REVIEW

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Opiate abuse, innate immunity, and bacterial infectious diseases

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Abstract

The first line of defense against invading bacteria is provided by the innate immune system. Morphine and other opiates can immediately disrupt the body's first line of defense against harmful external bacteria. Opiate, for example morphine, abuse degrades physical and physiologic barriers, and modulates phagocytic cells (macrophages, neutrophils) and, nonspecific cytotoxic T cells (γδ T), natural killer cells, and dendritic cells, that are functionally important for carrying out a rapid immune reaction to invading pathogens. *In vitro* studies with innate immune cells from experimental animals and humans and *in vivo* studies with animal models have shown that opiate abuse impairs innate immunity and is responsible for increased susceptibility to bacterial infection. However, to better understand the complex interactions between opiates, innate immunity, and bacterial infection and develop novel approaches to treat and even prevent bacterial infection in the opiate-abuse population, there is an urgent need to fill the numerous gaps in our understanding of the cellular and molecular mechanisms by which opiate abuse increases susceptibility to bacterial infection.

Key words: opiate, morphine, innate immunity, macrophages, neutrophils, dentritic cells, NK cells, γδ T lymphocytes, cytokines, bacterial infection.

Abbreviations: NK – natural killer, DCs – dendritic cells, AMs – alveolar macrophages, APCs – antigen-presenting cells.

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INTRODUCTION

Drug use and abuse have generated a major public health problem and a controversial social issue. Heroin, morphine, and other opiate analgesics continue to be commonly abused in the USA [72]. Opiate drugs are also among the most widely prescribed drugs for pain management. Opioid addicts are prone to both bacterial and viral infections. The high incidence of AIDS, hepatitis, tuberculosis, and bacterial pneumonia among long-term opiate users suggests altered resistance to infectious diseases and increased susceptibility to opportunistic infections [17, 51, 87, 93]. Opiate abuse has been determined to be a significant risk factor for the development of infectious diseases. However, the mechanism by which opiate abuse affects host resistance to bacterial infection has not been well characterized.

The first line of defense against invading pathogens is provided by the innate immune system, which acts nonspecifically and with no memory toward a few highly conserved antigens present on microorganisms. The innate immune system not only provides initial protection against microorganisms, but also stimulates the adaptive immune response. The innate immune system has a much more important and fundamental role in host defense. Clonal expansion of lymphocytes in response to infection takes 3–5 days for sufficient numbers of clones to be produced and to differentiate into effector cells, which allows more than enough time for most pathogens to damage the host [1]. The effector mechanisms of innate immunity are activated immediately after infection and rapidly control the replication of the infecting pathogen. The innate defenses include physical and physiologic barriers, phagocytic cells

(macrophages, neutrophils) and, to a lesser extent, certain nonspecific cytotoxic T cells (γδ T), natural killer (NK) cells, and dendritic cells (DCs), which are saddled with the responsibility of carrying out this rapid immune reaction to invading pathogens [54]. Morphine and other opiates can immediately disrupt the body's first line of defense against harmful external pathogens. This mini-review will focus on the current understanding of the mechanisms by which morphine abuse affects innate immunity and increases susceptibility to bacterial infection.

OPIOID ABUSE AND INNATE IMMUNITY

Physical barriers: epithelia

The epithelium of the human body forms an interface between the internal milieu and the external environment. Skin and mucosal epithelium function as a mechanical barrier to the invasion of microbial pathogens. The epithelium contributes to the host's defense in several ways, including providing a physical barrier and producing chemokines, cytokines, antimicrobial peptides, protease inhibitors, and surfactant proteins. The recognition systems used by epithelial cells to sense microbial exposure do have a certain degree of specificity and serve to mount a quick and efficient response to deal with a certain pathogen. Relatively little is known about the effect of morphine on the physical barriers of innate immunity. Previous studies have shown that chronic morphine exposure increases the penetration of microorganisms across the epithelial lining of the gastrointestinal (GI) tract. Those studies suggest that morphine may modulate the permeability of the GI tract epithelium [31, 45]. Opiate addicts are at increased risk of progressive chronic renal failure and the development of human immunodeficiency virus (HIV)-associated nephropathy. Higher concentrations of morphine triggered glomerular epithelial cell apoptosis. Free-radical scavengers as well as antioxidants inhibit morphine-induced pro-apoptotic effects, suggesting that the effects of morphine are mediated through oxidative stress [57]. A recent study showed that morphine increases intestinal ulcer formation and degradation of the host defense barrier. Morphine-treated mice exhibit mucosal ulcer formation in the ileum and in the upper third of the colon. Morphine induces intestinal injury through nitric oxide (NO) generation. The degradation of the host defense barrier correlates with macrophage injury, but not intestinal injury [25]. However, the specific cellular and molecular mechanisms underlying morphine's effects on the epithelial barrier still need to be elucidated.

