MINIREVIEW

The Role of Endogenous Opioids and Their Receptors in the Immune System (43309B)

DANIEL J. J. CARR[1]

Department of Physiology and Biophysics, University of Alabama at Birmingham, Birmingham, Alabama 35294-0005

The production and action of opioid peptides in the immune system has been extensively studied beginning in the years 1979–1980 (1–3). The purpose of this review is to identify and highlight the accomplishments to date categorized into three major themes: (i) the action of opioids on immunocompetence *in vitro* and *in vivo*; (ii) the production of opioid peptides by cells of the immune system (immunocytes); and (iii) the identification and characterization of opioid receptors on immunocytes. Opioid peptides can be categorized into three groups: (i) the endorphins, which are encoded by the pro-opiomelanocortin (POMC) gene; (ii) the enkephalins, which are encoded by proenkephalin; and (iii) the dynorphins, which are encoded by prodynorphin. As will be described in this review, the modulation by these peptides of immunocompetence is mediated through membrane bound receptors classified into four types: δ, ϵ, κ, and μ. Whereas the δ-, κ-, and μ-classes of receptors are sensitive to broad and selective opioid antagonists, the ϵ-like receptor is specific for β-endorphin. However, it would appear that the ϵ-like receptor does, in fact, share some features in common with other opioid receptors of immune and neuroendocrine origin. The role these molecules have in immune homeostasis and in the production of opioids by immunocytes implicates these peptides as important signal molecules within the immune system and between the immune and neuroendocrine systems.

Biological Consequences of Opioid Administration *In Vitro* and *In Vivo*

Lymphocyte proliferation in response to mitogens is a widely used and useful technique to assess "general" immunocompetence. Early work described the ability of β-endorphin to enhance lymphocyte proliferation to T cell mitogens, concanavalin A (Con A), and phytohemagglutinin, but not to B cell mitogens (4). However, other opioid peptides, such as α-endorphin or [D-Ala^2,Met^5]enkephalin, were ineffective. Moreover, the antagonist naloxone was unable to block the effect mediated by β-endorphin, indicating that the interaction of β-endorphin with the lymphocyte membrane receptor is not through a "classical" opioid receptor-ligand interaction. Confirmation of the original finding is shown both *in vitro* and *in vivo*. Specifically, both acetylated and nonacetylated β-endorphin$_{1\text{-}31}$ and truncated forms of β-endorphin (β-endorphin$_{6\text{-}31}$ and β-endorphin$_{18\text{-}31}$) enhance Con A-induced T cell proliferation, whereas β-endorphin$_{1\text{-}27}$, Met-enkephalin, and α- and γ-endorphin are without effect (5). Similarly, intravenous administration of β-endorphin (but not Met-enkephalin or γ-endorphin) augment spleen cell proliferation to Con A in a time- and dose-dependent manner (6). Interestingly, the truncated forms of β-endorphin are consistently not as effective in potentiating the mitogenic response compared with the intact 31-residue peptide, implicating that the opioid active portion of the molecule (N-terminal portion) is a requirement to achieve maximal effect (5). However, the observation that β-endorphin$_{1\text{-}27}$ is inactive suggests that the C-terminal portion of the peptide is required for biological activity. A conformational analysis of β-endorphin may have clarified some of these observations. Specifically, β-turns reportedly occur only in the C-terminal end in the 27–31 segment of β-endorphin (7), whereas a stable α-helix, present from residues 14–24, produces a strong amphiphilic surface on the molecule (8). In the absence of the N (residues 1–11)- and C (residues 27–31)-terminal ends, the percentage of α-helix increases, suggesting that the N- and C-terminal portions are important in maintaining the amphiphilic

---

[1] To whom correspondence and requests for reprints should be addressed at Department of Microbiology, Immunology, and Parasitology, LSU Medical Center, 1901 Perdido Street, New Orleans, LA 70112-1393.

0037-9727/91/1982-0710$3.00/0

nature of the peptide and thus removing the hydrophobic domain from exposure to water (8). This structural conformation promotes interactions with lipid moieties (8). Therefore, removal of either end could dramatically alter the amphiphilic nature of the peptide and thus alter the ability of the peptide to interact with the immunocyte receptor binding site. The data suggest that the C-terminal end is crucial for this modulatory effect. Another segment of the β-endorphin molecule which may be important in regulating immune responses is the α-helix domain, residues 14–24. This segment of β-endorphin has been shown to specifically bind to calmodulin, a known regulator of adenylate cyclase (9). Since increases in $[cAMP]_i$ have thus far been reported to inhibit T cell activation (10, 11), modulation of adenylate cyclase by β-endorphin$_{14\text{-}24}$ through interactions with calmodulin is another possible pathway in the potentiation of mitogen-induced lymphocyte proliferation.

Other opioid pathways (naloxone-reversible) regulate lymphocyte proliferation as well. For example, the μ-class opioid ligands morphine and [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (DAGO) enhance mitogen-stimulated lymphocyte proliferation *in vitro*, which in the case of DAGO is naloxone reversible (12–14). However, *in vivo* administration of morphine attenuates lymphocyte proliferation to mitogen (15–17). The mechanism of suppression by morphine *in vivo* is presently unclear, although corticosterone has been implicated (18). Specifically, morphine has been proposed to be a pharmacologic stressor that mimics behavioral stress (15). Adrenalectomized animals undergoing morphine treatment are only slightly immunosuppressed, implying that the adrenals are a source of the morphine-mediated immunosuppression (17). Similar to μ-class opioid ligands, but in the absence of mitogen, δ-selective agonists increase [^{3}H]thymidine uptake in lymphocytes that is blocked by the δ-selective antagonist ICI-174864 (19). Collectively, the effects of opioid compounds on lymphocyte proliferation indicate that two pathways are operational, (i) a β-endorphin specific pathway and (ii) a naloxone-sensitive opioid pathway. It would also seem these pathways share either (i) intracellular signal transduction pathways or (ii) a common structural component of a(the) receptor complex that is coupled to similar signal transduction molecules.

Opioid peptides regulate antibody production *in vitro* and *in vivo* as well. In 1982, a study reported that α-endorphin *in vitro* significantly depressed primary antibody production to sheep red blood cells at 500 n*M* (20). Both Met- and Leu-enkephalin are moderate inhibitors, whereas β- and γ-endorphin are not effective. In addition, naloxone is capable of blocking the α-endorphin-mediated suppression. These results have subsequently been confirmed using α-endorphin and measuring the primary antibody response to ovalbumin (21). In this study, it was found that the tyrosine at the N-terminal end of α-endorphin is necessary for the observed response, suggesting that the effect is mediated through a naloxone-sensitive opioid receptor. Similarly, μ-class and κ-class selective opioid agonists also inhibit antibody production to sheep red blood cells which is reversible using class-selective antagonists (22). Interestingly, β-endorphin, but not [Met]-enkephalin, suppresses the IgG, but not IgA or IgM, production to *Staphylococcus aureus* in a naloxone-reversible fashion (23). The ability of some opioid peptides to regulate antibody production while others are inactive is not due to different opioid receptor classes on these cells, since both β-endorphin and Met-enkephalin act on μ- and δ-class opioid receptors with near equal affinities. However, one possible explanation may reside with the time of expression of the opioid receptor on the lymphocytes, which may be correlated with whether the immune reaction is primary or secondary. Specifically, *in vivo* administration of β-endorphin inhibits the primary antibody response to keyhole-limpet hemocyanin, but enhances the secondary response (6). Similarly, α- and β-endorphin enhance the secondary response to tetanus toxoid at the nanomolar and picomolar levels (24). The effect of opioids on antibody production is also dependent on the lymphocyte populations tested. Specifically, Con A-stimulated Peyer's patch lymphocyte immunoglobulin production is suppressed in the presence of oxymorphindole (δ-selective agonist) or β-endorphin (25). However, these compounds have no effect on Con A-elicited immunoglobulin production by splenic lymphocytes.

