathetic nerve fibers in inflamed paws. Chemical ablation of these fibers has been shown to abolish intrinsic opioid analgesia (Rittner et al. 2005). In summary, CRF and IL-1 β, as well as sympathetic neuron-derived NA, can act on their respective receptors on leukocytes to release opioid peptides (Fig. 2). Leukocytes are able to exert analgesic effects by releasing opioid peptides, which bind to opioid receptors of the nociceptors in the periphery.

Exogenous opioids

Of the exogenous opioids, morphine the principal alkaloid of opium have been most extensively studied. Gates and Tschudi were the first to successfully synthesize the complete molecule of morphine in 1952 (Gylbert 1973). The overall structure of morphine consists of two planes, with the first plane containing a benzene ring, an oxide ring, and a carboxylic ring, while the second plane contains a carbocyclic ring, an ethenamine ring, O_2 and N (Gylbert

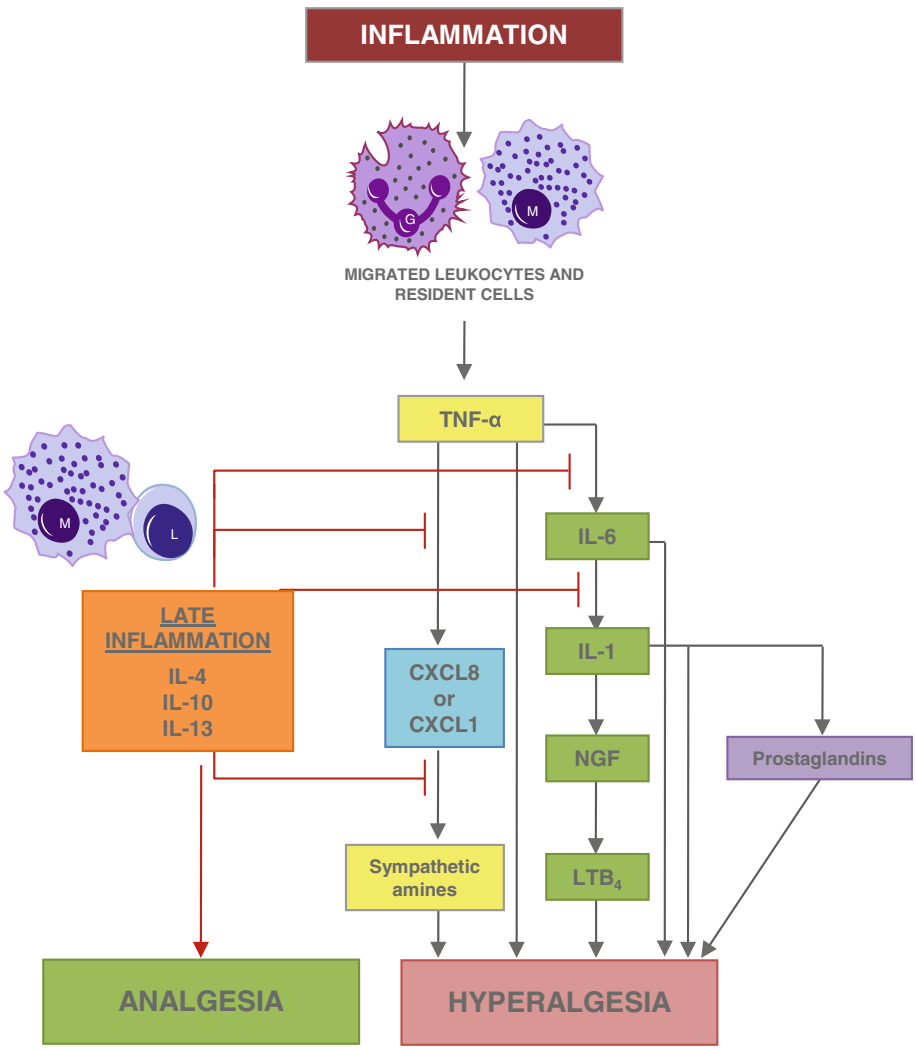

Fig. 1 Hyperalgesic and analgesic mechanisms in inflammation. In early inflammation, leukocytes, e.g., granulocytes (*G*) and monocytes (*M*), migrate into the inflamed tissue. Here, these leukocytes as well as resident cells release cytokines including TNF-α, interleukins (*ILs*), chemokines [CXC chemokine ligand 8 (CXCL8), CXCL1], NGF and secondary mediators, such as sympathetic amines, leukotriene B_4 (LTB_4) and prostaglandins, culminating in hyperalgesia. TNF-α, IL-6 and IL-1 can also have direct hyperalgesic effects on nociceptors. During ongoing late inflammation, lymphocytes (*L*) and monocytes/macrophages (*M*) start producing anti-inflammatory cytokines, such as IL-4, IL-10 and IL-13. These cytokines inhibit the proinflammatory cytokines, such as TNF-α, IL-1 and IL-6, and block the cascade, resulting in analgesia

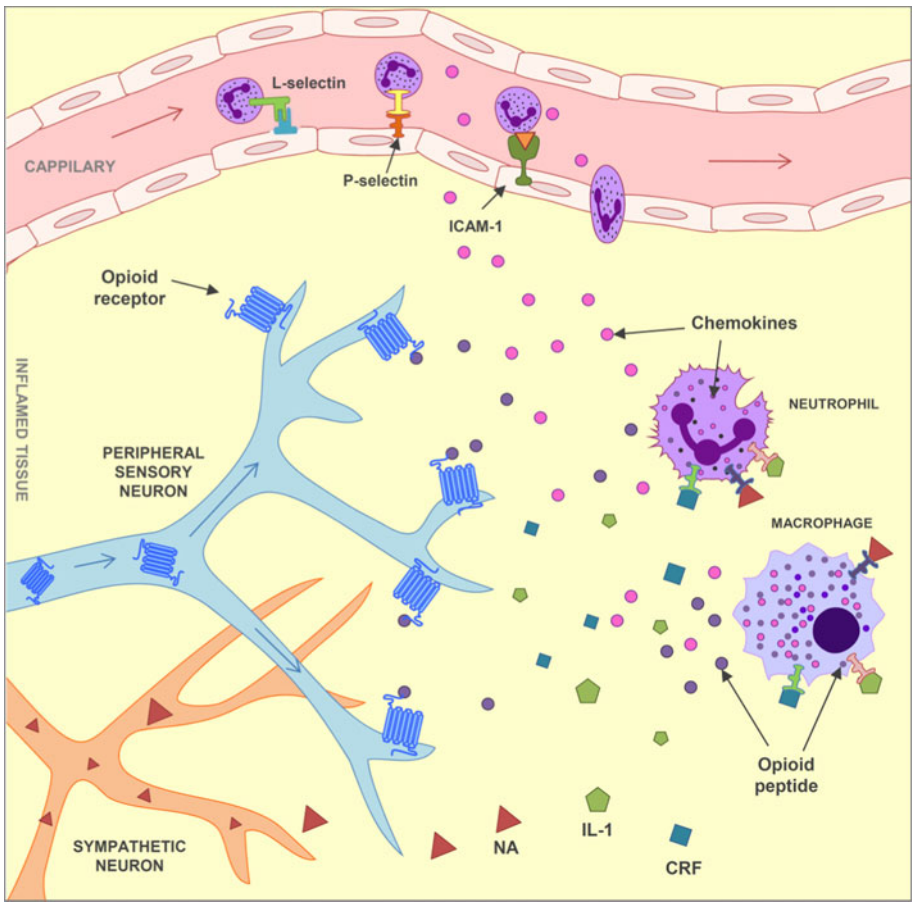

Fig. 2 Inflammation-induced migration of opioid-producing leukocytes and opioid secretion. Resident macrophages of the inflamed tissue release chemokine gradient to recruit neutrophils form the bloodstream. Chemokine secretion leads to upregulation of adhesion molecules (P-selectin, ICAM-1 etc.) on the capillary endothelium, which facilitates neutrophil rolling, adhesion and extravasation. Once extravasated, leukocytes can be stimulated by release of corticotropin-releasing factor (CRF), interleukin-1β (IL-1) and/or noradrenaline (NA). CRF, IL-1 and NA (derived from sympathetic neurons) elicit opioid release by activating their respective receptors on leukocytes. Opioids bind to peripheral opioid receptors (produced in the dorsal root ganglia and are transported to peripheral endings of sensory neurons) causing analgesia by inhibiting the excitability of these neurons. Opioid agonists have easier access to neuronal opioid receptors during inflammation, because inflammation disrupts the perineurium (normally a rather impermeable sheath encasing peripheral nerve fibers). *Arrow* in the blood vessel and sensory neuron indicates the direction of the events

1973). Morphine is metabolized in the liver via *N*-dealkylation and glucoronidation at the third position morphine-3-glucuronide (M3G) or the sixth position morphine-6-glucuronide (M6G). Although M3G is the most common metabolite (accounting for 50% of the metabolites produced), they exert no biological activity when bound to MOR. In contrast, M6G, although less prevalent (accounting for 10% of the metabolites produced), can induce an analgesic effect upon binding to the MOR (Dahan et al. 2008).