Cellular components and receptors

Phagocytes: neutrophils and macrophages. Eradication of microbial pathogens by neutrophils is a complex multi-step process. First, neutrophils are rapidly recruited from the circulation and bone marrow reserves to sites of microbial invasion by host- and pathogen--derived stimuli. Second, recruitment of neutrophils to the sites of infection and subsequent cell activation results in elimination of the invading microbes and inflammation. Morphine-treated animals showed a transient reduction in chemotaxis toward a complement-derived chemotactic factor [41]. Morphine also suppresses chemokine-mediated neutrophil migration [27, 49]. Studies from our laboratory have shown that in a novel mouse model of chronic morphine treatment followed by intranasal infection with *Streptococcus pneumoniae*, morphine treatment significantly delays the entry of neutrophils into the alveolar compartment, resulting in significantly fewer neutrophils in lung tissue and bronchial alveolar lavage fluid in the early phase of infection. Morphine treatment-induced compromised neutrophil migration may be a mechanism responsible for the increase in bacterial burden and severity of infection seen in morphine-treated mice infected with *S. pneumoniae* [87]. Interestingly, morphine exposure to human neutrophils *in vitro* did not decrease chemotaxis of adult neutrophils, but did modestly impair chemotaxis of newborn neutrophils. After exposure to morphine sulfate, adult neutrophils showed no difference in interleukin (IL)-8 receptor expression, whereas newborn neutrophils expressed fewer IL-8 receptors. Newborn neutrophils had reduced chemotaxis toward IL-8. Morphine treatment further decreased their chemotactic function. The differential effect may be explained in part by the reduction of IL-8 receptors of newborn neutrophils after morphine treatment [92].

Neutrophils are fundamentally important phagocytes in the defense against microorganisms, such as bacteria and fungi, by eliminating them through a process known as phagocytosis. During phagocytosis, neutrophils produce reactive oxygen species (ROS), including superoxide, hydrogen peroxide, and hypochlorous acid, and release cytotoxic granule components into pathogen-containing phagocytic vacuoles. Morphine inhibited neutrophil respiratory burst and inhibited the scavenging of oxygen free radicals [12]. Morphine treatment greatly reduces the phagocytic activities of rat peripheral blood polymorphonuclear and primate polymorphonuclear cells [41, 43, 49]. Furthermore, morphine treatment caused a significant time- and dose--dependent decrease in complement (CD11b, CD35) and Fcγ-receptor (CD16) expression. The NO- and μ_3-opiate receptor-dependent mechanisms mediated by morphine suppressed complement receptor expression, phagocytosis, and respiratory burst in neutrophils [91]. Morphine also inhibits nuclear factor (NF)-κB activation in human neutrophils and monocytes by an NO--coupled mechanism [90].

Macrophages play a central role in innate and adaptive immunity. In the classic acute inflammatory response, blood monocytes enter the infected tissue and differentiate into macrophages shortly after the recruit-

ment of neutrophils. Encountering bacteria, macrophages can initiate phagocytosis with or without opsonization and subsequently release cytokines, tumor necrosis factor (TNF)-α, IL-1, IL-6, and other products which orchestrate host cellular defense. The inflammatory response of the host is also critical for the interruption and resolution of the infection process. During a dynamic inflammatory response, the macrophage population will display a variety of functional patterns, depending on the balance of macrophage-modulating ligands present in the tissue microenvironment. Macrophages also play a critical liaison role in the communication between the innate and adaptive immune systems. Macrophages can display antigen-presenting activity and phenotype and the inflammatory milieu created by macrophages can significantly impact the maturation of myeloid DCs and thus influence the nature of the adaptive immune response that will be elicited. Morphine significantly alters a number of macrophage functions in humans, rodents, monkeys, and swine, including phagocytosis, apoptosis, migration, tumoricidal activity, NO production, superoxide formation, and cytokine and chemokine expression.

Chronic and acute exposure to morphine results in inhibition of phagocytosis in both human and rodent macrophages through the μ-opioid receptors [16, 64]. These results are further corroborated by data from our laboratory demonstrating that morphine-mediated inhibition of phagocytosis was completely abolished in μ--opioid receptor knockout mice [65, 67]. Moreover, a recent study showed that the inhibitory effect of opioids on phagocytosis by murine peritoneal macrophages was mediated by μ and δ_2 receptors, suggesting the μ and δ receptors interact by an unknown cooperative mechanism in such a way that binding of an agonist or antagonist by one receptor subtype will affect the properties of other subtypes [10, 42, 83].