Natural killer (NK) activity mediated predominately by large granular lymphocytes is also regulated by opioid compounds. β-endorphin and Met-enkephalin augment NK activity in a dose-dependent (peaking at 10 n*M*) and naloxone-reversible fashion (26, 27). α-Endorphin, γ-endorphin, Leu-enkephalin, and morphine are ineffective or slightly suppressive (26, 28). Another study found that nonopioid fragments of β-endorphin (peptide segments 2–16, 2–17, and 6–17) enhance NK activity at picomolar levels through a naloxone-reversible pathway (29). This data would suggest that the N terminus tyrosine is not required to mediate the effect. Moreover, the results suggest that unique opioid binding sites occur on these cells compared with those on neuronal tissue (the N terminus tyrosine is a requirement for opioid activity in neural tissue). Class-selective opioid agonists for δ-, κ-, and μ-classes have recently been shown to modulate NK activity both positively and negatively, depending on the responder population (30). Thus, the opioid peptides, including DAGO, [D-Ser^2]-Leu-enkephalin, and dynorphin$_{1\text{-}13}$, act as "fine tuners," enhancing lower NK responder populations and suppressing high NK responder populations at a concentration range of 10^{-8}–

10^{-12} *M*. However, another recent report has shown that the μ-selective ligands DAGO and 2-(*p*-ethoxybenzyl)-1-[*N*,*N*-diethylamino]ethyl-5-isothio cyanatobenzimidazole suppress NK activity (14). The results *in vitro* are complicated by data obtained *in vivo*. When employing an inescapable footshock stress paradigm that is known to elicit endogenous opioid peptide release, a decrease in NK activity has been reported (31). Likewise, systemic administration of morphine (30–50 mg/kg) results in suppression. Both these effects are blocked by the long-acting antagonist naltrexone. However, systemic administration of *N*-methylmorphine, which is incapable of crossing the "blood-brain barrier," does not produce any measurable suppression, indicating the involvement of central (brain) opioid receptors in the morphine-induced immunosuppression (32). Subsequent studies have mapped the immunosuppressive qualities of morphine to the periaqueductal gray matter of the mesencephalin (33). Based on the above results, the relationship of opioids and NK activity remains somewhat controversial. Nevertheless, opioid pathways play a significant immunoregulatory role *in vivo*. This idea has most recently been demonstrated using naloxone. The administration of naloxone to mice enhances NK activity and antigen-specific antibody production in a dose-dependent and stereoselective manner (34). Similarly, clinical studies have also implicated opioids with immunocompetence. Specifically, a recent study correlated low NK activity with low circulating levels of plasma β-endorphin and predicted higher illness morbidity (35). Likewise, another study showed that Met-enkephalin inhibits tumor metastasis and potentiates splenic NK activity in mice immunized with the murine B-16 melanoma (36).

Neutrophils and monocytes and macrophages are important immune effector cells in the phagocytosis of foreign matter such as bacteria, as well as in the processing and presentation of antigen by macrophages. Both cell types are affected by opioid compounds. Specifically, β-endorphin, morphine, and Met-enkephalin stimulate granulocyte and mononuclear cell chemotaxis (37, 38). These effects are reversed by naloxone; in one investigation, the antagonist inhibition was stereoselective (38). In another study using fragments of β-endorphin, both N- and C-terminal peptides stimulated chemotaxis of monocytes (39). Moreover, only the N-terminal-mediated effects were naloxone reversible. Morphine, β-endorphin, and Met-enkephalin also effect respiratory burst activity of mononuclear cells. Whereas resting levels of O_2^- and H_2O_2 are significantly enhanced in these cells (40, 41), zymosan- and phorbol myristate acetate-induced respiratory burst activity is significantly suppressed by opioid peptides (40). The opioid-mediated effects are blocked by naloxone and cannot be mimicked with *N*-acetylated β-endorphin, indicating the involvement of non-β-endorphin-specific opioid receptors (40). Interestingly, morphine (10^{-7} *M*), but not DAGO, DADLE, or dynorphin$_{1\text{-}9}$, inhibits granulocyte aggregation (42), whereas β-endorphin and Met-enkephalin (10^{-7}–10^{-14} *M*) enhance neutrophil adherence to serum-coated glass (43). The inhibitory effect by morphine is completely naloxone-reversible (42), whereas the action of the opioid peptides on neutrophil adherence is only partially naloxone-reversible (43). The observations showing that opioids suppress intracellular killing mechanisms of activated mononuclear cells (40), coupled with studies indicating opioid peptides suppress phagocytosis and reduce vimentin filament expression and surface expression of HLA-DR molecules (44), seem to indicate opioid molecules may be detrimental to granulocyte and monocyte function as well. In addition, the ability to present antigen in the context of major histocompatibility complex/human histocompatibility leukocyte antigens expression by antigen-presenting cells (e.g., macrophages) that is compromised by opioid peptides correlates with the immunosuppression mediated by opioid peptides in the primary antibody response (20–23).

The fact that opioids enhance the secondary antibody response may be linked to other immunocyte-mediated events. For example, Met-enkephalin and β-endorphin have been shown to augment γ-interferon production by mononuclear cells (45) and purified large granular lymphocytes (46), although heterogeneity among donor lymphocytes has been reported (47). Likewise, β-endorphin has been shown to enhance interleukin IL 2 production by Con A-stimulated lymphocytes (48) or by IL-1-stimulated EL-4 lymphoid cells (49). Similarly, β-endorphin and Leu-enkephalin have been shown to potentiate IL-1 production by bone-marrow macrophages concomitantly stimulated with lipopolysaccharide (LPS) (50). The enhanced production of these cytokines by opioid peptides is, in most cases, reversed by naloxone (46, 49, 50). The findings that β-endorphin and Met-enkephalin augment the generation of cytotoxic T lymphocytes through a naloxone-sensitive pathway (51) may also be related to the opioid-mediated effects on cytokine production.

Collectively, the data leaves little doubt that opioid compounds regulate immunocompetence and immune homeostasis (Table I). These effects are mediated through both β-endorphin-specific, ϵ-like opioid receptors and opioid receptors that exhibit ligand preferences similar to the δ-, κ-, and μ-class opioid receptors found in the central nervous system. However, in some instances, opioids both suppress and enhance the same immunological function, e.g., antibody production (52), NK activity (30), superoxide anion release by neutrophils (53), and mitogen-stimulated lymphocyte proliferation (54). The reasons for these differences remain to be determined, although some explanations

Table I. Opioid Receptor-Mediated Biological Effects on Various Immune Parameters *In Vitro*

Immune function	Opioid receptor class[a]	Effect	Reference
Mitogen-induced lymphocyte proliferation	ε-Like	Enhancement	4–6
	μ	Enhancement	13, 14
Unstimulated lymphocyte proliferation	δ	Enhancement	19
Primary antibody production	Opioid	Suppression	6, 20, 21, 23
	μ, κ	Suppression	22
Secondary antibody production	Opioid	Enhancement	6, 24
Natural killer activity	Opioid	Enhancement	26–29
	μ, δ, κ	Enhancement	30
	μ, δ, κ	Suppression	14, 30
Monocyte-granulocyte chemotaxis	Opioid	Enhancement	37–39
γ-Interferon production	Opioid	Enhancement	45–47
Interleukin 1 production	Opioid	Enhancement	50
	Opioid	Suppression	
Interleukin 2 production	ε-Like	Enhancement	48
	Opioid	Enhancement	49
Cytotoxic T lymphocyte generation	Opioid	Enhancement	51

[a] The immunomodulatory effect elicited by the opioid drug acts through a specific class of opioid receptor. These classes include δ, ε, κ, and μ. Where the word "opioid" is used, it refers to a naloxone-sensitive binding site which has not yet been categorized into a particular opioid receptor class.

have been provided, including species and strain differences, cell preparation techniques, and ligands employed (30, 53, 54). It is anticipated that future work will incorporate these findings into relevant therapeutic protocols to alleviate or reverse pathophysiologic states. In one such case, Met-enkephalin was reportedly successful in reversing symptoms in acquired immune deficiency syndrome patients (55).