The synthetic morphine derivatives, fentanyl and heroin, have similar efficacy and addictive properties as morphine, yet these two drugs differ in their onset and duration of action. Rapid onset of fentanyl and heroin are attributed to their highly lipophilic profiles making these drugs readily available crossing the blood–brain barrier.

Classical targets of opioid action

Originally, before the discovery of opioid receptors on leukocytes, it was thought that opioids exert their effects solely through an indirect pathway by the binding of opioid receptors expressed in the central nervous system (CNS). One school of thought postulates that opioid receptors are expressed only in the CNS, and therefore the classical target cells of the opioid-mediated analgesia were neuronal cells of the CNS. Morphine's modulation of the pain mechanisms and immune system occurs through the activation of the hypothalamo–pituitary–adrenal axis (HPA) and the stress-responsive neuroendocrine pathway (Peterson et al. 1993).

Hypothalamo–pituitary–adrenal axis is a complex set of direct influences and feedback interactions among the

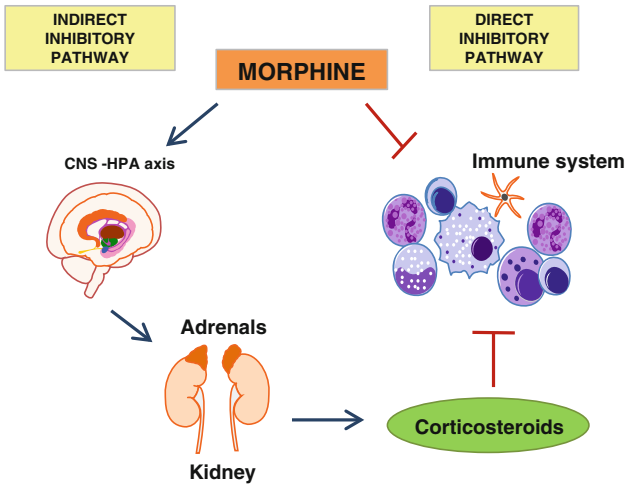

Fig. 3 Pathways of opiate-induced immune suppression. Morphine can modulate immune system via direct and indirect pathways. Indirectly, morphine acts on CNS and the hypothalamic–pituitary–adrenal (HPA) axis which leads to the release of corticosteroids, and immunosuppressive hormones which leads to suppression of the immune system. Direct inhibitory pathway requires the direct interaction of opioids on the cells of the immune system

hypothalamus, pituitary gland and the adrenal (or suprad-renal) glands (Fig. 3). The interactions among these organs constitutes the HPA axis, a major part of the neuroendocrine system that controls reactions to stress and, through release of stress hormones [corticosteroids (CORT)], exerts immunosuppressive effects.

However, more recently, studies supporting a direct role of opioids on immune system are gaining more acceptance primarily with the discovery of opioid receptors on the immune cells (Table 1). Opioid alkaloids and peptides such as morphine and the endogenous peptides, including β-endorphin and the dynorphin peptides, directly modulate the functions of lymphocytes and other cells involved in host defense and immunity (Bidlack et al. 2006). The concept of direct and indirect morphine action was first introduced through studies with morphine-dependent rodents. Findings by our group and others indicate that morphine-induced immunosuppression is mediated by the MOR and that, although some functions are amplified in the presence of CORT or sympathetic activation, the inhibition of IFN-gamma synthesis and modulation of macrophage-cytokine synthesis are CORT independent and only partially dependent on sympathetic activation (Peterson et al. 1987; Bryant et al. 1991; Casellas et al. 1991; Perez-Castrillon et al. 1992). Although the current research focus has shifted to direct effects of opioids on immune cells, when looking at in vivo models of drug abuse and immunomodulation, it is important to consider the role of stress mechanisms mediated by the HPA axis.

Opioid modulation of immune cell function

Research studies provide strong support that chronic morphine can indirectly modulate both adaptive and innate immune systems. Although, support for an indirect effect of morphine modulation is strong (Vallejo et al. 2004; Wang et al. 2005), recent focus has shifted to exploring how morphine may directly exert its suppressive effects on innate immune cells by binding to their opioid receptors. The multifaceted immunosuppressive actions of morphine add to the complexity of identifying targets of its inhibition.

Direct immunomodulation

Opioids and adaptive immune response

Modulation of adaptive immune system has first been observed in morphine-treated rodents where morphine treatment led to decreased splenic and thymic weight, resulting in reduced function of T cells and their precursors (Bryant et al. 1988a, b, 1991). Such morphologic changes indicate the magnitude of morphine's immunosuppressive capabilities.

Morphine and T cells Morphine has been observed to modulate various aspects of T-cell functions (see Table 3 for details). Murine opioid dependence models utilizing a slow-release morphine pellet have shown that chronic morphine treatment leads to a reduction in cell viability, proliferative response, T-helper cell function, as well as reduced CD4/CD8 population in vivo. Additionally, chronic morphine treatment in vitro (pharmacological doses that are consistent with those observed in drug abusers or patient on a moderate to severe pain management regimen) has been shown to significantly decrease the production of IL-1β, IL-2, TNF-α and IFN-γ from mouse splenocyte cultures, as well as to stimulate the production of anti-inflammatory cytokines, TGF-β1 and IL-10 (Pacifici et al. 2000). Furthermore, in vitro morphine treatment of PBMCs or splenocytes results in T-helper cell differentiation toward the Th2 lineage (Roy et al. 2001). Mechanistically, morphine treatment impairs mitogen-stimulated lymphocyte proliferation by interfering with transcriptional activation of the IL-2 gene (Roy et al. 1997), as well as interfering with IFN-γ promoter activity through two distinct cAMP-dependent pathways, specifically the NF-$\kappa\beta$ and AP-1/NFAT pathways (Wang et al. 2003). Low-dose morphine (10–100 nM) treatment of lymph node-derived T lymphocytes results in impaired Con A-induced proliferation and IL-2 and IFN-γ production, accompanied by an increase in apoptosis. These effects were abolished in the absence of MOR in MORKO

Table 3 Morphine suppresses immune cell function

Cell type	Morphine effect	References
PBMC	Suppressed activity	Carr et al. (1993)
	Th1 Th2 shift	Roy et al. (2001)
NK cells	↓ Superoxide production	Peterson et al. (1987)
	Suppressed activity	Carr et al. (1993)
	↓ Number	Carr et al. (1993)
T cells	↓ IFN-γ promoter activity via ↑ cAMP	Wang et al. (2003)
B cells	↓ Mitogenic responses of splenic B cells to LPS	Bhargava et al. (1994), Bryant et al. (1988a, b), Bussiere et al. (1992)
	↓ Numbers in mouse spleens	Lefkowitz et al. (2000)
Murine macrophages	↑ Phagocytosis ↑ intracellular bacterial growth ↓ respiratory burst activity ↓ chemotaxis	Tomei and Renaud (1997), Perez-Castrillon et al. (1992), Wang et al. (2005), Martin et al. (2010)
Murine splenocytes	↓ IL-1β, IL-2, TNF-α, IFN-γ,↑ TGF-β1, IL-10 production	Pacifici et al. (2000)
	Th1 Th2 shift	Roy et al. (2001)
Murine thymocytes	↓ Activation of IL-2 gene	Roy et al. (1997)

mice (Wang et al. 2001). Other investigators have also investigated the role of the mu-opioid receptor in morphine-induced immunosuppression and have noted that morphine-induced lymphoid organ atrophy and diminished NK cell activity were lost in MORKO mice following Con A stimulation, demonstrating the essential role of the mu-opioid receptor in morphine-mediated immune deficits (Gaveriaux-Ruff et al. 1998). Studies by Borner et al. 2008 using loperamide, a peripheral MOR agonist, have shown that activation of MOR in the periphery can lead to inhibition of CD3/28 mAb-induced interleukin-2 transcription in JURKAt cells, similar to fentanyl. These studies further support direct opioid-induced immunosuppression, by indicating that MOR activation in the periphery can lead to immunosuppressive effects.

