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COMMENTARY

MECHANISMS OF MORPHINE-INDUCED IMMUNOMODULATION

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The concept of opiates as immunomodulatory agents dates to the turn of this century [1, 2]. By the 1940s, many studies had been carried out on the effects of opiates on immune cells and on resistance of animals to infection [reviewed in Ref. 3]. Also, most of the infectious disease complications of intravenous opiate abuse were well recognized [4]. Several decades elapsed, however, before interest in this topic was rekindled by the observation that cell-mediated immune function is impaired in heroin addicts [5] and by reports in 1979 and 1980 that the major cell types involved in cell-mediated immunity—T-lymphocytes and mononuclear phagocytes—possess opiate receptors [6–8]. Thus, the stage was set in 1981, the outset of the acquired immunodeficiency syndrome (AIDS§) epidemic, for intensive research in this area of immunology, once it became established that injection drug use was a prominent risk factor for this devastating infectious disease [9].

In addition to the potential relevance of opiate-induced immunomodulation to AIDS, progress in this area has been spurred by developments in two related fields. First, major advances have been made in the field of opiate pharmacology, especially in the delineation of selective classes of opioid receptors [reviewed in Ref. 10]. Second, new concepts have been derived from the field of "psychoneuroimmunology" regarding bidirectional communication between the neuroendocrine and immune systems [reviewed in Refs. 11 and 12]. Studies in this multidisciplinary area of research have demonstrated a dynamic interaction between these two systems through chemical mediators, including endogenous opioid peptides. Not only have all three classes of endogenous opioid peptides been shown to affect immune cell function, but immunocytes have been demonstrated to be a source of several classes of opioid peptides [reviewed in Ref. 13].

The effects of exogenous opioids on immune cells have been studied as well. In this area of study ("pharmaconeuroimmunology"), the naturally occurring opiate alkaloid, morphine, has been investigated most extensively. Although a large body of evidence indicates that this opiate has multiple effects on the immune system (Table 1), and that these effects may compromise host defenses against a variety of microorganisms, relatively few studies have examined the mechanisms of morphine-induced immunomodulation. These studies are the focus of this article. As in most areas of research, it has become clear that there are more questions than answers and that much work needs to be done.

SITES OF MORPHINE ACTION

One of the major efforts in research on the mechanisms of opiate-induced immunomodulation has been to determine the primary site(s) of the action of morphine at an organ-system level (Fig. 1). Work with morphine-dependent rodents has demonstrated that one of the primary sites of action of morphine is within the central nervous system (CNS), with secondary effects on the immune system being mediated through a stress-responsive neuroendocrine pathway, i.e. the hypothalamo-pituitary-adrenal (HPA) axis. In addition to this indirect mechanism, studies with cell cultures from rodents, swine, and humans have shown that exposure of immune cells to morphine results in a variety of functional disturbances. Only recently have the mechanisms underlying these direct effects of morphine been ascertained.

CNS-mediated effects of morphine

In 1984, Shavit *et al.* [21] reported that splenic natural killer (NK) cell cytotoxicity against a tumor cell target was suppressed in rats subjected to inescapable footshock stress. The suppressed NK cell activity was blocked by administration of the opiate antagonist naltrexone, and similar suppression was induced by high doses of morphine. Subsequent studies by Shavit *et al.* [22] demonstrated that the morphine-induced suppression of NK activity was also blocked by naltrexone treatment, and that

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§ Abbreviations: AIDS, acquired immunodeficiency syndrome; HPA axis, hypothalamo-pituitary-adrenal axis; NK, natural killer; Con A, Concanavalin A; LPS, lipopolysaccharide; SRBC, sheep erythrocytes; PBMC, peripheral blood mononuclear cells; PHA, phytohemagglutinin; TGF, transforming growth factor; HIV, human immunodeficiency virus; and TNF, tumor necrosis factor.