The Production of Opioid Peptides by Immunocytes

Originally, it was found that substances that induced α-interferon, such as viruses, elicited the production of endorphin-like molecules (3, 56). Lymphocyte-derived, endorphin-like molecules could block [^{3}H] dihydromorphine binding to brain tissue and lymphocytes taken from virally induced mice stained positive using a specific antiserum to γ-endorphin (56). The production of endorphins or enkephalins by lymphocytes has subsequently been shown by various groups. Macrophages constitutively secrete β-endorphin (57); LPS stimulates production of α- or γ-endorphin by leukocytes (58); sheep red blood cells induce opioid-like molecules (M_r = 1300–2000), termed "myelopeptides," by bone marrow cells (59); corticotropin-releasing hormone (CRH) elicits the production of immunoreactive β-endorphin by peripheral blood mononuclear cells (60) and splenic and mesenteric lymph node lymphocytes (61); and phytohemagglutinin elicits the production of proenkephalin-derived peptides by lymphocytes (62). The CRH-elicited production of β-endorphin is reportedly mediated through IL-1 secreted from CD14$^+$ monocytes (63). Moreover, the CRH-elicited production of opioids by immunocytes is biologically active. Specifically, preincubation of splenic lymphocytes with CRH has been shown to enhance natural killer activity through naloxone- and naltrindole (δ-selective antagonist)-sensitive pathways (64). The CRH-mediated effect is time-dependent and requires macrophages. Taken together, the results point to a biological role for the production of opioid peptides by immune cells after CRH treatment, which may be important in paracrine or autocrine regulatory mechanisms.

Transcripts for the POMC gene have been identified in lymphocytes obtained from the antropyloric and duodenum mucosa (65) splenic lymphocytes from Newcastle disease virus-infected mice (66), phytohemagglutinin-stimulated peripheral blood, tonsillar lymphocytes (67), Epstein-Barr virus-infected B lymphocyte lines (67), and a T lymphocyte cell line derived from a patient with lymphoma (68). Proenkephalin mRNA has been isolated from T cell lines (69, 70), mastocytoma and macrophage cell lines (70), normal B and T lymphocytes obtained from the spleen, lymph nodes, and bone marrow (71), and CD4$^+$ thymocytes (72). Proenkephalin-derived peptides secreted by T helper cell lines have also been reported (73). However, these peptides are much larger (mol wt of 3,000-15,000) than neuroendocrine enkephalins and apparently are inactive in opioid-binding assays.

CRH up-regulates the expression of two truncated POMC transcripts (74). Using the polymerase chain reaction with sense primers homologous to the 5′ end of exons 1, 2, and 3 and internal to exon 3 and an antisense primer complementary to the 3′ end of exon 3 and the first-strand cDNA obtained from selectively reverse-transcribing lymphocyte poly(A$^+$) mRNA from unstimulated lymphocytes, a truncated exon 3 product

(549 bp) results (74). When lymphocytes are treated with CRH prior to polymerase chain reaction analysis, both a truncated (549 bp) and full-length (615 bp) exon 3 product are obtained. In neither treated nor untreated lymphocytes are products observed using selective primers for exons 1 and 2. From this study, it was concluded that (i) alternate splicing of the POMC mRNA exists or (ii) different transcription initiation sites on the POMC gene occur in lymphocytes. Exon 3, which contains the coding region for adrenocorticotropin and β-lipotropin (which encodes endorphin), has recently been sequenced in lymphocytes and found to be identical to the coding sequence from pituitary exon 3 (75).

Posttranslational processing of opioid peptides by immunocytes has also been studied. Splenic macrophages have been shown to secrete β-endorphin$_{1\text{-}31}$, *N*-acetyl-β-endorphin$_{1\text{-}31}$, and *N*-acetylated fragments of β-endorphin (β-endorphin$_{1\text{-}16}$, $_{1\text{-}17}$, and $_{1\text{-}27}$) (76). However, the predominant product appears to be the nonacetylated β-endorphin$_{1\text{-}31}$. Furthermore, processing of the endorphin molecule is reportedly dependent on the stimulus for induction. For example, CRH-treated lymphocytes produce β-endorphin, whereas LPS-stimulated lymphocytes secrete α- and γ-endorphin (77). The fact that immunocytes produce acetylated and nonacetylated forms of β-endorphin (76), as well as fragments thereof depending on the stimulus (77), suggests that these peptides may have an immunoregulatory role. In addition, since neutral endopeptidases can cleave opioid peptides to fragments including Y-G-G, which are reportedly endogenous opioid antagonists (78), these fragments may also be considered regulatory molecules relative to opioid peptides in the immune system.

The validity of biologically active, lymphocyte-derived opioids *in vivo* has been shown employing two different models. The first model used LPS-sensitive (C3He/FeJ) and -resistant (C3H/HeJ) inbred mice. The C3HeB/FeJ mice injected with LPS presented with the pathophysiologic symptoms of endotoxic shock (79). Moreover, lymphocytes obtained from these animals, but not those from the C3H/HeJ mice, stained positive for endorphin after LPS treatment. However, by isolating the immunoreactive endorphin from C3He/FeJ cultured, LPS-stimulated lymphocytes and administering it to the C3H/HeJ mice, symptoms associated with endotoxic shock were manifested in these animals (79). The second study used a hind paw inflammatory model. It was found that localized opioid receptor-mediated antinociception in peripheral inflamed tissue was due to the secretion of β-endorphin by infiltrating macrophages, lymphocytes, mast cells, and plasma cells (80). Taken together, these studies illustrate the biological relevance lymphocyte-derived opioids have as potent neuroendocrine signaling molecules.

Characterization of Opioid Receptors on Cells of the Immune System

Opioid receptors first identified in neuronal tissue (81–83) are designated into four distinctive classes—μ, δ, ε, and κ—depending on ligand preferences (84, 85). These receptors exhibit saturable and stereospecific binding with the δ-, κ-, and μ-receptors sensitive to antagonists, while the ε-receptor is insensitive to opioid antagonists. The δ-, κ-, and μ-receptors are coupled to G proteins (Gi and Go), which in the case of δ- and μ-receptors regulates adenylate cyclase (86–88). In general, neuronal opioid receptors (i) have affinities in the nanomolar range (89), (ii) are sensitive to cations and guanosine 5′-0-(3-thiotriphosphate) (89), (iii) are glycosylated (90), (iv) are sensitive to reducing agents (91), and (v) are intricately associated with lipid moieties (92).

The molecular characterization of immunocyte opioid receptors has only recently been undertaken. A high affinity (K_d = 3 n*M*), ε-like receptor initially described on cultured lymphocytes (93) was later studied using the EL-4 thymoma cell. Chemical cross-linking studies indicate that the ε-like receptor binding site has an apparent mol wt of 72,000 (94). Recent studies have confirmed the high affinity (K_d = 4 n*M*) nature of the ε-like binding site on splenocytes, with a discrepancy to the size of the receptor (95). Covalent cross-linking experiments of [^{125}I]β-endorphin to splenocytes that were subsequently resolved on polyacrylamide gels show the selective labeling of proteins at mol wt of 66,000, 57,000 and 44,000. The [^{125}I]β-endorphin can be displaced with *N*-acetylated-β-endorphin and C-terminal fragments of β-endorphin, but not by naloxone (95). The ε-like receptor is sensitive to cations and guanosine 5′-0-(3-thiotriphosphate) (96) and is coupled to a calcium uptake pathway in the early stages of lymphocyte activation (97). Interestingly, an earlier study using similar cross-linking techniques, polyacrylamide gel electrophoresis (PAGE), and [^{125}I]β-endorphin showed the labeling of proteins on splenocytes with mol wt of 50,000 and 34,000 (98), with the majority of the radioactive signal associated with the 50-kDa polypeptide. In addition, the labeling was blocked in the presence of excess, unlabeled naloxone. Subtracting the molecular weight of β-endorphin and cross-linking agent, the apparent size of the naloxone-sensitive binding site is 46 kDa. Photoaffinity-labeling brain opioid receptors with [^{125}I](D-Ala^2,*p*-N$_3$-Phe^4,Met$_5$) enkephalin shows the specific labeling of a polypeptide with a mol wt of 46,000 as determined by PAGE (99). The labeling can be blocked with μ, δ-, and κ-class-selective opioid ligands, but not with unrelated peptides, suggesting that the protein is a common structural element among μ-, δ-, and κ-brain opioid receptors. Taken together, the data indicate a similar-sized component of the putative opioid receptor complex from immune and neuroendocrine tissues. In addition, the similarity in size of one of the naloxone-insensitive receptor polypeptides (44 kDa) and the naloxone-sensitive receptor polypeptides (46 kDa) suggests that the naloxone-sensitive and -insensitive opioid receptors share a common structural polypeptide which may be involved in the intracellular signaling transduction pathway.