The mononuclear phagocyte is an important component of the host defense system against bacterial infection. Monocytes migrate to the site of infection to phagocytose and kill invading bacteria. Morphine decreases the migration of macrophages by diminishing chemotaxis [60]. A recent study showed that morphine decreased macrophage migration in both *in vivo* and *in vitro* studies. Morphine-treated mice showed a decrease in the migration of macrophages into the peritoneal cavity compared with control mice. In addition, peritoneal macrophages, as well as bone marrow cells harvested from morphine-treated mice, showed decreased migration across the filter of a modified Boyden chamber compared with control cells. Furthermore, morphine promotes HO-1 expression by macrophages, where increased HO-1 activity is associated with decreased macrophage migration [58].

Another potential mechanism by which morphine modulates macrophage function is morphine-induced macrophage apoptosis [4]. TNF-β, p38 mitogen-activated protein kinase phosphorylation, Fas-Fas ligand interaction, and heme oxygenase-1 may play critical roles in morphine's effect [73, 74]. Morphine also modulates HIV-1 gp160-induced murine macrophage and human monocyte apoptosis [36].

Morphine and opiates are able to induce ROS formation in several cells [75] and decrease the antioxidant defense system, including enzymes, superoxide dismutase, catalase and glutathione peroxidase, and antioxidants, glutathione, Se, and vitamins [4]. Morphine significantly inhibited lipopolysaccharide (LPS)-induced NO production in a naloxone-reversible manner [35]. The decrease in NO by morphine can be speculated to compromise bactericidal activity of macrophages and thereby increase bacterial burden and susceptibility to infection [23]. In contrast, several studies, including ours, demonstrate that morphine treatment, *in vitro* and *in vivo*, leads to increased production of superoxide and NO both in naïve and LPS-activated macrophages [78, 89]. Although it is not clear why studies investigating the effects of morphine on immune parameters have contrasting effects, it is clear that several factors, including the experimental model, strain, age of animal, sex, cell culture system, activation agent, and the route and concentrations of morphine administration, all contribute to the conflicting results observed with morphine on immune parameters. However, since the bactericidal function of mononuclear phagocytes is predominantly dependent upon their respiratory burst, a response in which oxygen is rapidly metabolized to provide a series of toxic intermediates, inhibition of this function by pharmacological doses of morphine will contribute to the increased susceptibility to infection seen in opiate addicts.

The molecular mechanism and signaling by which morphine modulates macrophage functions is not clear. Morphine uses multiple intracellular pathways to exert its generalized immunosuppression. The involvement of RelB in morphine-induced immunosuppression was investigated in mice deficient in RelB factor. RelB does not seem to be involved in the early steps of macrophage activation, such as chemotaxis, although it may have a role in morphine modulation of NO production. RelB is an important target for the modulation by morphine of proinflammatory and Th1 cytokines IL-1β, TNF-α, and IL-12, but not for anti-inflammatory and Th2 cytokines, for example IL-10 [46]. Our laboratory recently investigated how morphine modulated alveolar macrophage (AM) function in a murine drug-abuse and pulmonary *S. pneumoniae*-infection model. Using *in vivo* pharmacologically depleted AM mice and *in vitro* cell-infection approaches we found that morphine induces defects in the early response of AMs to *S. pneumoniae* by modulating Toll-like receptor (TLR)9-NF-κB signaling. Depletion of lung macrophages *in vivo* reduced neutrophil recruitment to lungs infected with *S. pnemoniae* in the early phase of infection (6 h after infection). These data suggest that resident AMs play a critical role in initiating a NF-κB-mediated response to *S. pneumoniae*. Morphine treatment inhibited the initiation of NF-κB activation in response to *S. pneumoni-*

ae in AMs, leading to impaired initiation of innate immune response to infection. Using TLR transfection cells and TLR9 shRNA-transfected AMs we found that NF-κB activity and MIP-2 production in response to *S. pneumoniae* were through TLR9 signaling at the very early stage of infection. Morphine treatment impaired the early TLR9-NF-κB signaling [88].