Table 1. Morphine effects on immune cells*

Cell type	Effect
T-lymphocytes	E-rosette marker (−)
	TH, TS markers (−)
	Mitogen-induced proliferation (−, +)
	Interferon-γ production (−)
B-lymphocytes	Antibody production (−)
Natural killer lymphocytes	Cytotoxicity (−, +, ±)
Lymphokine-activated killer cells	Cytotoxicity (−)
Monocytes/macrophages	Respiratory burst activity (−); phagocytosis (−); chemotaxis (−)
Polymorphonuclear leukocytes	Chemotaxis (−); respiratory burst activity (−); phagocytosis (−)

* Compiled from a number of *in vivo* and *in vitro* studies reviewed in Refs. 14–20. Key: (−) inhibition; (+) stimulation; and (±) no effect.

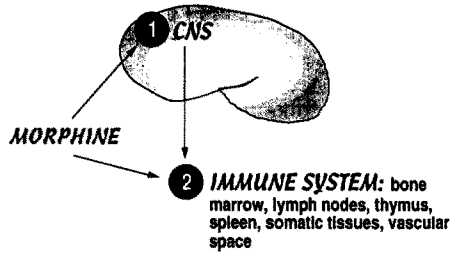

Fig. 1. Targets of opiate-induced alterations of the immune system. Research on the mechanisms whereby morphine affects immune cell function suggests that both indirect effects (i.e. via CNS neuroendocrine pathways) and direct interactions with immune cells are involved.

the immunosuppressive effect of morphine was associated with the development of tolerance after 14 days of morphine administration. These investigators went on to show that the NK-suppressive effect of morphine was mediated by opiate receptors in the brain [23]. In 1989, Weber and Pert [24] provided evidence that opiate receptors within the periaqueductal gray matter of the mesencephalon served as the primary target for morphine-induced NK cell suppression. Although the precise cell type within the brain and the efferent pathway involved in the effect of morphine on splenic NK lymphocytes were not determined, both groups of investigators [23, 24] proposed that signaling between the CNS and the immune system was relayed through neuroendocrine pathways, such as the HPA axis or sympathetic innervation of lymphoid organs.

Support for the hypothesis that the HPA axis is involved in morphine-induced immunomodulation has been provided by *in vivo* studies of the effects of morphine on T- and B-lymphocyte function. In 1979, Ho and Leung [25] demonstrated that lymphocytes from the peripheral blood and lymph nodes of morphine-addicted mice were significantly less responsive to the T-cell mitogen concanavalin A (Con A), a phenomenon that was blocked by simultaneous treatment of mice with naloxone. These investigators speculated that the mechanism by which morphine exerted its immunosuppressive effect involved the neuroendocrine system [25]. In a series of more recent studies using implantation of a 75-mg morphine pellet in C3H/HeN mice as a model of chronic morphine treatment, Bryant *et al.* [26–28] demonstrated that the HPA axis plays an important role not only in morphine-induced suppression of the proliferative response of splenic T-cells to Con A but also in impaired splenic B-cell responsiveness to lipopolysaccharide (LPS) *in vitro*. Additionally, the HPA axis was found to be involved in morphine-induced inhibition of delayed type hypersensitivity and graft vs host responses *in vivo* [29]. In the animal model of Bryant *et al.*, morphine-pelleted mice were found to have significant elevations of serum corticosterone associated with adrenal hypertrophy, as well as marked reductions in spleen and thymus weights. In adrenalectomized mice, the morphine-induced effects on splenic and thymus size and on immune cell function were abrogated. Effects similar to adrenalectomy were found in mice given a glucocorticoid receptor antagonist [28]. While the exact sequence of events in the glucocorticoid-mediated effects on immune cells were not determined in these studies, Bryant *et al.* [30] have suggested that the macrophage and certain of its products may play a pivotal role.