Early pharmacological studies on other (naloxone-sensitive) immunocyte opioid receptors illustrate that granulocytes and monocytes (100, 101), as well as lymphocytes (102, 103), possess δ-, κ-, and μ-opioid binding sites. Although an accurate determination as to the affinity and the number of sites per cell cannot be obtained from the data presented, these early studies have helped establish a precedence for neuropeptide receptors on immune cells. More recent binding studies have identified high affinity (K_d in the nanomolar range), saturable, κ-class opioid receptors on EL-4 thymoma (104) and P388d$_1$ macrophage (105) cell lines. Although the κ-type opioid binding sites on EL-4 thymoma cells do not exhibit stereospecificity, class-selective properties for ligand interaction are observed (104). The κ-like opioid receptor binding site on the P388d$_1$ cell is sensitive to κ-class ligands such as trans-(±)-3,4-dichloro-*N*-methyl-*N*[-2-(1-pyrolidinyl)cyclohexyl]-benzene acetamide methane sulphonate (U-50488), but not DAGO (μ-selective) or Met-enkephalinamide (δ-selective). In addition, this binding site shows enantioselectivity using derivatives of U-50488 (106). Similarly, a μ-like opioid binding site is found on mitogen-activated lymphocytes using [^{3}H]naloxone (107). [^{3}H] Naloxone binding to these cells can be displaced by using excess unlabeled morphine sulphate or naloxone, but not Met- or Leu-enkephalin or β-endorphin. Furthermore, the binding of [^{3}H]naloxone is inhibited in the presence of guanosine 5′-0-(3-thiotriphosphate) and Na^+ (107). In addition, the binding capacity of lymphocytes for [^{3}H]naloxone is reduced in aged animals, although the affinity remains constant (108).

Structurally, immunocyte opioid receptors have been studied using immunoaffinity purification techniques. The immunoaffinity-purified immunocyte receptor has been described as a complex composed of four polypeptide chains with mol wt of 70,000, 56,000–58,000, 46,000, and 31,000 (109). The entire complex has a mol wt of >350,000, as determined by PAGE (110). A similar subunit structure has also been identified for both brain (109) and NG108-15 neuroblastoma × glioma hybrid cells (111). However, the opioid receptor complex from NG108-15 cells has a mol wt of 210,000, as determined by PAGE (111) or radiation inactivation analysis (112). Likewise, covalently labeling brain opioid receptors with radiolabeled ligands followed by molecular exclusion chromatography and PAGE show a complex with a mol wt of 380,000 (113). However, the number of subunits the opioid receptor complex consists of is presently unclear. Specifically, the 58-kDa and 70-kDa polypeptides are believed to be mature and immature forms of the same protein, whereas the 31-kDa subunit may be a stable degradative product of one of the other polypeptides (14, 110). Therefore, only the 46,000- and 58,000–70,000-mol wt subunits are accountable as complex constituents.

The sizes of the class-specific opioid binding sites have recently been investigated using radiolabeled, site-directed acylating agents and class-selective ligands. The δ-class selective opioid drug [^{3}H]cis-(+)-3-methyl-fentanylisothiocyanate (SUPERFIT) labels a protein on immunocyte membranes with an apparent mol wt of 58,000 under nonreducing conditions and 70,000 under reducing conditions (114). The labeling is specific, since excess unlabeled δ-selective ligands (SUPERFIT or DADLE) or naloxone can displace [^{3}H]SUPERFIT binding, while DAGO (μ-selective) cannot appreciably inhibit binding. These results are similar to those using [^{3}H]SUPERFIT labeling of δ-class opioid receptors on NG108-15 cells (115).

The μ-class selective opioid compound [^{3}H]2-(*p*-ethoxy-benzyl)-1-[*N,N*-diethylamino]ethyl-5-isothio cyanatobenzimidazole (BIT) has been used to identify the binding site of μ-class opioid receptors on immunocytes. Similar to the δ-class opioid receptor, [^{3}H]BIT-labeled protein from immune cells has a mol wt of 58,000 under nonreducing conditions and 70,000 under reducing conditions (14). However, [^{3}H]BIT-labeled proteins from brain extracts migrate at a mol wt of 54,000 under nonreducing conditions (14). These size differences may be due to glycosylation anomalies. In addition, the κ-selective ligand, ($5\alpha,7\alpha,8\beta$)-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro (4,5)-dec-8-yl] benzeneacetamide (U-69593) can partially displace [^{3}H]BIT binding to immunocyte, but not brain, opioid receptors (14). However, this observation is not repeated using dynorphin A, indicating a unique feature of U-69593 for μ-like opioid binding sites on immune cells. Interestingly, the δ-selective opioid agonist oximorphindole augments [^{3}H]BIT binding to both brain and immunocyte opioid receptors, while the δ-selective antagonist naltrindole (116) blocks [^{3}H]BIT binding (14). However, neither SUPERFIT nor Leu-enkephalin affect binding of [^{3}H]BIT to immunocyte receptors (Fig. 1). The observation showing that oxymorphindole augments binding of [^{3}H]BIT to brain and immunocyte opioid receptors suggests a possible coupling of μ/δ-binding sites or -receptor complexes. Other investigations have also found evidence for a μ/δ-opioid receptor complex in neuronal tissue (117–120).

Unlike the immunocyte μ- and δ-binding sites, the κ-class binding site is smaller in size (106). The κ-selective acylating agent [^{3}H](1S,2S)-(−)-trans-2-isothio cyanato-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]

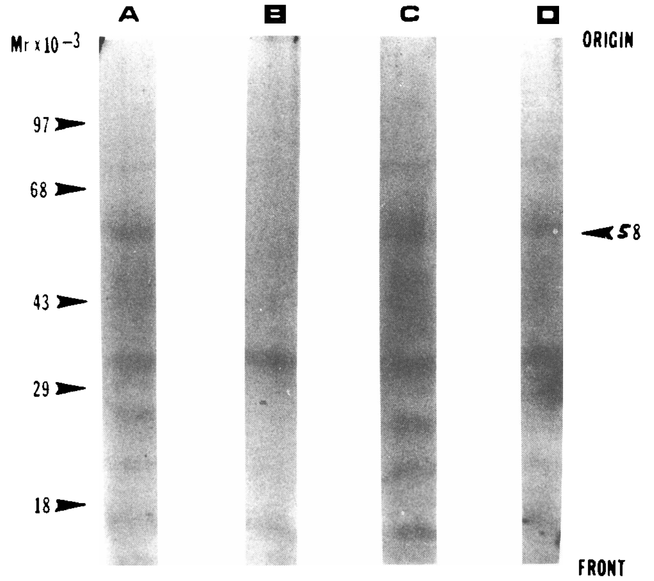

Figure 1. Autoradiogram of *in situ*-labeled P388d$_1$ cells specifically bound to [^{3}H]BIT electrophoresed on polyacrylamide gels under nonreducing conditions. P388d$_1$ cells (2 mg/ml) were incubated with [^{3}H]BIT (50 n*M*, sp act 31.4 Ci/mmol) in the presence or absence of various unlabeled ligands and subsequently electrophoresed on a 10% polyacrylamide gel. The resultant gel was subjected to autoradiography (21 days). Lane A, [^{3}H]BIT only. Lane B, [^{3}H]BIT + unlabeled BIT (2 μM). Lane C, [^{3}H]BIT + unlabeled SUPERFIT (2 μM). Lane D, [^{3}H]BIT + unlabeled Leu-enkephalin (10 μM).

benzeneacetamide ([−]-BD 166) specifically labels a protein from P388d^1 macrophage cell membranes with a mol wt of 38,000 under nonreducing conditions and 42,000 under reducing conditions, as determined by PAGE (106). The active enantiomer (−) of U-50488 is able to block the labeling, whereas the inactive enantiomer (+) of U-50488 is unable to displace specific binding. In comparison, κ-opioid receptors isolated from frog brain membranes and resolved on polyacrylamide gels have a mol wt of 65,000 and 43,000 under reducing conditions (121). Moreover, a monoclonal antibody recognizing κ- but not δ- or μ-opioid receptors binds to a 45,000- mol wt protein by Western blot analysis (122). Therefore, the data suggest immunocyte- and neuronal-derived κ-opioid receptors may share a common binding subunit, i.e., a 42,000–45,000-mol wt polypeptide. A summary comparing the size and affinities of neuronal and immune opioid receptor binding sites is shown in Table II.