Morphine and B cells In contrast to T-cell research, work on morphine's effect on function of B cells is limited. First findings reported by Bussiere et al. indicated that morphine pellet implantation in vivo suppressed the primary antibody response in multiple strains of mice. These findings were further supported by several other groups that found that implantation in vivo morphine treatment led to reduction of the mitogenic responses of splenic B cells to bacterial lipopolysaccharide (LPS) (Bryant et al. 1988a, b; Bussiere et al. 1992; Bhargava et al. 1994). Formation of antibody response requires interaction of macrophages, T cells and B cells; therefore, modulation of antibody producing capacity by morphine may be mediated by affecting any of the three cell types (Eisenstein and Hilburger 1998). Bussiere et al. also found that morphine's inhibition of antibody responses could be restored with the addition of untreated macrophages or with the addition of macrophage cytokines [IL-1, IL-6 or interferon-γ (IFN-γ)], suggesting that the morphine-induced suppression was due in part to a deficit of macrophage activity (Bussiere et al. 1993). Furthermore, it was demonstrated that morphine's modulation of antibody responses was T-cell dependent, but not to a T cell-independent antigen, suggesting that morphine did not directly affect B-cell function (Weber et al. 1987; Eisenstein and Hilburger 1998).

Opioids and innate immune response

The modulation of innate immune system has been observed at several levels. Morphine treatment modulates leukocyte recruitment, cytokine secretion and bacterial clearance by decreasing the proliferative capacity of macrophage progenitor cells (morphine treatment inhibits the number of macrophages that are available to respond to an infection) (Roy et al. 2006). In addition, morphine delays leukocyte migration, which affects the numbers of phagocytes recruited to the site of infection and ultimately suppresses the capacity of macrophages to ingest opsonized pathogens (Casellas et al. 1991; Szabo et al. 1993; Tomei and Renaud 1997). Collectively, these findings suggest that the macrophage is a key cellular target for the suppressive effects of morphine on the antibody response (Bussiere et al. 1993). Although morphine modulates both innate and adaptive immune systems, defects in innate immunity seem to have broader consequences, with modulation of macrophage functions playing an essential role. Therefore, examining morphine-mediated modulation of macrophage processes will be the main focus of our discussion.

Morphine and macrophages Our laboratory first demonstrated that morphine modulation of several immune functions was attributable to the mu-opioid receptor, including macrophage phagocytosis and secretion of TNF-α, since these effects were abolished in morphine-treated MORKO mice (Roy et al. 1998a).

Macrophages form the first line of defense against pathogens, and play an essential role in innate immunity through their phagocytic and bactericidal roles, as well as through their ability to recruit other cells to the site of infection. Therefore, any defects in macrophage function can be detrimental to the host. Macrophages have been at the center of several studies due to the significant role they play in morphine-mediated immune suppression.

Morphine modulation of macrophage phagocytosis Morphine treatment leads to suppression of peritoneal macrophage phagocytosis, as well as inhibition of respiratory burst activity and chemotaxis (Perez-Castrillon et al. 1992). Due to inhibition of phagocytosis, bacteria are inadequately removed and, since respiratory burst is inhibited, morphine attenuates bacterial killing, which together with inhibited phagocytosis leads to increased bacteremia and bacterial escape from latency as shown by our group and others (Bhaskaran et al. 2001; Wang et al. 2005; Lugo-Chinchilla et al. 2006). Human studies and rodent models of drug abuse indicate that morphine impairs the ability to eradicate infection by inhibiting phagocytosis. In vivo models of morphine abuse have shown that morphine inhibits phagocytosis by non-elicited and elicited macrophages in a naltrexone reversible manner, indicating the involvement of classical opioid receptors (Rojavin et al. 1993a, b). Subsequent in vitro studies indicate that morphine inhibits Fcγ receptor (FcgR)-mediated phagocytosis essential for internalization of extracellular pathogens, and that inhibition of phagocytosis occurs through μ- and δ-opioid receptors (Szabo et al. 1993; Tomassini et al. 2004). Studies by our group confirmed that morphine-mediated inhibition of phagocytosis was abolished in MOR knockout (MORKO) mice, adding further evidence for the role of MOR in these functions (Roy et al. 1998a). Activation of MOR in the periphery via loperamide has been shown to lead to inhibition of clearance of *Giardia lamblia* leading to increased trophozoite loads (Andersen et al. 2006). Additionally, it was observed that in vitro administration of endogenous opioid peptides such as leu- and met-enkephalin (delta receptor agonists) are able to inhibit phagocytosis of opsonized sheep red blood cells (Casellas et al. 1991).

In addition to inhibiting macrophage phagocytosis, several studies support that in vivo morphine treatment (opioid dependent model) attenuates bacterial killing as evident by increased bacterial loads or sepsis (Wang et al. 2005; Hilburger et al. 1997). In mice, chronic morphine treatment in a drug abuse model has been shown to modulate bacterial killing by inhibition of NO release (Menzebach et al. 2004; Bhaskaran et al. 2007). Our laboratory's previous data and several other studies indicate that chronic morphine (drug abuse model), by inhibition of NO release, increases susceptibility to bacterial infection, resulting in bacteremia and bacterial invasion of the CNS (Wang et al. 2005; Asakura et al. 2006; Bhaskaran et al. 2007). A recent study by (Singh and Singal 2007) notes that morphine administration has a dose-dependent biphasic modulation in *Leishmania donovani*-infected mice and peritoneal macrophages in vitro, via a NO-dependent mechanism. Furthermore, it was demonstrated that morphine administration in the nanomolar range was protective against *L. donovani* infection, while morphine concentrations in the micromolar range led to augmented parasite growth in macrophages.

In addition, morphine has been implicated in the inhibition of superoxide production. Several groups, studying morphine's effect on infection examined superoxide release as a mechanism of bacterial killing, noticed that morphine treatment in vitro (doses that mimic levels in drug abusers) inhibited superoxide production in neutrophils and macrophages (Sharp et al. 1985; Simpkins et al. 1986; Welters et al. 2000). In addition to exogenous opioids, endogenous opioids had similar inhibitory effects, where pretreatment with endogenous opioid peptides, leucine or methionine enkephalins reduced the neutrophil's ability to generate superoxide production in response to the *Escherichia coli* product, *N*-formyl methionyl leucyl phenylalanine (FMLP) (Sharp et al. 1985; Simpkins et al. 1986). Morphine-mediated suppression of superoxide production was reproduced in human peripheral mononuclear cells in studies done by Peterson et al. (1987, 1989), which examined respiratory burst activity in response to phorbol myristate acetate (PMA).

In addition to inhibition of bacterial clearance, morphine treatment in vivo (opioid dependent model) leads to inhibition of macrophage recruitment and function during an innate immune response. A study carried out by Grimm et al. (1998b) showed a significant decrease in macrophage chemotaxis when cells were preincubated with morphine or met-enkephalin. They concluded that morphine's inhibition of subsequent macrophage chemotaxis occurred upon direct binding to the macrophage MOR, and that this activation of MOR led to the phosphorylation and desensitization of chemokine receptors, CCR1, CCR2, CXCR1 and CXCR2. Desensitized chemokine receptors are therefore unable to elicit a response when their ligands are present.

In the presence of the endotoxin LPS, suppression of cytokines IL-6 and TNF-α was seen following morphine treatment (Roy et al. 1998b). The transcription factor NFκB, responsible for up-regulation of several cytokines

including IL-6, TNF-α, NO and IL-10, was also suppressed following morphine treatment.