Consistent with the hypothesis that morphine-induced immunomodulation is mediated indirectly via the HPA axis, Sei *et al.* [31] have reported that thymic hypoplasia and phenotypic changes of thymocytes in morphine-pelleted C57BL/6J mice are glucocorticoid dependent. T-cell-dependent antibody responses also have been shown to be suppressed by implanation of a 75-mg morphine pellet in mice [32, 33], and such effects on humoral immune responses appear to involve the triggering of the HPA axis [34]. All of these findings are compatible with high concentrations of morphine being localized to the hypothalamus [35] and with the well-recognized effects of morphine on the HPA axis [36].

In contrast to the findings in studies using the chronic (75-mg morphine pellet) C3H/HeN mouse model, Bayer *et al.* [37] found in a rat model of acute morphine administration that the HPA axis is

not involved in opiate-induced immunosuppression. In this study, morphine-induced impairment of splenic NK cell activity and of peripheral blood lymphocyte responsiveness to Con A was blocked by naltrexone treatment; however, serum corticosterone levels were not reduced in animals receiving morphine plus naltrexone. These investigators suggested that in addition to activation of the HPA axis, other factors appeared necessary to elicit the immunosuppressive effects observed in morphine-treated rats [37]. Subsequent studies by this group of investigators [38] revealed that the immunosuppressive effects of acute administration of morphine on rat immune cell function are not due to a direct effect of morphine on immune cells.

Evidence for a glucocorticoid-independent mechanism of morphine-induced immunomodulation has also been provided by Sei *et al.* [39] in C57BL/6J mice treated with 75-mg morphine pellets. Using an assay of calcium mobilization in response to Con A, these investigators found that morphine-induced inhibition of intracellular [Ca^{2+}] uptake by splenic B-cells was not altered by adrenalectomy, even though naltrexone blocked this morphine-induced effect on B-cells. Intriguingly, morphine-induced inhibition of [Ca^{2+}] uptake by Con A-stimulated CD4 splenic lymphocytes was abolished by adrenalectomy; however, naltrexone did not block the action of morphine on this lymphocyte population [39]. To further complicate matters, Bussiere *et al.* [40] recently found that the *in vivo* effect of morphine on the primary antibody response of murine splenic B-cells *in vitro* varies markedly among mouse strains and that both classical (naloxone-reversible) and non-classical (naloxone-insensitive) opioid receptor mechanisms are operable.

Direct effects of morphine on immune cells

Although direct effects of morphine on immune-cell function have not been found in some studies, a considerable literature derived from *in vitro* culture systems indicates potential for such direct effects *in vivo*. Following the initial reports that T-lymphocytes and mononuclear phagocytes possess specific (naloxone-inhibitable) opiate receptors [6–8], several groups of investigators have further characterized the nature of these receptors. In studies with the relatively nonselective opiate antagonist [^{3}H]-naloxone, Mehrishi and Mills [41] found evidence of a μ-type opioid receptor in lymphocytes from the peripheral blood of healthy humans. Madden *et al.* [42] determined that human T-lymphocytes have a low-affinity binding site for naloxone with a K_D of 50.6 ± 2.4 nM. The bound naloxone was partially displaceable not only by morphine but also by β-endorphine and δ opioid receptor agonists [42]. Ovadia *et al.* [43], however, found that rat splenocytes had [^{3}H]naloxone binding sites that displaced with morphine but not with opioid peptides. In this study, the binding of naloxone was shown to be sensitive to Na^+ and to guanosine 5′-*O*-(3-thiotriphosphate) (GTPγS), suggesting that a GTP-binding regulatory protein that couples receptors to adenylate cyclase is involved in opiate binding to lymphocytes. Similar results were obtained in a later study with [^{3}H]-etorphine [44], a ligand with broad opioid receptor specificity.