Natural killer cells and dendritic cells. NK cells are an important component of innate resistance to viruses, bacteria, certain parasites, and tumors. NK cells are by definition capable of lysing their target cells without prior exposure or priming. NK cells kill infected or malignant cells through the release of perforin and granzymes from granular storage compartments and through binding of the death receptors Fas and TRAIL-R on target cells through their respective NK-cell ligands [77]. Both acute and chronic administration of morphine affects the functioning of human, monkey, and rodent NK cells. Acute morphine treatment in rats resulted in markedly decreased splenic NK-cell cytotoxicity [21]. In addition to the acute effect, chronic exposure to morphine also suppressed NK cell activity. NK cells from different compartments of the immune system seem to have varying sensitivities to morphine treatment. Splenic NK cells and NK cells from the peripheral blood were more susceptible than lymph node cell--derived NK cells. Such differences could be attributed to either inherent variations in the NK cell population in these compartments or to the bioavailability of morphine at these sites [9]. Morphine's action on NK cells is an indirect effect mediated by morphine acting through central nervous system (CNS) receptors. This conclusion is supported by the failure of systemic administration of N-methyl morphine, a quaternary morphine analog which does not readily diffuse across the blood brain barrier, to inhibit NK cells' cytolytic activity [44]. The α and β adrenergic pathways have been shown to be involved in morphine-mediated suppression of NK cells [22]. Morphine-induced immunomodulation of NK cells were mediated through the CNS dopaminergic mechanisms. Both systemic and central administration of 7-OH-DPAT (a dopamine D_3 receptor agonist) attenuate the suppressive effect of morphine on NK cells, suggesting that 7-OH-DPAT antagonizes morphine's suppressive influence on the immune status by preventing morphine-induced elevations of dopamine [69]. The authors also demonstrated that site-specific microinjection of morphine activates dopamine-receptive neurons in the shell of the nucleus accumbens which in turn induces suppression of splenic NK cell activity. However, how μ-opioid receptor activation in the nucleus accumbens affects other parameters of immune status and different immune compartments (e.g. spleen vs. blood) remains to be determined [69]. Their more recent study showed that morphine-induced reductions of splenic NK cell activity are mediated peripherally by NPY Y_1 receptors, whereas morphine's effects on splenic T and B cell proliferative responses to concanavalin A and LPS are mediated by peripheral β-adrenoceptors. The adrenal medulla appears to be the source of catecholamines that activate β-adrenoceptors, which inhibits splenocyte proliferative responses, whereas the peripheral source of NPY that modulates NK activity via Y_1 receptors is not the adrenal medulla, but possibly sympathetic nerves such as those that innervate the spleen. The results of the current and prior studies further suggest that morphine administration induces the activation of dopamine D_1 receptors in the nucleus accumbens shell, leading to the suppression of splenic NK activity as a result of increased NPY release from sympathetic nerves [70]. Corticosteroid level in blood increased in a time-dependent manner following morphine administration, and corticosteroids have been implicated as a possible indirect mechanism by which morphine modulates NK cell activity. Since enhanced glucocorticoid release also increases mesolimbic dopaminergic signaling, it is not clear if the morphine-induced increase in dopamine is a direct effect or a consequence of a morphine-induced increase in hypothalamic-pituitary-adrenal activation. Clearly, more studies are needed to understand how morphine interacts with all of these different pathways that modulate NK cell activity.

DCs, together with NK cells, are considered to be players in the development of innate immunity. DCs are the single most central player in all immune responses, both innate and adaptive. The decision to activate an appropriate immune response is made by unique DCs, the most professional of antigen-presenting cells (APCs), which control the responses of several types of lymphocytes and play a central role in the transition between innate and adaptive immunity. Increased secretion of inflammatory endogenous mediators such as cytokines and arachidonic acid-derived lipid mediators, also termed eicosanoids, can activate APCs, particularly DCs, which in turn induce an adaptive immune response [79]. An *in vitro* study has shown that morphine exerts a stimulatory effect on human myeloid DCs. Immature DCs and LPS--matured DCs expressed similar levels of μ-, κ-, and δ-opioid receptors. Morphine modified LPS-induced DC maturation. Morphine-treated DCs showed increased expression levels of HLA-DR, CD86, and CD83 compared with DCs differentiated in the absence of morphine. Morphine consistently reduced LPS-induced IL-10 and increased LPS-induced IL-12 (p70) production. Increased IL-12 secretion by morphine treatment is mediated through p38 MAPK. Morphine enhances the T cell stimulatory capacity of LPS-induced matured DCs. The results from this study suggest that morphine's influence on DCs has no negative effect on the host's immune response to bacterial infection [48]. Overall, little is known about how opioid abuse regulates DC function, and more studies clearly need to be conducted to clarify this issue.