Morphine and neutrophils Although it has been observed for some time that chronically administered morphine modulates neutrophil chemotaxis and function, controversy still exists in determining which mechanisms are at play. A growing body of literature supports morphine's suppressive effects on recruitment and immune functions of neutrophils during an innate immune response. Exogenous opioid treatment of peripheral human blood neutrophils leads to inhibition of IL-8-induced chemotaxis (Grimm et al. 1998a). Conversely, Simpkins et al. (1984) reported an increase in neutrophil chemotaxis following endogenous opioid (β-endorphin) treatment. The discrepancy of the latter finding may in part be explained by the differences in affinity of morphine and β-endorphins to the mu-opioid receptors on immune cells. Furthermore, acute morphine treatment in vivo (opioid-dependent model) leads to inhibition of neutrophil cytokines involved in regulation of wound healing (Martin et al. 2010). In a wound healing model, it was demonstrated that morphine treatment (opioid-dependent model) resulted in a significant delay and reduction in both neutrophil and macrophage recruitment to the wound site (Martin et al. 2010). The delay and reduction in neutrophil reduction was attributed to altered early expression of keratinocyte-derived cytokine and was independent of macrophage inflammatory protein-2 expression, whereas suppression of macrophage infiltration was attributed to suppressed levels of the potent macrophage chemoattractant, called monocyte chemotactic protein-1.

Taken together, the complexity by which morphine acts as an immunosuppressor on migration and functional activity of innate immune responders, particularly neutrophils and macrophages, poses a compromising environment that proves detrimental to the hosts' ability to eradicate pathogens.

Autocrine and paracrine opioid signaling

Endogenous opioids are capable of paracrine and autocrine signaling. Cells of the CNS and immune system are capable of generating endogenous opioids. Interestingly, exogenous opioids are capable of acting directly on the immune cells, as well as indirectly by activating the HPA axis.

It is accepted that inflammatory mediators released from leukocytes contribute to the generation of pain. However, it is less well known that immune cells also produce mediators that can effectively counteract pain. These include anti-inflammatory cytokines and opioid peptides (Machelska 2007). Physiological pain triggers a warning mechanism, which functions to minimize tissue damage. During the inflammatory response, various pro-inflammatory and pro-analgesic mediators are released to activate specialized peripheral pain signaling sensory neurons ("nociceptors"). Trigeminal and dorsal root ganglia (DRG) contain nociceptor cell bodies, which give rise to myelinated Aδ and unmyelinated C fibers. Peripheral terminals of Aδ and C fibers transduce and propagate noxious stimuli from peripheral tissues (such as skin, muscles, joints and viscera) to the dorsal horn of the spinal cord and thereafter to the brain. At the spinal and supraspinal sites, the integration of signals from pro-analgesic neurotransmitters, environmental and cognitive factors eventually results in the sensation of pain (Woolf and Salter 2000). Inflammation in the periphery leads to increased synthesis and axonal transport of opioid receptors in DRG neurons, resulting in upregulation of their surface expression and enhanced G-protein coupling at peripheral nerve terminals (Ji et al. 1995; Mousa et al. 2001b). This is followed by disruption in the perineurial barrier, allowing opioids to access their respective receptors and modulate the pain signals emanating from the site of inflammation (Antonijevic et al. 1995).

Another way by which leukocytes are able to control inflammatory pain is by recruiting other opioid-containing leukocytes to the site of inflammation. During the inflammatory response, leukocytes are recruited to the site of infection through chemokines, cytokines and upregulation of adhesion molecules. Studies by Machelska et al. (1998, 2002) have shown that pretreatment of rats with selectin blocker (fucoidin) or selective antibodies against ICAM-1, integrins (α_4 and $\beta_{2)}$ or chemokines (CXCL1 and CXCL2/3) lead to a substantial decrease in the number of opioid-containing immune cells accumulating in inflamed tissue, and consequently abolish endogenous peripheral opioid analgesia. In addition, the migration of opioid-containing leukocytes into injured tissues appears to be modulated by mechanisms involving signaling from the CNS. (Schmitt et al. 2003), have shown that intrathecally injected morphine, in a dose-dependent manner, significantly decreases the number of β-endorphin-containing leukocytes in inflamed rat paws and attenuates peripheral endogenous analgesia. These findings indicate that effective central inhibition of pain signals inhibit the recruitment of opioid-containing leukocytes to injured tissues (Machelska 2007).

These studies support a paracrine role of endogenous opioids in the regulation of pain through either leukocyte-mediated opioid release signaling via the nociceptors, or through the central opioid mechanisms utilized to limit opioid secretion at the inflammatory site. Paracrine and autocrine signaling of opioid-containing leukocytes is important in immune suppression. Leukocyte chemotaxis and key immune functions are significantly impaired in the

presence of opioids. By secreting opioids, leukocytes can inhibit their own immune functions as well as those of other leukocytes present at the inflammatory site. Opioid-mediated inhibition of cytokine and chemokine release inhibits further recruitment to the site of inflammation leading to reduced inflammatory signals and potential pain reduction. Therefore, opioids released from leukocytes can modulate pain by acting through nociceptors and DRG, as well as by inhibiting inflammation.

Clinical use of opioids and disease

Clinical opioids

Opioids remain the gold standard for chronic pain management, in spite of the adverse side effects resulting from their use. A number of opioids are available for clinical use, including morphine, hydromorphone, levorphanol, oxymorphone, methadone, meperidine, oxycodone and fentanyl (Inturrisi 2002). Opioids are often prescribed for management of cancer pain, post-operative pain, as well as chronic pain in individuals with late-stage HIV. Morphine and fentanyl are often used to alleviate severe pain, while codeine is used for milder pain. Other examples of opioids prescribed to relieve pain include propoxyphene (Darvon); hydromorphone (Dilaudid) and meperidine (Demerol), which are used less often because of their side effects. In addition to their effective pain-relieving properties, some of these medications can be used to relieve severe diarrhea (for example, Lomotil, also known as diphenoxylate) or severe coughs (codeine) (Lustman et al. 1987; Wee et al. 2011). Methadone and buprenorphine, are synthetic opioids that are used for treatment of addiction. They eliminate withdrawal symptoms and relieve craving. Methadone has been used successfully for more than 30 years to treat people addicted to heroin as well as opiates, while buprenorphine has been approved more recently for treating addiction to heroin and other opiates.

Naltrexone and naloxone are opioid receptor blockers, which are clinically used to prevent relapse and treat overdose (respectively). Naltrexone is a long-acting opioid receptor blocker that can be employed to help prevent relapse. It is not widely used, however, because of poor compliance, except by highly motivated individuals (e.g., physicians at risk of losing their medical license). This medication is only used in patients who have already been detoxified, since it can produce severe withdrawal symptoms in a person continuing to abuse opioids.

Studies examining morphine's effect vary greatly in terms of doses and concentrations used. Pharmacological doses that are consistent with those observed in drug abusers or patient on a moderate to severe pain management regimen have been observed to lead to morphine plasma levels of 11–1,440 ng/ml in cancer patients using morphine for pain management at a dose of 2.5–90 mg every 4 h (Aherne et al. 1979). Therefore, physiological concentrations used in most animal or in vitro studies, to mimic physiological doses, range from 10 nM to 1 μM.

Opioids and infection

Morphine's immunosuppressive effects have been observed for centuries. Recently, as the prescription of opioid-based pain relievers began to rise, opportunistic infections have followed the same trend (Compton and Volkow 2006; Wang et al. 2008). The prevalence of opioid use and abuse is undisputed, and has impacted a wide range of individuals in both the drug abuse population as well as the patients in clinical settings.

Immunosuppression in opioid abusers has been observed clinically and anecdotally. Although clinical studies examining opioid-mediated immune suppression are limited, animal studies indicate morphine's immunosuppressive abilities through increased incidence of many bacterial and viral infections. Several groups show that intravenous drug abusers have a greater incidence of infection than non-abusers (Hussey and Katz 1950; Louria et al. 1967). The documentation that opioids, such as morphine, has the potential to modulate immunity is consistent with their ability to alter immune responsiveness to microbial agents (Cabral 2006). Extensive research in the area of morphine-induced immune suppression noted that opioid addicts present with high prevalence of tuberculosis, bacterial pneumonias, abscesses, CNS infections as well as viral hepatitis A, B and C, and high rate of HIV infections (Louria et al. 1967; Reichman et al. 1979; Haverkos and Lange 1990).