The opioid receptor binding site on murine T- and B-lymphocytes has been investigated by Radulescu *et al.* [45]. Using highly selective opioid receptor ligands, these investigators delineated a μ-class binding site of 58 kDa, similar to that of the μ-site for neuronal tissue. Furthermore, the opioid receptor in T-lymphocytes, but not in B-lymphocytes, appeared to be coupled to calcium-uptake pathways [45]. From the results of their studies, these investigators also suggested the possibility that murine lymphocytes have distinct, yet interacting, μ-, δ-, and κ-opioid binding domains [45]. In a similar context, Taub *et al.* [46] demonstrated that the μ-selective agonists morphine and Tyr-D-Ala-Gly-*N*-Me-Phe-Gly-ol (DAMGE), as well two κ-selective agonists, inhibit the capacity of splenic lymphocytes from immunized mice to generate antibodies to sheep erythrocytes (SRBC) *in vitro*. In this lymphocyte culture system, however, opioid-mediated regulation occurred by way of classical μ and κ receptor–ligand pathways, without apparent cross-reactivity [46]. Recent studies by Rogers *et al.* [47] of murine splenic T-cell proliferative responses and of the secondary antibody response of mouse splenocytes to SRBC have demonstrated direct immunosuppressive effects of both μ receptor agonists (morphine and DAMGE) and κ agonists. As was found in their *in vivo* studies [40], marked differences in these *in vitro* effects of opioids were observed among mouse strains [47]. In ongoing studies of human T-lymphocytes, Madden *et al.* [48] have reported provocative evidence for a high-affinity binding site for [^{3}H]naloxone within the interior of these cells.

In addition to studies of the direct effects of morphine on lymphocytes, this opiate also has been shown to affect the function of mononuclear phagocytes via an opioid-receptor mechanism. Phagocytosis of immunoglobulin G-coated SRBC by mouse peritoneal macrophages [49], chemotaxis by human blood monocytes [50], and generation of superoxide ("respiratory burst activity") by blood monocytes from humans [51] have all been shown to be suppressed by *in vitro* exposure to morphine. In all of these studies, the functional effects of morphine were blocked by naloxone.

In our laboratory, research has focused on the involvement of cytokines in the mechanism of morphine-induced immunomodulation. Initial studies indicated that exposure of human peripheral blood mononuclear cells (PBMC) to morphine resulted in the suppressed production of the lymphokine interferon (IFN)-γ upon stimulation of PBMC cultures with Con A or a viral antigen [52]. The mechanism of this naloxone-inhibitable process appeared to involve an interaction of morphine with monocytes as the primary target cells in the PBMC cultures, with secondary inhibition of IFN-γ production by lymphocytes occurring as a result of suppressive monocyte products—reactive oxygen intermediates and prostaglandin E_2 [52]. Elliott *et al.* [53] also have reported that peritoneal macrophages from morphine-dependent rats release enhanced amounts of prostaglandin E_2; these

investigators suggested that this arachidonic acid metabolite is involved in the immunosuppressive effects of morphine.

Recently, we have shown that incorporation of morphine in cultures of LPS- or phytohemagglutinin (PHA)-stimulated PBMC results in the amplified release of another cytokine, transforming growth factor (TGF)-β [54]. Since TGF-β is known to have diverse immunologic effects, we suggested that morphine-induced augmentation of TGF-β release could explain some of the immunomodulatory properties of morphine. For example, morphine-induced suppression of monocyte respiratory burst activity [51] could be related to an enhanced release of TGF-β. Also, the increased growth of human immunodeficiency virus (HIV)-1 in human PBMC cocultures containing morphine [55, 56] could be due to a morphine-induced potentiation of TGF-β release, since this cytokine is capable of stimulating HIV-1 replication in PBMC cocultures [57].

We also have demonstrated that morphine treatment of PBMC results in the suppressed release of bioactive tumor necrosis factor (TNF) when these cells are stimulated with LPS or PHA [58]. This suppressive effect on PHA-stimulated TNF release was blocked by naloxone, whereas the suppressive effect of morphine on the response to LPS was not. The results of this study suggested that the mechanism of morphine-induced suppression of TNF release is counteracted by the potentiating effect of morphine on TGF-β release [58].