Table II. The Molecular Size of Opioid Receptor Binding Sites of Neural and Immune Origin[a]

Class	Immune (mol wt)	Neural (mol wt)	Reference
ϵ-Like	72,000	Unknown	94
	66,000, 57,000		95
δ	58,000		105, 114, 123
		58,000	89, 115
μ	58,000		14
		58,000	124
κ	42,000		106
		65,000, 43,000	122
Opioid[b]	46,000		98
		46,000	99

[a] The size of the opioid receptor binding sites was determined using affinity-labeling techniques of the binding sites subsequently resolved using sodium dodecyl sulphate polyacrylamide gels.

[b] Opioid class refers to an undetermined type of naloxone-sensitive opioid-binding protein that may be shared among the receptor classes (96).

Opioid receptors immunoaffinity purified from immunocyte membrane preparations bind [^{125}I]-β-endorphin, [^{3}H]SUPERFIT, and [^{3}H]Met-enkephalinamide with specificity, although the affinity is reduced (123). The reduction in affinity for opioid ligands by purified receptor has been observed by others (121, 125, 126). However, reconstitution of these purified receptors with acidic lipids results in a high affinity ligand-receptor interaction. The observations showing that lipid moieties play an important role in opioid ligand binding profiles and are intricately associated with opioid receptors (92, 127) may in part be an explanation for the differences observed of ligand preferences between neural and immunocyte opioid receptors (14, 107).

The activation of immunocyte opioid receptors stimulates a variety of transduction signaling pathways. For example, endogenous opioid peptides such as Met-enkephalin and β-endorphin have been shown to induce a rise in cGMP (128), yet suppress adenylase cyclase activity (129), through naloxone-sensitive pathways at physiological concentrations. However, other investigations indicate opioid peptides can augment or suppress cAMP production depending on the source of the immunocytes (donor) and the concentration of ligand (130, 131). Ionic conductance pathways are also modulated by opioid peptides. For example, β-endorphin suppresses mitogen-stimulated K^+ currents in Jurkat cells (132) and blocks K^+ channel activity of T-enriched lymphocytes (98, 133). Studying another signal transduction pathway, lymphocyte μ-receptor activation by DAGO or BIT augments Ca^{2+} uptake in T-enriched, but not B-enriched, lymphocytes (14). In some instances, the signaling pathways following opioid receptor activation in immunocytes parallel those seen after neuronal opioid receptor activation (e.g., suppression in cAMP production) (89). Collectively, the data illustrate both unique and shared features of the opioid receptors on cells of the immune system compared with their neuroendocrine counterparts.

Summary

Opioid peptides appear to be dynamic signaling molecules that are produced within the immune system and are active regulators of an immune response. Furthermore, the receptors for these peptides occurring on immunocyte membranes share characteristics with neuronal opioid receptors, including molecular size, immunogenicity, and the use of specific intracellular signaling pathways. Recent studies of the interaction of opioids with cytokines have indicated that opioid peptides are intimately involved within the immune system. Specifically, opioids, including 2-*n*-pentyloxy-2-phenyl-4-methyl-morpholine, naloxone, and β-endorphin, have been shown to interact with IL-2 receptors (134) and regulate production of IL-1 and IL-2 (48–50, 135). Conversely, IL-1 has been shown to up-regulate opioid peptide binding in brain tissue (136). Furthermore, the induction of IL-1 by opioids has also been identified in the invertebrate Mytilus, indicating the evolutionary conservation of this relationship (137). These results seem to typify the intricate association between the immune and neuroendocrine systems through opioid pathways. It is predicted that future endeavors will use this relationship to diagnose and treat specific diseases that have at their basis neuroendocrine and immunologic imbalances.

I am grateful to Dr. J. Edwin Blalock for his support. In addition, the author is greatly indebted to Drs. Kenner C. Rice and Brian R. DeCosta for providing the [^{3}H]BIT and SUPERFIT in the studies investigating the size and specificity of opioid receptor binding sites. The author would like to thank Dr. Joe Dillon for his critical evaluation of the manuscript and acknowledges Diane Weigent for the typing of the manuscript.

---

1. Wybran J, Appelboom T, Famaey J-P, Govaerts A. Suggestive evidence for receptors for morphine and methionine-enkephalin on normal human blood T lymphocytes. J Immunol **123**:1068–1070, 1979.
2. McDonough RJ, Madden JJ, Falek A, Shafer DA, Pline M, Gordon D, Bokos P, Kuehnle JC, Mendelson J. Alteration of T and null lymphocyte frequencies in the peripheral blood of human opiate addicts: In vivo evidence for opiate receptor sites on T lymphocytes. J Immunol **125**:2539–2543, 1980.
3. Blalock JE, Smith EM. Human leukocyte interferon: Structural and biological relatedness to adrenocorticotropic hormone and endorphins. Proc Natl Acad Sci USA **77**:5972–5974, 1980.
4. Gilman SC, Schwartz JM, Milner RJ, Bloom FE, Feldman JD. β-endorphin enhances lymphocyte proliferative responses. Proc Natl Acad Sci USA **79**:4226–4230, 1982.
5. Gilmore W, Weiner LP. The opioid specificity of beta-endorphin enhancement of murine lymphocyte proliferation. Immunopharmacology **17**:19–30, 1989.
6. Kusnicov AW, Husband AJ, King MG, Smith R. Modulation of mitogen-induced spleen cell proliferation and the antibody-forming cell response by beta-endorphin in vivo. Peptides **10**:473–479, 1989.
7. Hammonds RG, Hammonds AS, Ling N, Puett D. β-endorphin and deletion peptides. J Biol Chem **257**:2990–2995, 1982.
8. Zetta L, Consonni R, DeMarco A, Longhi R, Mansera E, Vecchio G. Opioid peptides in micellular systems: Conformational analysis by CD and by one-dimensional and two-dimensional H-NMR spectroscopy. Boipolymers **30**:899–909, 1990.
9. Giedroc DP, Puett D, Ling N, Staros JV. Demonstration by covalent crosslinking of a specific interaction between β-endorphin and calmodulin. J Biol Chem **258**:16–19, 1983.
10. Hadden JW, Coffey RG. Cyclic nucleotides in mitogen-induced lymphocyte proliferation. Immunol Today **3**:299–304, 1982.
11. Lerner A, Jacobson B, Miller RA. Cyclic AMP concentrations modulate both calcium flux and hydrolysis of phosphoinositol phosphates in mouse T lymphocytes. J Immunol **140**:936–940, 1988.
12. Bocchini G, Bonanno G, Canevari A. Influence of morphine on human peripheral blood T-lymphocytes. Drug Alcohol Depend **11**:233–237, 1983.
13. Bidlack JM, Hemmick LM. Morphine enhancement of mitogen-induced T-cell proliferation. In: Quirion F, Jhamandas K, Gianoulakis C, Eds. The International Narcotics Research Conference (INRC) '89. New York: Alan R. Liss, p405, 1990.
14. Radulescu RT, DeCosta BR, Jacobson AE, Rice KC, Blalock JE, Carr DJJ. Biochemical and functional characterization of an opioid binding site on cells of the immune system. Progress NeuroEndocrinImmunology **4**:166–179, 1991.
15. Bryant HV, Bernton EW, Holaday JW. Immunosuppressive effects of chronic morphine treatment in mice. Life Sci **41**:1731–1738, 1987.
16. Bayer BM, Daussin S, Hernandez M, Irvin L. Morphine inhibition of lymphocyte activity is mediated by an opioid dependent mechanism. Neuropharmacology **29**:369–374, 1990.
17. Bryant HV, Roudebush RE. Suppressive effects of morphine pellet implants on in vivo parameters of immune function. J Pharmacol Exp Ther **255**:410–414, 1990.
18. Bryant HV, Bernton EW, Holaday JW. Morphine-induced immunosuppression. Involvement of glucocorticoids and prolactin. In: Harris L, Ed. Problems of Drug Dependence. NIDA Res Monograph **90**:323, 1990.
19. Hucklebridge FH, Hudspith BN, Lydyard PM, Brostoff J. Stimulation of human peripheral lymphocytes by methionine enkephalin and δ-selective analogues. Immunopharmacology **19**:87–91, 1990.
20. Johnson HM, Smith EM, Torres BA, Blalock JE. Regulation of the in vitro antibody response by neuroendocrine hormones. Proc Natl Acad Sci USA **79**:4171–4174, 1982.
21. Heijnen CJ, Bevers C, Kavelaars A, Ballieux RE. Effect of α-endorphin on the antigen-induced primary antibody response of human blood B cells in vitro. J Immunol **136**:213–216, 1986.
22. Taub DD, Eisenstein TK, Geller EB, Adler MW, Rogers TJ. Immunomodulatory activity of μ- and κ-selective opioid agonists. Proc Natl Acad Sci USA **88**:360–364, 1991.
23. Morgan EL, McClurg MR, Janda JA. Suppression of human B lymphocyte activation by β-endorphin. J Neuroimmunol **28**:209–218, 1990.
24. Munn NA, Lum LG. Immunoregulatory effects of α-endorphin, β-endorphin, methionine-enkephalin, adrenocorticotropic hormone on anti-tetanus toxoid antibody synthesis by human lymphocytes. Clin Immunol Immunopathol **52**:376–385, 1989.
25. Carr DJJ, Radulescu RT, DeCosta BR, Rice KC, Blalock JE. Differential effect of opioids on immunoglobulin production by lymphocytes isolated from Peyer's patches and spleen. Life Sci **47**:1059–1069, 1990.
26. Mathews PM, Froelich CJ, Sibbitt WL, Bankhurst AD. Enhancement of natural cytotoxicity by β-endorphin. J Immunol **130**:1658–1662, 1983.
27. Kay N, Allen J, Morley JE. Endorphins stimulate normal human peripheral blood lymphocyte natural killer activity. Life Sci **35**:53–59, 1984.
28. Faith RE, Liang HJ, Murgo AJ, Plotnikoff NP. Neuroimmunomodulation with enkephalins: Enhancement in human natural killer (NK) cell activity in vitro. Clin Immunol Immunopathol **31**:412–418, 1984.
29. Kay N, Morley JE, van Ree JM. Enhancement of human lymphocyte natural killing function by non-opioid fragments of β-endorphin. Life Sci **40**:1083–1087, 1987.