To date, most studies examining opioid-induced immune suppression have been utilizing cohorts of drug abusers, where main confounders lie in the fact that opioid abusers often engage in risk behaviors (e.g., contaminated needles/syringes) as well as use multiple drugs of abuse (such as alcohol, nicotine, cocaine, etc.) exerting their own additional immunosuppressive effects. Effects of risky behavior that drug abusers engage in are also a co-factor in clinical studies of this kind. Therefore, infectious complications in iv drug users are very frequent, but cannot easily be distinguished from risk behavior or risk environment. Studies examining the effects of opioids in the clinical setting are scarce. The difficulty in designing clinical studies of opioid use lies in obtaining a patient population which is not immunocompromised, yet is undergoing opioid-based therapies for chronic pain. Therefore, most studies in patients with chronic (non-malignant) pain report

infectious complications in relation to opioid use; however, their incidence is usually not considered to be drug related. Limitations of studies examining clinical relevance of opioid use and abuse have been extensively described by several groups (Rittner et al. 2010; Brack et al. 2011) and therefore will not be a focus of this review. Although these confounders of direct opioid inhibition of immune function may play a role in clinical studies, a wide body of evidence in mammalian models indicates that opioids alone are capable of direct immunomodualtory effects.

Several groups indicate a connection between intravenous opioid use and increased incidence of infections in humans. (McCoy et al. 2004) examined the prevalence of HIV-1 and HCV among injection drug users in Miami, Florida. The results of multivariate analyses indicated a direct correlation between years of heroin use and HCV infection. Furthermore, retrospective studies as well as seroepidemiological analyses indicate that injection users of opioids, such as heroin, have an increased incidence of disease including that attributable to HIV infection (Horsburgh et al. 1989; Nemoto et al. 1990; Joe et al. 1991; Spittal et al. 2003).

A seminal study by (Tubaro et al. 1983) observed that following single daily injections of morphine, given 24–72 h prior to iv injection of fungus *Candida albicans*, resulted in increased lethality in mice from the organism. This study demonstrated that morphine was able to increase the number of viable *C. albicans* in the kidney in a dose-dependent manner. More recent studies indicate similar results, where mice implanted with slow-release morphine pellet presented with sepsis, which was manifested by increased bacterial loads in liver, spleen and peritoneal cavities (Hilburger et al. 1997). Additionally, our laboratory has shown that in vivo chronic morphine treatment followed by intranasal inoculation with *Streptococcus pneumoniae* markedly delayed neutrophil recruitment and increased bacterial burden in the lung, spleen and blood, with a subsequent increase in mortality (Wang et al. 2005). Morphine's immunosuppressive effects were first noted in its ability to increase susceptibility to infection, as well as accelerate the rate of their progression. Interestingly, *S. pneumoniae* is one of the most common diagnoses among opiate abusers; it is responsible for more than 25% of all cases of pneumonia and is still associated with an overall mortality rate of 23% among hospitalized patients. Drug abuse has been determined to be a significant risk factor for the development of community-acquired pneumonia. *Pneumococcal* clearance requires the cooperation of both innate and adaptive immunity. Epidemiological data suggest that HIV-positive drug abusers progress to symptomatic AIDS more rapidly than those who do not use drugs; therefore, additional longitudinal studies addressing the enhancement of disease in immunocompromised individuals are warranted (Cabral 2006).

The studies that have been reviewed suggest that illicit drugs act, at least, as cofactors that can increase the severity of infection by microbial agents through altering host resistance. This decrease in host resistance may be a consequence of immunosuppressive action on the activities of macrophages, T lymphocytes and NK cells. The mechanisms by which these drugs increase susceptibility to infection have not been fully delineated. Considering previously discussed studies, a convergent mode of action by which drugs of abuse affect immunity and increase susceptibility to infection appears to be that they affect cytokine and chemokine expression and, in so doing, alter the homeostatic balance of proinflammatory versus anti-inflammatory mediators. The documented evidence that illicit drugs alter antimicrobial activity in vivo and in vitro indicates that their use presents a potential risk of decreased resistance to infections in humans.

Conclusion

This review summarizes the current understanding of the roles opioids play in neuro-immunity. We delineate opioid receptor functions and distributions as well as the role of endogenous and exogenous opioids on the immunomodualtory and analgesic mechanisms. Immunosuppressive effects of opioids have been observed in several different models as signaling and acting directly through immune cells or acting via the HPA axis. The ability of the immune cells to produce opioid peptides, as well as their expression of opioid receptors, has led to an interesting paradigm shift. Original thoughts of opioids being secreted by and acting solely on the nervous system have recently been diverted to investigation of opioid secreting leukocytes and the role they play in modulation of traditional pain mechanisms. In spite of a multitude of research conducted in this field, a gap in the understanding of mechanisms underlying these processes still exists. Although many advances have been made in understanding the effects of endogenous and exogenous opioids on immune responses, the real clinical relevance of these effects is not completely clear. Enhancing our knowledge and understanding of opioid-mediated immune suppression and mechanisms involved in these processes is essential to the development of new and improved therapies for chronic pain management.

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References

Aherne GW, Piall EM, Twycross RG (1979) Serum morphine concentration after oral administration of diamorphine hydrochloride and morphine sulphate. Br J Clin Pharmacol 8:577–580

Akil H, Watson SJ, Young E, Lewis ME, Khachaturian H, Walker JM (1984) Endogenous opioids: biology and function. Annu Rev Neurosci 7:223–255

Andersen YS, Gillin FD, Eckmann L (2006) Adaptive immunity-dependent intestinal hypermotility contributes to host defense against *Giardia* spp. Infect Immun 74:2473–2476

Antonijevic I, Mousa SA, Schafer M, Stein C (1995) Perineurial defect and peripheral opioid analgesia in inflammation. J Neurosci 15:165–172

Asakura H, Kawamoto K, Igimi S, Yamamoto S, Makino S (2006) Enhancement of mice susceptibility to infection with Listeria monocytogenes by the treatment of morphine. Microbiol Immunol 50:543–547

Bhargava HN, Thomas PT, Thorat S, House RV (1994) Effects of morphine tolerance and abstinence on cellular immune function. Brain Res 642:1–10

Bhaskaran M, Kapasi AA, Reddy K, Singhal PC (2007) Morphine priming rescues high-dose morphine-induced biological perturbations. J Infect Dis 195:1860–1869

Bhaskaran M, Reddy K, Sharma S, Singh J, Radhakrishnan N, Kapasi A, Singhal PC (2001) Morphine-induced degradation of the host defense barrier: role of macrophage injury. J Infect Dis 184: 1524–1531

Bidlack JM, Khimich M, Parkhill AL, Sumagin S, Sun B, Tipton CM (2006) Opioid receptors and signaling on cells from the immune system. J Neuroimmune Pharmacol 1:260–269

Bloch B, Bugnon C, Fellman D, Lenys D (1978) Immunocytochemical evidence that the same neurons in the human infundibular nucleus are stained with anti-endorphins and antisera of other related peptides. Neurosci Lett 10:147–152

Bloom F, Battenberg E, Rossier J, Ling N, Leppaluoto J, Vargo TM, Guillemin R (1977) Endorphins are located in the intermediate and anterior lobes of the pituitary gland, not in the neurohypophysis. Life Sci 20:43–47

Borner C, Kraus J, Bedini A, Schraven B, Hollt V (2008) T-cell receptor/CD28-mediated activation of human T lymphocytes induces expression of functional mu-opioid receptors. Mol Pharmacol 74:496–504

Brack A, Rittner HL, Stein C (2011) Immunosuppressive effects of opioids-clinical relevance. J Neuroimmune Pharmacol 6:490–502

Bryant HU, Bernton EW, Holaday JW (1988a) Morphine pellet-induced immunomodulation in mice: temporal relationships. J Pharmacol Exp Ther 245:913–920

Bryant HU, Yoburn BC, Inturrisi CE, Bernton EW, Holaday JW (1988b) Morphine-induced immunomodulation is not related to serum morphine concentrations. Eur J Pharmacol 149:165–169

Bryant HU, Bernton EW, Kenner JR, Holaday JW (1991) Role of adrenal cortical activation in the immunosuppressive effects of chronic morphine treatment. Endocrinology 128:3253–3258

Bunzow JR, Saez C, Mortrud M, Bouvier C, Williams JT, Low M, Grandy DK (1994) 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 347:284–288

Bussiere JL, Adler MW, Rogers TJ, Eisenstein TK (1993) Cytokine reversal of morphine-induced suppression of the antibody response. J Pharmacol Exp Ther 264:591–597

Bussiere JL, Adler MW, Rogers TJ, Eisenstein TK (1992) Differential effects of morphine and naltrexone on the antibody response in various mouse strains. Immunopharmacol Immunotoxicol 14:657–673