γδ T cells. γδ T cells represent only a small fraction of T cells in the lymphoid organs of humans and mice (1–5%). γδ T cells function in an innate manner. As "the first line of defense", "regulatory cells", or "the bridge between innate and adaptive response", γδ T cells have a prominent role in the innate response; however, infor-

mation about the underlying mechanisms remains scarce. At the functional level, γδ T cells rapidly produce a variety of cytokines and usually exert potent cytotoxic activity toward bacteria as well as tumor cells [7]. A recent study by Olin et al. [56] showed that chronic morphine administration to pigs prior to BCG vaccination significantly impaired γδ T lymphocytes' proliferative response to antigen. In this study, γδ T lymphocytes from morphine-treated and control animal groups were incubated with *Mycobacterium*-infected monocytes. Administration of morphine resulted in γδ T cells' innate cytolytic activity being significantly suppressed compared with the control animal group. Antigen-directed cytolytic activity is an essential function for the elimination of intracellular pathogens. γδ T lymphocytes are capable of specific immunity against *Mycobacterium*. In particular, γδ T lymphocytes can lyse mycobacteria--infected monocytes. Morphine significantly decreased γδ T lymphocytes' antigen-driven cytolytic activity, an important defense against intracellular pathogens, including *Mycobacterium*-infected monocytes. Therefore, systemic administration of opiates modulates γδ T lymphocytes' ability to respond to *Mycobacterium*, which may have profound impact on BCG-induced immunity.

Toll-like receptors. Innate immunity relies on signaling by TLRs to alert the immune system of the presence of invading bacteria. TLR activation leads to the release of cytokines that allow for effective innate and adaptive immune responses. Mechanisms to recognize pathogens by the cellular components of innate immunity are therefore considered essential to mount a protective response by the innate immune system. Cells of the innate immune system, including phagocytes, DCs, and epithelial cells, use so-called pattern-recognition molecules to bind to conserved molecular patterns that are present on microorganisms. Pattern-recognition molecules can be present in secretions, circulating in soluble form, such as mannose-binding lectin, or they can be transmembrane molecules that mediate direct cellular responses to microbial exposure. The TLRs constitute an intensely studied family of pattern-recognition receptors that are represented by various members in almost all cells of the body. Their expression has been intensely studied on DCs, and it is now recognized that TLRs help to shape the adaptive immune response by directing the way that DCs instruct T cells. Epithelial cells also express a variety of TLRs that may help them to mount an adequate response to microbial exposure. Activation of TLRs on epithelial cells has now been shown to be involved in the regulation of expression of a variety of genes, including those encoding cytokines, chemokines, and antimicrobial peptides [85]. TLR2 recognizes a broad range of ligands, such as bacterial lipopeptides, yeast zymosan, parasite and viral proteins, and lipoteichoic acid from Gram-positive bacteria [80]. TLR4 recognizes mannose from yeast, host heat-shock proteins, fibrinogen, and envelope proteins from viruses, and pneumolysin from *S. pneumoniae*, but is mostly known as the LPS receptor [33]. TLR5 detects a conserved domain on flagellin monomers, the main structural protein that forms the flagella on Gram-negative bacteria [30]. TLR9 recognizes nucleic acids such as hypomethylated CpG, motifs which are common amongst prokaryotic DNA and absent in eukaryotic genomes [14]. TLR3, TLR7, and TLR8 recognize nucleic acids like TLR9, but only single-stranded and double-stranded RNA rather than DNA [15, 71, 84].