30. Oleson DR, Johnson DR. Regulation of human natural cytotoxicity by enkephalins and selective opiate antagonists. Brain Behav Immun **2**:171–186, 1988.
31. Shavit Y, Lewis JW, Terman GW, Gale RP, Liebeskind JC. Opioid peptides mediate the suppressive effect of stress on natural killer cell cytotoxicity. Science **223**:188–190, 1984.
32. Shavit Y, Depaulis A, Martin FC, Terman GW, Pechnick RN, Zane CJ, Gale RP, Liebeskind JC. Involvement of brain opiate receptors in the immune-suppressive effect of morphine. Proc Natl Acad Sci USA **83**:7114–7117, 1986.
33. Weber RJ, Pert A. The periaqueductal gray matter mediates opiate-induced immunosuppression. Science **245**:188–190, 1989.
34. Carr DJJ, Blalock JE. Naloxone administration in vivo stereoselectively alters antigen-dependent and antigen-independent immune responses. Psychoneuroendocrinology (in press).
35. Levy SM, Fernstrom J, Herberman RB, Whiteside T, Lee J, Ward M, Massoudi M. Persistently low natural killer cell activity and circulating levels of plasma β-endorphin: Risk factors for infectious disease. Life Sci **48**:107–116, 1991.
36. Faith RE, Murgo AJ. Inhibition of pulmonary metastasis and enhancement of natural killer cell activity by methionine-enkephalin. Brain Behav Immun **2**:114–122, 1988.
37. Van Epps DE, Saland L. β-endorphin and met-enkephalin stimulate human peripheral blood mononuclear cell chemotaxis. J Immunol **132**:3046–3053, 1984.
38. Marcoli M, Ricevoti G, Mazzone A, Pasotti D, Lecchini S, Frigo GM. A stereoselective blockade by naloxone by opioid and non-opioid-induced granulocyte activation. Int J Immunopharmacol **11**:57–61, 1989.
39. Sacedote P, Panerai AE. Analysis of the β-endorphin structure-related activity on human monocyte chemotaxis: Importance of the N- and C-terminal. Peptides **10**:565–569, 1989.
40. Peterson PK, Sharp B, Bekker G, Brummitt C, Keane WF. Opioid-mediated suppression of cultured peripheral blood mononuclear cell respiratory burst activity. J Immunol **138**:3907–3912, 1987.
41. Nagy JT, Foris G, Fulop T, Paragh G, Plotnikoff N. Activation of the lipoxygenase pathway in the methionine enkephalin-induced respiratory burst in human polymorphonuclear leukocytes. Life Sci **42**:2299–2306, 1988.
42. Mazzone A, Ricevuti G, Pasotti D, Fioravanti A, Marcoli M, Lecchini S, Notario A, Frigo GM. Peptide opioids and morphine effects on inflammatory process. Inflammation **14**:717–726, 1990.
43. Van Epps DE, Kutvirt SL. Modulation of human neutrophil adherence by β-endorphin and met-enkephalin. J Neuroimmunol **15**:219–228, 1987.
44. Prieto J, Subira ML, Castilla A, Arroyo JL, Serrano M. Opioid peptides modulate the organization of vimentin filaments, phagocytic activity, and expression of surface molecules in monocytes. Scand J Immunol **29**:391–398, 1989.
45. Brown SL, Van Epps DE. Opioid peptides modulate production of interferon γ by human mononuclear cells. Cell Immunol **103**:19–26, 1986.
46. Mandler RN, Biddison WE, Mandler R, Serrate SA. β-endorphin augments the cytolytic activity and interferon production of natural killer cells. J Immunol **136**:934–939, 1986.
47. Brummitt CF, Sharp BM, Bekker G, Keane WF, Peterson PK. Modulatory effects of β-endorphin on interferon-γ production by cultured peripheral blood mononuclear cells: Heterogeneity among donors and the influence of culture medium. Brain Behav Immun **2**:187–197, 1988.
48. Gilmore W, Weiner LP. β-endorphin enhances interleukin-2 (IL-2) production in murine lymphocytes. J Neuroimmunol **18**:125–138, 1988.
49. Bessler H, Sztein MB, Serrate SA. β-endorphin modulation of IL-1-induced IL-2 production. Immunopharmacology **19**:5–14, 1990.
50. Apte RN, Durum SK, Oppenheim JJ. Opioids modulate interleukin-1 production and secretion by bone-marrow macrophages. Immunol Lett **24**:141–148, 1990.
51. Carr DJJ, Klimpel GR. Enhancement of the generation of cytotoxic T cells by endogenous opiates. J Neuroimmunol **12**:75–87, 1986.
52. Rowland RRR, Chukwuocha R, Tokuda S. Modulation of the in vitro immune response by met-enkephalin. Brain Behav Immun **1**:342–349, 1987.
53. Marotti T, Suerko V, Hrsak I. Modulation of superoxide anion release from human polymorphonuclear cells by met- and leu-enkephalin. Brain Behav Immun **4**:13–22, 1990.
54. Millar DB, Hough CJ, Mazorow DL, Gootenberg JE. β-endorphin's modulation of lymphocyte proliferation is dose, donor, and time-dependent. Brain Behav Immun **4**:232–242, 1990.
55. Plotnikoff NP, Miller GC, Nimeh N, Wybran J. Methionine-enkephalin (MEK): Enhancement of cytotoxic T cells in ARC patients. Presented at the Fourth International Conference on AIDS, Stockholm, Sweden, June 12–16, 1988.
56. Smith EM, Blalock JE. Human lymphocyte production of corticotropin and endorphin-like substances: Association with leukocyte interferon. Proc Natl Acad Sci USA **78**:7530–7534, 1981.
57. Lolait SJ, Lim ATW, Toh BH, Funder JW. Immunoreactive β-endorphin in a subpopulation of mouse spleen macrophages. J Clin Invest **73**:277–280, 1984.
58. Harbour-McMenamin D, Smith EM, Blalock JE. Bacterial lipopolysaccharide induction of leukocyte-derived corticotropin and endorphins. Infect Immun **48**:813–817, 1985.
59. Petrov RV, Mikhailova SS, Zakharova LA, Vasilenko AM, Katlinsky AV. Myelopeptides-bone marrow mediators with immunostimulating and endorphin-like activity. Scand J Immunol **24**:237–243, 1986.
60. Smith EM, Morrill AC, Meyer WJ III, Blalock JE. Corticotropin releasing factor induction of leukocyte-derived immunoreactive ACTH and endorphins. Nature **322**:881–882, 1986.
61. Kavelaars A, Berkenbosch F, Croiset G, Ballieux RE, Heijnen CJ. Induction of β-endorphin secretion by lymphocytes after subcutaneous administration of corticotropin releasing factor. Endocrinology **126**:759–764, 1990.
62. Padros MR, Vindrola O, Zunszain P, Fainboin L, Finkielman S, Nahmod VE. Mitogenic activation of the human lymphocytes induces the release of proenkephalin-derived peptides. Life Sci **45**:1805–1811, 1989.
63. Kavelaars A, Ballieux RE, Heijnen CJ. The role of IL-1 in the corticotropin-releasing factor and arginine-vasopressin-induced secretion of immunoreactive β-endorphin by human peripheral blood mononuclear cells. J Immunol **142**:2338–2342, 1989.
64. Carr DJJ, DeCosta BR, Jacobson AE, Rice KC, Blalock JE. Corticotropin-releasing hormone augments natural killer cell activity through a naloxone-sensitive pathway. J Neuroimmunol **28**:53–61, 1990.
65. Endo Y, Sakato T, Watanabe S. Identification of proopiomelanocortin-producing cells in the rat pyloric antrum and duodenum by in situ mRNA-cDNA hybridization. Biomed Res **6**:253–256, 1985.
66. Westly HJ, Kleiss AJ, Kelly KW, Wong PKY, Yuen P-H. Newcastle disease virus-infected splenocytes express the proopiomelanocortin gene. J Exp Med **163**:1589–1594, 1986.
67. Oates EL, Allway GP, Armstrong GR, Boyajian RA, Kehil JH, Prabhakar BS. Human lymphocytes produce pro-opiomelanocortin gene-related transcripts. J Biol Chem **263**:10041–10044, 1988.
68. Buzzetti R, McLoughlin L, Lavender PM, Clark AJL, Rees LH. Expression of pro-opiomelanocortin gene and quantification of