Cabot PJ, Carter L, Gaiddon C, Zhang Q, Schafer M, Loeffler JP, Stein C (1997) Immune cell-derived beta-endorphin. Production, release, and control of inflammatory pain in rats. J Clin Invest 100:142–148

Cabral GA (2006) Drugs of abuse, immune modulation, and AIDS. J Neuroimmune Pharmacol 1:280–295

Carr DJJ, DeCosta BR, Jacobson AE, Rice KC, Edwin Blalock J (1991) Enantioselective kappa opioid binding sites on the macrophage cell line, P388d1. Life Sci 49:45–51

Casellas AM, Guardiola H, Renaud FL (1991) Inhibition by opioids of phagocytosis in peritoneal macrophages. Neuropeptides 18:35–40

Chao CC, Gekker G, Hu S, Sheng WS, Shark KB, Bu DF, Archer S, Bidlack JM, Peterson PK (1996) Kappa Opioid Receptors in Human Microglia Downregulate Human Immunodeficiency Virus 1 Expression. Proc Natl Acad Sci USA 93:8051–8056

Choi HS, Kim CS, Hwang CK, Song KY, Wang W, Qiu Y, Law PY, Wei LN, Loh HH (2006) The opioid ligand binding of human mu-opioid receptor is modulated by novel splice variants of the receptor. Biochem Biophys Res Commun 343:1132–1140

Chuang TK, Killam KF, Chuang LF, Kung HF, Sheng WS, Chao CC, Yu L, Chuang RY (1995) Mu Opioid Receptor Gene Expression in Immune Cells. Biochem Biophys Res Commun 216:922–930

Compton WM, Volkow ND (2006) Abuse of prescription drugs and the risk of addiction. Drug Alcohol Depend 83(Suppl 1):S4–S7

Dahan A, van Dorp E, Smith T, Yassen A (2008) Morphine-6-glucuronide (M6G) for postoperative pain relief. Eur J Pain 12:403–411

Eisenstein TK, Hilburger ME (1998) Opioid modulation of immune responses: effects on phagocyte and lymphoid cell populations. J Neuroimmunol 83:36–44

Elde R, Hokfelt T, Johansson O, Terenius L (1976) Immunohistochemical studies using antibodies to leucine-enkephalin: initial observations on the nervous system of the rat. Neuroscience 1:349–351

Evans CJ (2004) Secrets of the opium poppy revealed. Neuropharmacology 47(Suppl 1):293–299

Falke NE, Fischer EG, Martin R (1985) Stereospecific opiate binding in living human polymorphonuclear leucocytes. Cell Biol Int Rep 9:1041–1047

Gaveriaux C, Peluso J, Simonin F, Laforet J, Kieffer B (1995) Identification of kappa- and delta-opioid receptor transcripts in immune cells. FEBS Lett 369:272–276

Gaveriaux-Ruff C, Matthes HW, Peluso J, Kieffer BL (1998) Abolition of morphine-immunosuppression in mice lacking the mu-opioid receptor gene. Proc Natl Acad Sci USA 95:6326–6330

Glaum SR, Miller RJ, Hammond DL (1994) Inhibitory actions of delta 1-, delta 2-, and mu-opioid receptor agonists on excitatory transmission in lamina II neurons of adult rat spinal cord. J Neurosci 14:4965–4971

Goodsell DS (2005) The molecular perspective: morphine. Stem Cells 23:144–145

Grimm MC, Ben-Baruch A, Taub DD, Howard OM, Resau JH, Wang JM, Ali H, Richardson R, Snyderman R, Oppenheim JJ (1998a) Opiates transdeactivate chemokine receptors: delta and mu opiate receptor-mediated heterologous desensitization. J Exp Med 188:317–325

Grimm MC, Ben-Baruch A, Taub DD, Howard OM, Wang JM, Oppenheim JJ (1998b) Opiate inhibition of chemokine-induced chemotaxis. Ann N Y Acad Sci 840:9–20

Gylbert L (1973). The crystal and molecular structure of morphine hydrochloride trihydrate. Acta Crystallographica Section B 29(8):1630–1635

Hanner M, Moebius FF, Flandorfer A, Knaus HG, Striessnig J, Kempner E, Glossmann H (1996) Purification, molecular

cloning, and expression of the mammalian sigma1-binding site. Proc Natl Acad Sci USA 93:8072–8077
Haverkos HW, Lange WR (1990) From the alcohol, drug abuse, and mental health administration. Serious infections other than human immunodeficiency virus among intravenous drug abusers. J Infect Dis 161:894–902
Hilburger ME, Adler MW, Truant AL, Meissler JJ Jr, Satishchandran V, Rogers TJ, Eisenstein TK (1997) Morphine induces sepsis in mice. J Infect Dis 176:183–188
Hokfelt T, Elde R, Johansson O, Terenius L, Stein L (1977) The distribution of enkephalin-immunoreactive cell bodies in the rat central nervous system. Neurosci Lett 5:25–31
Horsburgh CR Jr, Anderson JR, Boyko EJ (1989) Increased incidence of infections in intravenous drug users. Infect Control Hosp Epidemiol 10:211–215
HUSSEY HH, KATZ S (1950) Infections resulting from narcotic addiction; report of 102 cases. Am J Med 9:186–193
Ignatowski TA, Bidlack JM (1998) Detection of kappa opioid receptors on mouse thymocyte phenotypic subpopulations as assessed by flow cytometry. J Pharmacol Exp Ther 284:298–306
Inturrisi CE (2002) Clinical pharmacology of opioids for pain. Clin J Pain 18:S3–S13
Jacobowitz DM, O'Donohue TL (1978) alpha-Melanocyte stimulating hormone: immunohistochemical identification and mapping in neurons of rat brain. Proc Natl Acad Sci USA 75:6300–6304
Ji RR, Zhang Q, Law PY, Low HH, Elde R, Hokfelt T (1995) Expression of mu-, delta-, and kappa-opioid receptor-like immunoreactivities in rat dorsal root ganglia after carrageenan-induced inflammation. J Neurosci 15:8156–8166
Joe GW, Knezek L, Watson D, Simpson DD (1991) Depression and decision-making among intravenous drug users. Psychol Rep 68:339–347
Khachaturian H, Lewis ME, Watson SJ (1982a) Immunocytochemical studies with antisera against leu-enkephalin and an enkephalin-precursor fragment (BAM-22P) in the rat brain. Life Sci 31:1879–1882
Khachaturian H, Watson SJ, Lewis ME, Coy D, Goldstein A, Akil H (1982b) Dynorphin immunocytochemistry in the rat central nervous system. Peptides 3:941–954
Kirst A, Wack C, Lutz WK, Eggert A, Kämpgen E, Fischer WH (2002) Expression of functional κ-opioid receptors on murine dendritic cells. Immunol Lett 84:41–48
Kohno T, Kumamoto E, Higashi H, Shimoji K, Yoshimura M (1999) Actions of opioids on excitatory and inhibitory transmission in substantia gelatinosa of adult rat spinal cord. J Physiol 518(Pt 3):803–813
Krieger DT, Liotta A, Brownstein MJ (1977) Presence of corticotropin in limbic system of normal and hypophysectomized rats. Brain Res 128:575–579
Kulkarni-Narla A, Walcheck B, Brown DR (2001) Opioid receptors on bone marrow neutrophils modulate chemotaxis and CD11b/CD18 expression. Eur J Pharmacol 414:289–294
Lefkowitz DL, Stuart R, Gnade BT, Roberts E, Lefkowitz SS (2000) Effects of a glyconutrient on macrophage functions. Int J Immunopharmacol 22:299–308
Liu H, Li H, Guo L, Li M, Li C, Wang S, Jiang W, Liu X, McNutt MA, Li G (2010) Mechanisms involved in phosphatidylinositol 3-kinase pathway mediated up-regulation of the mu opioid receptor in lymphocytes. Biochem Pharmacol 79:516–523
Lopker A, Abood LG, Hoss W, Lionetti FJ (1980) Stereoselective muscarinic acetylcholine and opiate receptors in human phagocytic leukocytes. Biochem Pharmacol 29:1361–1365
Lord JA, Waterfield AA, Hughes J, Kosterlitz HW (1977) Endogenous opioid peptides: multiple agonists and receptors. Nature 267:495–499
Louria DB, Hensle T, Rose J (1967) The major medical complications of heroin addiction. Ann Intern Med 67:1–22
Lugo-Chinchilla AM, Baez D, Velez M, Ildefonso C, Renaud FL (2006) Altered subcellular signaling in murine peritoneal macrophages upon chronic morphine exposure. J Neuroimmunol 176:86–94
Lustman F, Walters EG, Shroff NE, Akbar FA (1987) Diphenoxylate hydrochloride (Lomotil) in the treatment of acute diarrhoea. Br J Clin Pract 41:648–651
Machelska H, Cabot PJ, Mousa SA, Zhang Q, Stein C (1998) Pain control in inflammation governed by selectins. Nat Med 4:1425–1428
Machelska H, Mousa SA, Brack A, Schopohl JK, Rittner HL, Schafer M, Stein C (2002) Opioid control of inflammatory pain regulated by intercellular adhesion molecule-1. J Neurosci 22:5588–5596
Machelska H (2007) Targeting of opioid-producing leukocytes for pain control. Neuropeptides 41:355–363
Makarenkova VP, Esche C, Kost NV, Shurin GV, Rabin BS, Zozulya AA, Shurin MR (2001) Identification of delta- and mu-type opioid receptors on human and murine dendritic cells. J Neuroimmunol 117:68–77
Martin JL, Koodie L, Krishnan AG, Charboneau R, Barke RA, Roy S (2010) Chronic morphine administration delays wound healing by inhibiting immune cell recruitment to the wound site. Am J Pathol 176:786–799
Martin WR, Eades CG, Thompson JA, Huppler RE, Gilbert PE (1976) The effects of morphine- and nalorphine- like drugs in the nondependent and morphine-dependent chronic spinal dog. J Pharmacol Exp Ther 197:517–532
McCoy CB, Metsch LR, Collado-Mesa F, Arheart KL, Messiah SE, Katz D, Shapshak P (2004) The prevalence of human immunodeficiency virus type 1 and hepatitis C virus among injection drug users who use high risk inner-city locales in Miami, Florida. Mem Inst Oswaldo Cruz 99:789–793
Menzebach A, Hirsch J, Nost R, Mogk M, Hempelmann G, Welters ID (2004) Morphine inhibits complement receptor expression, phagocytosis and oxidative burst by a nitric oxide dependent mechanism. Anasthesiol Intensivmed Notfallmed Schmerzther 39:204–211
Meunier JC, Mollereau C, Toll L, Suaudeau C, Moisand C, Alvinerie P, Butour JL, Guillemot JC, Ferrara P, Monsarrat B (1995) Isolation and structure of the endogenous agonist of opioid receptor-like ORL1 receptor. Nature 377:532–535
Mizoguchi H, Watanabe C, Higashiya T, Takeda S, Moriyama K, Yonezawa A, Sato T, Komatsu T, Sakurada T, Sakurada S (2011) Involvement of mouse mu-opioid receptor splice variants in the spinal antinociception induced by the dermorphin tetrapeptide analog amidino-TAPA. Eur J Pharmacol 651:66–72
Mollereau C, Parmentier M, Mailleux P, Butour JL, Moisand C, Chalon P, Caput D, Vassart G, Meunier JC (1994) ORL1, a novel member of the opioid receptor family. Cloning, functional expression and localization. FEBS Lett 341:33–38
Monassier L, Bousquet P (2002) Sigma receptors: from discovery to highlights of their implications in the cardiovascular system. Fundam Clin Pharmacol 16:1–8
Moon HD, Li CH, Jennings BM (1973) Immunohistochemical and histochemical studies of pituitary beta-lipotrophs. Anat Rec 175:529–537
Mousa SA, Shakibaei M, Sitte N, Schafer M, Stein C (2004) Subcellular pathways of beta-endorphin synthesis, processing, and release from immunocytes in inflammatory pain. Endocrinology 145:1331–1341
Mousa SA, Zhang Q, Sitte N, Ji R, Stein C (2001) β-Endorphin-containing memory-cells and μ-opioid receptors undergo transport to peripheral inflamed tissue. J Neuroimmunol 115:71–78
Nemoto T, Brown LS Jr, Foster K, Chu A (1990) Behavioral risk factors of human immunodeficiency virus infection among intravenous drug users and implications for preventive interventions. AIDS Educ Prev 2:116–126