Morphine possesses immunomodulatory effects, but its mechanisms, especially in the TLR signaling pathway, are not totally understood. A recent study showed that the release of TNF and IL-6 from peptidoglycan (PGN, TLR2 stimulator)-stimulated monocytes was inhibited in a dose- and time-dependent manner by morphine. The nonspecific antagonist NLX and δ and κ receptor antagonists (NLT and nor-BNI, respectively) at concentrations of 10^{-5} M did not prevent the decrease in TNF and IL-6 production induced by morphine. Conversely, the morphine-inhibiting effect was reversed when monocytes were treated with the specific μ-opioid receptor antagonist at a concentration of 10^{-5} M, suggesting that μ-opioid receptors mediate morphine-induced TNF and IL-6 inhibition in PGN-stimulated monocytes. Furthermore, no IL-10 production was detected in the monocytic cell cultures after PGN stimulation with or without morphine pretreatment. These results indicate that IL-10 is not a factor for morphine--induced suppression of the production of TNF and IL-6 in cultured monocytes. Overall, this study demonstrates that there is an inhibitory effect of morphine on proinflammatory cytokine production in human monocytes after TLR2 stimulation and that this inhibition is mediated solely by the μ-opioid receptor. Direct monocyte--to-lymphocyte contact (peripheral blood mononuclear cells) alters the inhibitory effects of morphine observed on monocytes alone. IL-10 is not a factor for the inhibition of TNF and IL-6 production [5]. In our studies, stably transfected HEK 293 cells (HEK293-pcDNA3, HEK293-TLR2-YFP, HEK293-TLR4/MD2-CFP, and HEK293-TLR9-YFP) were challenged with *S. pneumoniae*. Our studies showed that *S. pneumoniae* infection resulted in a significant induction of NF-κB activity in HEK 293-TLR9 cells, but not in HEK293-TLR2 and HEK293-TLR4, following 2 h of cell infection. After 6 h of cell infection, *S. pneumoniae* infection led to significant induction of NF-κB activity in both HEK293--TLR9 and HEK293-TLR2 cells. Morphine treatment inhibited *S. pneumoniae*-induced NF-κB activity in HEK293-TLR9 cells following 2 h of cell infection. These data indicate that morphine treatment impaired innate immune defenses against *S. pneumoniae* by modulating TLR9-NF-κB signaling in the early stage of infection. However, TLR2 signaling may also participate in morphine's action at a later stage of infection because morphine also inhibits TLR2-induced NF-κB activation following 6 h of cell infection. To further confirm that morphine modulates TLR9-NF-κB signaling in AMs, the production of MIP-2 following *S. pneumoniae* infection was investigated using AMs transfected with TLR9-

-GFP shRNA plasmid DNA. A high level of TLR9 expression was seen in negative control shRNA-vector--transfected AMs. Morphine treatment decreased MIP-2 production following 4 h of infection. A significantly lower level of MIP-2 was shown in TLR9 shRNA plasmid DNA-transfected AMs. A noninfected baseline level of MIP-2 was detected in TLR9 shRNA plasmid DNA transfection-positive AMs (enriched by FACS cell sorting for GFP-expressing cells). These results further support the concept that TLR9-NF-κB signaling acts at an early stage in the host defense against pneumococcal infection and morphine treatment impairs this signaling [88].

Effectors factors: cytokines and chemokines

Effective host defense against bacterial infection is believed to be mainly mediated via phagocytosis by macrophages and recruited neutrophils [26]. Such defenses are orchestrated by a rapid inflammatory response after infection. Cytokines have been shown to be important soluble mediators responsible for coordinating this response [52]. Many cytokines are known to be involved in antibacterial defenses. TNF-α is capable of recruiting inflammatory cells to the site of infection, both directly and via up-regulation of adhesion molecules [53]. TNF-α has also been shown to stimulate the release of chemokines that are directly responsible for the chemotaxis of inflammatory cells. After recruitment of phagocytic cells, TNF can also promote antimicrobial activity by activating respiratory burst activity and increasing the degranulation of macrophages [18, 37]. These effects have been shown to be required for an effective *in vivo* host defense against a range of microorganisms, including *Klebsiella pneumoniae* and *Pneumocystis carinii* [39]. IL-1β shares several of TNF's activities, including the promotion of cell recruitment and the activation of macrophages at the site of infection [63]. In some situations the two cytokines act synergistically to exert their effects [55]. IL-1 may mediate similar functions as TNF-α and may facilitate the emigration of neutrophils in the lungs in the absence of TNF-α signaling. IL-6 has been ascribed to have both pro- and anti-inflammatory effects. IL-6 can activate monocytes [6] and it can synergize with TNF-α to increase the respiratory burst of neutrophils [50]. Morphine's effect on inflammatory cytokine synthesis and secretion is also an area that shows variable results depending on experimental conditions and morphine concentrations. Several groups have reported that chronic morphine treatment of animals *in vivo* and *in vitro* inhibits the production of IL-1, IL-6, and TNF-α [13, 19, 65]. In contrast, some groups have shown that morphine resulted in a significant elevation of IL-12 and TNF-α levels and central morphine administration elevated plasma levels of the proinflammatory cytokine IL-6 [34, 59]. We have shown that morphine produces a biphasic effect when administered *in vivo*. While low--dose morphine (nanomolar) promotes inflammatory cytokine synthesis, high-dose morphine (micromolar) inhibits cytokine synthesis. The potentiating effects were completely reversed by naloxone, but the inhibitory effects were not completely reversed. We have also show that both morphine-induced activation (low dose – acute) and inhibition of TNF-α production (high dose – chronic) were abolished in μ-opioid receptor knockout (MORKO) mice. In contrast, the inhibition of IL-1 and IL-6 synthesis by morphine was not abolished in the MORKO mice [66, 68]. We speculate that the observed elevation, depression, and no change in inflammatory cytokines can be seen following μ-opioid receptor activation depending on several factors, including the specific cell type, the activation state of the cell population, chronic vs. acute morphine administration, the concentration of agonist employed, and other factors.