Finally, morphine may also exert an immunomodulatory effect on immune cells by altering their response to cytokines. Roy *et al.* [59], for example, have shown recently that bone marrow cells from mice treated with 75-mg morphine pellets are less responsive to the cytokine macrophage colony-stimulating factor. This finding could be mimicked by direct exposure of bone marrow cells from untreated mice to morphine in culture, and the opioid peptide β-endorphin was even more potent in this regard.

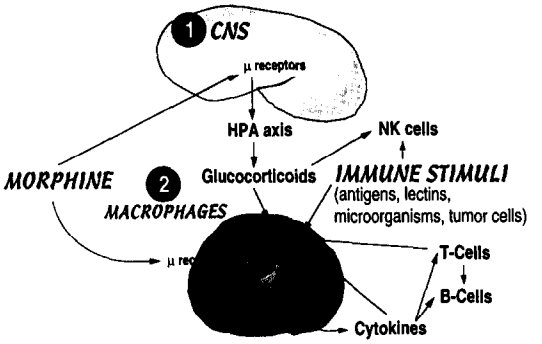

Fig. 2. Proposed mechanisms of morphine-induced immunomodulation. Morphine can act: (1) indirectly via triggering opiate receptors within the periaqueductal gray matter of the mesencephalon, resulting in activation of the HPA axis, with subsequent glucocorticoid-mediated effects on immune cells, or (2) directly by stimulating opiate receptors of immune cells. In this schema, modulation of macrophage production of cytokines in response to various stimuli plays a pivotal role in the action of morphine on immune cells. Morphine can also act via non-opiate (naloxone-insensitive) mechanisms and can have macrophage-independent effects on lymphocyte function.

CONCLUSIONS

It has become clear that morphine can alter a variety of immune responses via indirect (CNS-mediated) and direct effects on immune cells (Fig. 2). The finding that morphine can affect both immune cell and neuronal cell function is consistent with the view that the immune system and the brain are "connected". Following the lead of prodigious research on the nature of neuronal opioid receptors, investigators of opioid receptors on immunocytes have uncovered both similarities and unique features. The effects of opiate drugs within both the brain and the immune system appear to be inextricably linked to the function and regulation of endogenous opioid systems. Researchers in this field will no doubt determine the location of these receptors in immunocytes, their coupling mechanisms to second-messenger systems, and interactions between the different opioid receptor types. The recent cloning of a δ opioid receptor from neuronal cells [60] is likely to accelerate progress of this work. Other questions that need to be explored more fully are the cellular and molecular bases for interindividual variability in the effect of morphine on certain immune responses, the immune cell types which serve as primary targets for morphine, and the involvement of immune cell products (e.g. cytokines, free radicals, and arachidonic acid metabolites) in morphine-induced immunomodulation.

Now that it has been shown that chronic (75-mg morphine pellet) opiate administration in mice results in immunosuppression via a neuroendocrine pathway, questions regarding genetic-, gender-, and age-related influences are being examined. Given the multiplicity of confounding variables in human studies in this area of research, there is a need to extend these studies from rodents to other animal species. Indeed, such studies are presently underway in primate and swine models. Since the biological significance of opiate-induced immunomodulation is best appreciated in studies of infectious disease pathogenesis, research with animal models should increasingly relate mechanisms to infectious disease outcomes. Considering the overwhelming importance of AIDS and the link between this infectious disease and injection drug use, animal models of retroviral infection and of relevant opportunistic pathogens are increasingly being used. The results of some of these studies [reviewed in Ref. 61] are provocative in that primates or swine that are chronically maintained on morphine demonstrate benefit in terms of reduced pathogenesis of certain types of viral infection. Opiate withdrawal, on the other hand, may be highly deleterious due to stress-induced impairment of host defenses [61].

Research in this area of pharmaconeuroimmunology has implications beyond the possible relationship to AIDS. Effects on immunocompetence should be an area of concern in the development of

all novel opiate-related pharmaceuticals [29]. Studies of the mechanisms of opiate-induced immunomodulation may also lead to new therapies in areas such as transplantation and autoimmune disease, as well as in protection against host-mediated inflammatory injury associated with infectious agents.

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