Nestler EJ (2001) Molecular basis of long-term plasticity underlying addiction. Nat Rev Neurosci 2:119–128

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

Pan L, Xu J, Yu R, Xu MM, Pan YX, Pasternak GW (2005) Identification and characterization of six new alternatively spliced variants of the human mu opioid receptor gene. Oprm. Neuroscience 133:209–220

Pan YX (2005) Diversity and complexity of the mu opioid receptor gene: alternative pre-mRNA splicing and promoters. DNA Cell Biol 24:736–750

Pelletier G (1980) Ultrastructural localization of a fragment (16 K) of the common precursor for adrenocorticotropin (ACTH) and beta-lipotropin (beta-LPH) in the rat hypothalamus. Neurosci Lett 16:85–90

Pelletier G, Leclerc R, Labrie F, Cote J, Chretien M, Lis M (1977) Immunohistochemical localization of beta-lipotropic hormone in the pituitary gland. Endocrinology 100:770–776

Perez-Castrillon JL, Perez-Arellano JL, Garcia-Palomo JD, Jimenez-Lopez A, De Castro S (1992) Opioids depress in vitro human monocyte chemotaxis. Immunopharmacology 23:57–61

Peterson PK, Gekker G, Brummitt C, Pentel P, Bullock M, Simpson M, Hitt J, Sharp B (1989) Suppression of human peripheral blood mononuclear cell function by methadone and morphine. J Infect Dis 159:480–487

Peterson PK, Molitor TW, Chao CC (1993) Mechanisms of morphine-induced immunomodulation. Biochem Pharmacol 46:343–348

Peterson PK, Sharp B, Gekker G, Brummitt C, Keane WF (1987) Opioid-mediated suppression of cultured peripheral blood mononuclear cell respiratory burst activity. J Immunol 138:3907–3912

Przewlocki R, Hassan AH, Lason W, Epplen C, Herz A, Stein C (1992) Gene expression and localization of opioid peptides in immune cells of inflamed tissue: functional role in antinociception. Neuroscience 48:491–500

Reichman LB, Felton CP, Edsall JR (1979) Drug dependence, a possible new risk factor for tuberculosis disease. Arch Intern Med 139:337–339

Rittner HL, Brack A, Machelska H, Mousa SA, Bauer M, Schafer M, Stein C (2001) Opioid peptide-expressing leukocytes: identification, recruitment, and simultaneously increasing inhibition of inflammatory pain. Anesthesiology 95:500–508

Rittner HL, Machelska H, Stein C (2005) Leukocytes in the regulation of pain and analgesia. J Leukoc Biol 78:1215–1222

Rittner HL, Roewer N, Brack A (2010) The clinical (ir)relevance of opioid-induced immune suppression. Curr Opin Anaesthesiol 23:588–592

Rojavin M, Szabo I, Bussiere JL, Rogers TJ, Adler MW, Eisenstein TK (1993) Morphine treatment in vitro or in vivo decreases phagocytic functions of murine macrophages. Life Sci 53: 997–1006

Roy S, Balasubramanian S, Sumandeep S, Charboneau R, Wang J, Melnyk D, Beilman GJ, Vatassery R, Barke RA (2001) Morphine directs T cells toward T(H2) differentiation. Surgery 130:304–309

Roy S, Barke RA, Loh HH (1998a) MU-opioid receptor-knockout mice: role of mu-opioid receptor in morphine mediated immune functions. Brain Res Mol Brain Res 61:190–194

Roy S, Cain KJ, Charboneau RG, Barke RA (1998b) Morphine accelerates the progression of sepsis in an experimental sepsis model. Adv Exp Med Biol 437:21–31

Roy S, Chapin RB, Cain KJ, Charboneau RG, Ramakrishnan S, Barke RA (1997) Morphine inhibits transcriptional activation of IL-2 in mouse thymocytes. Cell Immunol 179:1–9

Roy S, Wang J, Kelschenbach J, Koodie L, Martin J (2006) Modulation of Immune Function by Morphine: Implications for Susceptibility to Infection. JNIP 1:77–89

Schafer M, Mousa SA, Zhang Q, Carter L, Stein C (1996) Expression of corticotropin-releasing factor in inflamed tissue is required for intrinsic peripheral opioid analgesia. Proc Natl Acad Sci USA 93:6096–6100