Chemokines are a group of small cytokine-like polypeptides which control leukocyte trafficking during homeostasis as well as during inflammation and are necessary for the linkage between innate and adaptive immunity. All cell types, including structure cells, have the potential to actively participate in chemokine production. Opioid and chemokine receptors are members of the G protein-coupled 7-transmembrane-segment receptor family. Chuang's group demonstrated that the oligomerization of the chemokine receptor CCR5 and opioid receptors on the cell membrane of human or monkey lymphocytes may modulate receptor functions. Also, little is known about how morphine modulates chemokines in monocytes/macrophages. Morphine enhances HIV R5 strain infection of macrophages through the down-regulation of MIP-1β production and up-regulation of CCR5 receptor expression and may have an important role in HIV immunopathogenesis [29]. However, the molecular mechanism by which morphine modulates these chemokines is still controversial. We have shown morphine's effect on chemokine production in a *S. pneumonia* lung-infection model. In the early stage of infection, morphine inhibited MIP-2 and KC synthesis, two key chemokines essential for neutrophil recruitment and clearance of the infectious pathogens. However, morphine treatment resulted in a compensatory increase in these inflammatory cytokines and chemokines during the late phase of *S. pneumoniae* lung infection, causing an increase in lung tissue damage [87]. The effect morphine has on proinflammatory cytokines and chemokines under conditions of bacterial infection is complex; outcomes depend on the interaction and balance between morphine, host defense, and bacterial load.

Overall, as summarized in Table 1, morphine abuse causes defects in epithelial cells, phagocytic cells (macrophages, neutrophils), nonspecific cytotoxic T cells (γδ T), NK cells, and DCs, which are functionally cellular components to carry out rapid immune responses to invading pathogens.

OPIOID ABUSE AND BACTERIAL INFECTION

Infections are among the most serious complications of drug use. Drug use plays a major role in disease etiol-

Table 1. Effect of morphine on innate immunity

Cell type	Parameter studied	Opioid treatment	Effects
Physical and physiologic barriers			
epithelia	1. permeability of the GI tact epithelium	*in vivo*	increased
	2. apoptosis	*in vitro*	increased
Phagocytic cells			
neutrophils	1. neutrophil migration	*in vitro/in vivo*	decreased
	2. phagocytosis	*in vitro*	decreased
	3. CD11, CD35, CD16 expression	*in vitro*	decreased
	4. CXCR2 expression	*in vivo*	decreased
	5. NF-κB activity	*in vitro*	decreased
macrophages	1. phagocytosis	*in vitro*	decreased
	2. migration/chemotaxis	*in vitro/in vivo*	decreased
	3. apoptosis	*in vitro*	increased
	4. IL-1, IL-6, TNF-α production	*in vitro/in vivo*	decreased
	5. NO production	*in vitro*	decreased
		in vitro/in vivo	increased
	6. TLR9-NF-κB	*in vivo*	decreased
	7. TLR2-TNF	*in vivo*	decreased
NK cells	cytotoxicity	*in vitro/in vivo*	decreased
DCs	1. HLA-DR, CD86, CD83	*in vitro*	increased
	2. LPS induced IL-10	*in vitro*	decreased
Nonspecific cytotoxic			
γδ T cells	1. antigen driven cytolytic activity	*in vitro/in vivo*	decreased
	2. LPS induced IL-12	*in vitro/in vivo*	increased

ogy, such as the transmission of HIV, sexually transmitted diseases, and viral hepatitis. In addition to these viral infections, drug users risk acquiring a variety of bacterial infections.

Bacterial infections in opiates abusers

It is now recognized that opiate abusers are a high-risk population in terms of bacterial infection [20]. Numerous clinical studies have shown that immunocompromise resulting from prolonged drug use contributes to the increased risk of respiratory tract infection. There is actually a 10-fold increase in the risk of community-acquired pneumonia in drug users [8]. Overall, respiratory tract infections caused by *S. pneumoniae*, *S. aureus*, *K. pneumoniae*, *Mycobacterium*, *Staphylococcus*, *Haemophilus*, and other bacteria are among the most common diagnoses of this population [32, 61]. Endovascular infections, including infective endocarditis, septic thrombophlebitis, mycotic aneurysms, and sepsis, are among the most serious complications of injection-drug use. Infective endocarditis in injection-drug users is most commonly due to *Staphylococcus aureus*, *Streptococcus*, and *Pseudomonas* species [47, 82]. Injection-drug users are susceptible to musculoskeletal infections, including septic arthritis and osteomyelitis, in uncommon places such as the sternoclavicular and sacroiliac joints in addition to more usual sites such as the vertebral spine and knee. *S. aureus* is the most frequently isolated pathogen in this population [3]. Skin and soft-tissue infections caused by spore-forming bacteria in injecting drug users and *S. aureus* and group A *Streptococcus* in severe soft tissue infections are related to injection-drug use [28] .