adrenocorticotropic hormone-like immunoreactivity in human normal peripheral mononuclear cells and lymphoid and myeloid malignancies. J Clin Invest **83**:733–737, 1989.

69. Zurawski G, Benedik M, Kamb BJ, Abrams JS, Zurawski SM, Lee FD. Activation of mouse T-helper cells induces abundant preproenkephalin mRNA synthesis. Science **232**:772–775, 1986.
70. Martin J, Prystowsky MB, Angeletti RH. Preproenkephalin mRNA in T-cells, macrophages, and mast cells. J Neurosci Res **18**:82–87, 1987.
71. Rosen H, Behar O, Abramsky O, Ovadia H. Regulated expression of proenkephalin A in normal lymphocytes. J Immunol **143**:3703–3707, 1989.
72. Linner KM, Beyer HS, Sharp BM. Induction of the messenger ribonucleic acid for proenkephalin A in cultured murine CD4-positive thymocytes. Endocrinology **128**:717–724, 1991.
73. Roth KA, Lorenz RG, Unahue RA, Weaver CT. Nonopiate active proenkephalin-derived peptides are secreted by T helper cells. FASEB J **3**:2401–2407, 1989.
74. Galin FS, LeBoeuf RD, Blalock JE. Corticotropin-releasing factor upregulates expression of two truncated pro-opiomelanocortin transcripts in murine lymphocytes. J Neuroimmunol **31**:51–58, 1991.
75. Galin FS, LeBoeuf RD, Blalock JE. A lymphocyte mRNA encodes the adrenocorticotropin/β-lipoprotein region of the pro-opiomelanocortin gene. Progr Neuroendocrinol **3**:205–208, 1990.
76. Lolait SJ, Clements JA, Markwick AJ, Cheng C, McNally M, Smith AI, Funder JW. Pro-opiomelanocortin messenger ribonucleic acid and posttranslational processing of beta endorphin in spleen macrophages. J Clin Invest **77**:1776–1779, 1986.
77. Harbour DV, Smith EM, Blalock JE. Novel processing pathway for proopiomelaocortin in lymphocytes: Endotoxin induction of a new prohormone-cleaving enzyme. J Neurosci Res **18**:95–101, 1987.
78. Stefano GB, Shipp MA, Scharrer B. A possible immunoregulatory function for [Met]-enkephalin-Arg^6-Phe^7 involving human and invertebrate granulocytes. J Neuroimmunol **31**:97–104, 1991.
79. Harbour DV, Smith EM, Blalock JE. Splenic lymphocyte production of an endorphin during endotoxic shock. Brain Behav Immun **1**:123–133, 1987.
80. Stein C, Hassan AHS, Przewloci R, Gramsch C, Peter K, Herz A. Opioids from immunocytes interact with receptors on sensory nerves to inhibit nociception in inflammation. Proc Natl Acad Sci USA **87**:5935–5939, 1990.
81. Pert CB, Snyder SH. Opiate receptors: Its demonstration in nervous tissue. Science **179**:1011–1014, 1973.
82. Simon EJ, Hiller JM, Edelman I. Stereospecific binding of the potent narcotic analgesic [^{3}H]etorphine to rat brain homogenates. Proc Natl Acad Sci USA **70**:1947–1949, 1973.
83. Terenius L. Stereospecific interaction between narcotic analgesics and a synaptic plasma membrane fraction of rat cerebral cortex. Acta Pharmacol Toxicol **32**:377–384, 1973.
84. Lord JAH, Waterfield AA, Hughes J, Kosterlitz HW. Endogenous opioid peptides: Multiple agonists and receptors. Nature **267**:495–498, 1977.
85. Loh HH, Tseng LF, Wei E, Li CH. β-endorphin is a potent analgesic agent. Proc Natl Acad Sci USA **73**:2895–2898, 1976.
86. Koski G, Klee WA. Opiates inhibit adenylate cyclase by stimulating GTP hydrolysis. Proc Natl Acad Sci USA **78**:4309–4313, 1981.
87. Ueda H, Harada H, Nozaki M, Katada T, Ui M, Satoh M, Takagi H. Reconstitution of rat brain μ opioid receptors with purified guanine nucleotide-binding regulatory proteins Gi and Go. Proc Natl Acad Sci USA **85**:7013–7017, 1988.
88. Allgaier C, Daschmann B, Sieverling J, Hertting G. Presynaptic β-opioid receptors on noradrenergic nerve terminals couple to G proteins and interact with the α_2-adrenoceptors. J Neurochem **53**:1629–1635, 1989.
89. Simonds WF. The molecular basis of opioid receptor function. Endocrine Rev **9**:200–212, 1988.
90. Gioannini T, Foucaud B, Hiller JM, Hatten ME, Simon EJ. Lectin binding of solubilized opiate receptors: Evidence for their glycoprotein nature. Biochem Biophys Res Commun **105**:1128–1134, 1982.
91. Kamikubo K, Murase H, Muragama M, Matsuda M, Miura K. Evidence for disulfide bonds in membrane-bound and solubilized opioid receptors. J Neurochem **50**:503–509, 1988.
92. Lee NM, Smith AP. A protein-lipid model of the opiate receptor. Life Sci **26**:1459–1464, 1980.
93. Hazum E, Chang K-J, Cuatrecases P. Specific nonopiate receptors for β-endorphin. Science **205**:1033–1035, 1979.
94. Schweigerer L, Schmidt W, Teschemacher H, Gramsch C. β-endorphin: Surface binding and internalization in thymoma cells. Proc Natl Acad Sci USA **82**:5751–5755, 1985.
95. Shahabi NA, Linner KM, Sharp BM. Murine splenocytes express a naloxone-insensitive binding site for β-endorphin. Endocrinology **126**:1442–1448, 1990.
96. Shahabi NA, Peterson PK, Sharp BM. β-endorphin binding to naloxone-insensitive sites on a human mononuclear cell line (U937): Effects of cations and guanosine triphosphate. Endocrinology **126**:3006–3015, 1990.
97. Hemmick LM, Bidlack JM. β-endorphin modulation of mitogen-stimulated calcium uptake by rat hymocytes. Life Sci **41**:1971–1978, 1987.
98. Carr DJJ, Bubien JK, Woods WT, Blalock JE. Opioid receptors on murine splenocytes. Possible coupling to K$^+$ channels. Ann NY Acad Sci **540**:694–697, 1988.
99. Yeung CWT. Photoaffinity labeling of opioid receptor of rat brain membranes with ^{125}I(D-Ala^2,p-N$_3$-Phe^4-Met^5)enkephalin. Arch Biochem Biophys **254**:81–91, 1987.
100. Lopker A, Abood LG, Hoss W, Lionetti FJ. Stereoselective muscarinic acetylcholine and opiate receptors in human phagocytic leukocytes. Biochem Pharmacol **29**:1361–1365, 1980.
101. Falke NE, Fischer EG, Martin R. Stereospecific opiate binding in living human polymorphonuclear leukocytes. Cell Biol Int Rep **9**:1041–1047, 1985.
102. Ausiello CM, Roda LG. Leu-enkephalin binding to cultured human T lymphocytes. Cell Biol Int Rep **8**:97–106, 1984.
103. Mehrishi JN, Mills IH. Opiate receptors on lymphocytes and platelets in man. Clin Immunol Immunopathol **27**:240–249, 1983.
104. Fiorica E, Spector S. Opioid binding site in EL-4 thymoma cell line. Life Sci **42**:199–206, 1988.
105. Carr DJJ, DeCosta BR, Kim C-H, Jacobson AE, Guarcello V, Rice KC, Blalock KE. Opioid receptors on cells of the immune system: Evidence for δ- and κ-classes. J Endocrinol **122**:161–168, 1989.
106. Carr DJJ, DeCosta BR, Jacobson AE, Rice KC, Blalock JE. Enantioselective kappa opioid binding sites on the macrophage cell line, P388d$_1$. Life Sci **49**:45–51, 1991.
107. Ovadia H, Nitsan P, Abramsky O. Characterization of opiate binding sites on membranes of rat lymphocytes. J Neuroimmunol **21**:93–102, 1989.
108. Levay G, Gaspar E, Nyakas C, Endroczi E. Age-related changes in adrenocorticoid and opioid receptor capacity of thymus-derived lymphocytes in rats. Exp Clin Endocrinol **94**:262–268, 1989.
109. Carr DJJ, Blalock JE. A molecular basis for intersystem communication between the immune and neuroendocrine systems. Int Rev Immunol **4**:213–228, 1989.
110. Carr DJJ. Neuroendocrinimmology: Intersystem communication by the immune and neuroendocrine systems. In: Muller