Schmitt TK, Mousa SA, Brack A, Schmidt DK, Rittner HL, Welte M, Schafer M, Stein C (2003) Modulation of peripheral endogenous opioid analgesia by central afferent blockade. Anesthesiology 98:195–202

Sedqi M, Roy S, Ramakrishnan S, Elde R, Loh HH (1995) Complementary DNA cloning of a mu-opioid receptor from rat peritoneal macrophages. Biochem Biophys Res Commun 209:563–574

Sedqi M, Roy S, Ramakrishnan S, Loh HH (1996) Expression cloning of a full-length cDNA encoding delta opioid receptor from mouse thymocytes. J Neuroimmunol 65:167–170

Sharp BM, Keane WF, Suh HJ, Gekker G, Tsukayama D, Peterson PK (1985) Opioid peptides rapidly stimulate superoxide production by human polymorphonuclear leukocytes and macrophages. Endocrinology 117:793–795

Sharp BM, Roy S, Bidlack JM (1998) Evidence for opioid receptors on cells involved in host defense and the immune system. J Neuroimmunol 83:45–56

Simpkins CO, Alailima ST, Tate EA, Johnson M (1986) The effect of enkephalins and prostaglandins on O-2 release by neutrophils. J Surg Res 41:645–652

Simpkins CO, Dickey CA, Fink MP (1984) Human neutrophil migration is enhanced by beta-endorphin. Life Sci 34:2251–2255

Singh PP, Singal P (2007) Morphine-induced neuroimmunomodulation in murine visceral leishmaniasis: the role(s) of cytokines and nitric oxide. J Neuroimmune Pharmacol 2:338–351

Spittal PM, Bruneau J, Craib KJ, Miller C, Lamothe F, Weber AE, Li K, Tyndall MW, O'Shaughnessy MV, Schechter MT (2003) Surviving the sex trade: a comparison of HIV risk behaviours among street-involved women in two Canadian cities who inject drugs. AIDS Care 15:187–195

Stefano GB, Digenis A, Spector S, Leung MK, Bilfinger TV, Makman MH, Scharrer B, Abumrad NN (1993) Opiate-like substances in an invertebrate, an opiate receptor on invertebrate and human immunocytes, and a role in immunosuppression. Proc Natl Acad Sci USA 90:11099–11103

Stein C (1995) The control of pain in peripheral tissue by opioids. N Engl J Med 332:1685–1690

Stiene-Martin A, Knapp PE, Martin K, Gurwell JA, Ryan S, Thornton SR, Smith FL, Hauser KF (2001) Opioid system diversity in developing neurons, astroglia, and oligodendroglia in the subventricular zone and striatum: impact on gliogenesis in vivo. Glia 36:78–88

Suzuki S, Chuang LF, Yau P, Doi RH, Chuang RY (2002) Interactions of opioid and chemokine receptors: oligomerization of mu, kappa, and delta with CCR5 on immune cells. Exp Cell Res 280:192–200

Suzuki S, Chuang LF, Doi RH, Bidlack JM, Chuang RY (2001) Kappa-opioid receptors on lymphocytes of a human lymphocytic cell line: morphine-induced up-regulation as evidenced by competitive RT-PCR and indirect immunofluorescence. Int Immunopharmacol 1:1733–1742

Szabo I, Rojavin M, Bussiere JL, Eisenstein TK, Adler MW, Rogers TJ (1993) Suppression of peritoneal macrophage phagocytosis of Candida albicans by opioids. J Pharmacol Exp Ther 267:703–706

Tomassini N, Renaud F, Roy S, Loh HH (2004) Morphine inhibits Fc-mediated phagocytosis through mu and delta opioid receptors. J Neuroimmunol 147:131–133

Tomei EZ, Renaud FL (1997) Effect of morphine on Fc-mediated phagocytosis by murine macrophages in vitro. J Neuroimmunol 74:111–116

Tubaro E, Borelli G, Croce C, Cavallo G, Santiangeli C (1983) Effect of morphine on resistance to infection. J Infect Dis 148:656–666

Uhl GR, Kuhar MJ, Synder SH (1978) Enkephalin-containing pathway: amygdaloid efferents in the stria terminalis. Brain Res 149:223–228

Vallejo R, de Leon-Casasola O, Benyamin R (2004) Opioid therapy and immunosuppression: a review. Am J Ther 11:354–365

van Rijn RM, Whistler JL, Waldhoer M (2010) Opioid-receptor-heteromer-specific trafficking and pharmacology. Curr Opin Pharmacol 10:73–79

Waldhoer M, Bartlett SE, Whistler JL (2004) Opioid receptors. Annu Rev Biochem 73:953–990

Wang J, Barke RA, Charboneau R, Loh HH, Roy S (2003) Morphine negatively regulates interferon-gamma promoter activity in activated murine T cells through two distinct cyclic AMP-dependent pathways. J Biol Chem 278:37622–37631

Wang J, Barke RA, Charboneau R, Roy S (2005) Morphine impairs host innate immune response and increases susceptibility to Streptococcus pneumoniae lung infection. J Immunol 174:426–434

Wang J, Barke RA, Ma J, Charboneau R, Roy S (2008) Opiate abuse, innate immunity, and bacterial infectious diseases. Arch Immunol Ther Exp (Warsz) 56:299–309

Wang J, Charboneau R, Balasubramanian S, Barke RA, Loh HH, Roy S (2001) Morphine modulates lymph node-derived T lymphocyte function: role of caspase-3, -8, and nitric oxide. J Leukoc Biol 70:527–536

Watson SJ, Akil H, Ghazarossian VE, Goldstein A (1981) Dynorphin immunocytochemical localization in brain and peripheral nervous system: preliminary studies. Proc Natl Acad Sci USA 78:1260–1263

Weber E, Roth KA, Barchas JD (1982) Immunohistochemical distribution of alpha-neo-endorphin/dynorphin neuronal systems in rat brain: evidence for colocalization. Proc Natl Acad Sci USA 79:3062–3066

Weber E, Evans CJ, Barchas JD (1983) Multiple endogenous ligands for opioid receptors. Trends Neurosci 6:333–336

Weber RJ, Ikejiri B, Rice KC, Pert A, Hagan AA (1987) Opiate receptor mediated regulation of the immune response in vivo. NIDA Res Monogr 76:341–348

Wee, B., Browning, J., Adams, A., Benson, D., Howard, P., Klepping, G., Molassiotis, A., Taylor, D., 2011. Management of chronic cough in patients receiving palliative care: review of evidence and recommendations by a task group of the Association for Palliative Medicine of Great Britain and Ireland. Palliat Med 1–8

Welters ID, Menzebach A, Goumon Y, Langefeld TW, Teschemacher H, Hempelmann G, Stefano GB (2000) Morphine suppresses complement receptor expression, phagocytosis, and respiratory burst in neutrophils by a nitric oxide and mu(3) opiate receptor-dependent mechanism. J Neuroimmunol 111:139–145

Wick MJ, Minnerath SR, Roy S, Ramakrishnan S, Loh HH (1996) Differential expression of opioid receptor genes in human lymphoid cell lines and peripheral blood lymphocytes. J Neuroimmunol 64:29–36

Woolf CJ, Salter MW (2000) Neuronal plasticity: increasing the gain in pain. Science 288:1765–1769

Woolf CJ, Ma Q (2007) Nociceptors—Noxious Stimulus Detectors. Neuron 55:353–364

Wuster M, Schulz R, Herz A (1979) Specificity of opioids towards the mu-, delta- and epsilon-opiate receptors. Neurosci Lett 15:193–198

Xu J, Xu M, Rossi GC, Pasternak GW, Pan YX (2011) Identification and characterization of seven new exon 11-associated splice variants of the rat mu opioid receptor gene, OPRM1. Mol Pain 7:9

Yoshimura M, North RA (1983) Substantia gelatinosa neurones hyperpolarized in vitro by enkephalin. Nature 305:529–530

Zagon IS, Gibo DM, McLaughlin PJ (1991) Zeta (zeta), a growth-related opioid receptor in developing rat cerebellum: identification and characterization. Brain Res 551:28–35

Zagon IS, Verderame MF, Allen SS, McLaughlin PJ (2000) Cloning, sequencing, chromosomal location, and function of cDNAs encoding an opioid growth factor receptor (OGFr) in humans. Brain Res 856:75–83