Opiate abuse affects bacterial infection in animal models

Experimental animal studies showed that opioid treatment causes increased susceptibility and mortality to various bacterial infections, including *S. pneumoniae* [87, 88], *K. pneumoniae* [86], acute *Toxoplasma gondii* [11], oral *Salmonella typhimurium* [24], *Candida albicans* [81], *Acinetobacter baumannii* [40], *S. aureus* [38], and *Listeria monocytogenes* [2]. Although opiate addiction has been identified as a risk factor for clinical tuberculosis [17], an *in vitro* infection study, contrary to other bacterial infection studies, showed that morphine stimulated the phagocytosis of *M. tuberculosis* by human microglial cells, the resident macrophages of the brain. The stimulatory effect of morphine was blocked by naloxone and the μ-opiate receptor-selective antagonist β-funaltrexamine. Also, morphine induced an increase in phagocytic activity which was markedly inhibited by pertussis toxin and was unaffected by cholera toxin. This suggests that the mechanism of morphine's stimulatory effect on microglial cell phagocytosis involves the μ-opiate receptors [62]. Also, a recent study reported a protective effect of morphine on murine tuberculosis. Morphine exerted a suppression of intravenous *M. tuberculosis* infection in mice. Furthermore, *in vitro* morphine lacked direct antimycobacterial effects, as assessed by a radiometric BACTEC method. In infected macrophages, morphine

increased bacteria killing, an effect that was blocked by naloxone and aminoguanidine [76].

Based on most animal model studies it is clear that opiate abuse is correlated with a high risk of bacterial infections. However, there is an urgent need to fill the abundant gaps in our understanding of the cellular and molecular mechanisms by which opiate abuse increases susceptibility to bacterial infections. Further studies are needed to fully understand the impacts of opioid abuse on innate and adaptive immunity. Understanding the cellular and molecular mechanisms by which opiate abuse impairs the innate immune response to bacterial infection is important for gaining insights into developing therapeutic intervention to control bacterial infection in the drug-abusing population. For example, using *in vivo* pharmacological AM-depletion and *in vitro* cell--infection approaches, our studies indicate that resident AMs are an essential cell population that participates in morphine's effect. During the early stage of *S. pneumoniae* infection, inhibition of TLR9-NF-κB signaling and decreased bacterial clearance play a vital role in the morphine-induced delay in the initiation of the innate immune response to *S. pneumoniae* infection. Our results suggest that AMs and TLR9-NF-κB signaling may be promising therapeutic targets in *S. pneumoniae*--infected opiate abuser [88].

CONCLUSION AND OUTLOOK

A substantial amount of research has been performed on the immunomodulatory effects of opioid abuse, but studies investigating how opiate abuse compromises the host innate immune response to bacteria and increases opportunistic bacterial infection were relatively limited until recently. Recent advances in animal infection model studies, including ours, enable further insights into the interactions between innate immunity and abused opiates, as well invasive bacteria. The most recent advances, using drug abuse and bacterial infection animal model *in vivo* infection studies, have focused our attention on specific components of the host innate immune response, enabling further insight into the often overlooked analysis of the basic host immune interactions with bacterial infection. Although there is accumulating evidence to implicate individual components of innate immunity, such as cytokines (TNF-α, IL-1, IL-6), chemokines (MIP-2, KC), TLRs, macrophages, and neutrophils, to bacterial infection, we are still some distance away from determining how these insights might be exploited clinically. It is evident that the interactions between opiates, bacterial infection, and host defenses are highly complex. As we begin to understand the pathology of infection at the cellular and molecular levels, we increase the prospect of developing novel approaches to the treatment and even prevention of bacterial infection in the opiate-abusing population. Moreover, opiates are commonly used in clinical medicine as an effective pain reliever. Morphine, an active metabolite of heroin, is an important analgesic for post-operative and terminal cancer patients. Caution is warranted in prescribing these drugs, since little is known about if and when they compromise immune function. There is an urgent need to fill the numerous gaps in our understanding of the mechanisms by which analgesic morphine affects immunity.

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