EE, MacLeod RM, Eds. Neuroendocrine Perspectives. New York: Springer-Verlag, Vol **9**: p163, 1991.

111. Carr DJJ, Bost KL, Blalock JE. An antibody to a peptide specified by an RNA that is complementary to γ-endorphin mRNA recognizes an opiate receptor. J Neuroimmunol **12**:329–337, 1986.
112. McLawhon RW, Ellory JC, Dawson G. Molecular size of opiate (enkephalin) receptors in neuroblastoma-glioma hybrid cells as determined by radiation inactivation analysis. J Biol Chem **258**:2102–2105, 1983.
113. Zukin RS, Kream RM. Chemical crosslinking of a solubilized enkephalin macromolecular complex. Proc Natl Acad Sci USA **76**:1593–1597, 1979.
114. Carr DJJ, Kim C-H, DeCosta B, Jacobson AE, Rice KC, Blalock JE. Evidence for a δ-class opioid receptor on cells of the immune system. Cell Immunol **116**:44–51, 1988.
115. Carr DJJ, DeCosta B, Jacobson AE, Bost KL, Rice KC, Blalock JE. Immunoaffinity-purified opiate receptor specifically binds the δ-class opiate receptor ligand, cis-(+)-3-methylfentanylisothiocyanate, SUPERFIT. FEBS Lett **224**:272–276, 1987.
116. Portoghese PS. Bivalent ligands and the message-address concept in the design of selective opioid receptor antagonists. TIPS **10**:230–235, 1989.
117. Bowen WD, Gentleman S, Herkenham M, Pert CB. Interconcerting μ and δ forms of the opiate receptor in rat striatal patches. Proc Natl Acad Sci USA **78**:4818–4822, 1981.
118. Rothman RB, Long JB, Bykov V, Jacobson AE, Rice KC, Holaday JW. β-FNA binds irreversibly to the opiate receptor complex: In vivo and in vitro evidence. J Pharmacol Exp Ther **247**:405–416, 1988.
119. Schoffelmeer ANM, Rice KC, Heijna NJ, Hogenboom F, Mulder AH. Fentanyl isothiocyanate reveals the existence of physically associated μ- and δ-opioid receptors mediating inhibition of adenylate cyclase in rat neostriatum. Eur J Pharmacol **149**:179–182, 1988.
120. Schoffelmeer ANM, Yao Y-H, Gioannini TL, Hiller JM, Ofri D, Roques BP, Simon EJ. Crosslinking of human [^{125}I]β-endorphin to opioid receptors in rat striatal membranes: Biochemical evidence for the existence of a mu/delta opioid receptor complex. J Pharmacol Exp Ther **253**:419–426, 1990.
121. Simon J, Benylie S, Hepp J, Varga E, Medrihradszky K, Borsodi A, Wollemann M. Method for isolation of kappa-opioid binding sites by dynorphin affinity chromatography. J Neurosci Res **25**:549–555, 1990.
122. Maderspach K, Nemeth K, Simon J, Benyhe S, Szucs M, Wollemann M. A monoclonal antibody recognizing κ- but not μ- and δ-opioid receptors. J Neurochem **56**:1897–1904, 1991.
123. Carr DJJ, DeCosta BR, Kim C-H, Jacobson AE, Bost KL, Rice KC, Blalock JE. Anti-opioid receptor antibody recognition of a binding site on brain and leukocyte opioid receptors. Neuroendocrinology **51**:552–560, 1990.
124. Cho TM, Hasegawa J-I, Ge B-L, Loh HH. Purification to apparent homogeneity of a mu-type opioid receptor from rat brain. Proc Natl Acad Sci USA **83**:4138–4142, 1986.
125. Cabon F, Cupo A, Ruiz-Gayo M, Baumann NA, Zalc B. Pharmacological and molecular properties of opioid binding sites synthesized in a cell-free translation system. J Neurosci Res **26**:242–250, 1990.
126. Scheideler MA, Zukin RS. Reconstitution of solubilized delta-opioid receptor binding sites in lipid vesicles. J Biol Chem **265**:15176–15182, 1990.
127. Remmers AE, Nordby G, Meczihradsky F. Modulation of opioid receptor binding by cis and trans fatty acids. J Neurochem **55**:1993–2000, 1990.
128. Foris G, Medgyesi GA, Hauck M. Bidirectional effect of met-enkephalin on macrophage effector functions. Mol Cell Biochem **69**:127–137, 1986.
129. Carr DJJ, Bost KL, Blalock JE. The production of antibodies which recognize opiate receptors on murine leukocytes. Life Sci **42**:2615–2624, 1988.
130. Fulop T, Kekessy D, Foris G. Impaired coupling of naloxone sensitive opiate receptors to adenylate cyclase in PMNLs of aged male subjects. Int J Immunopharmacol **9**:651–657, 1987.
131. Kavellars A, Ballieux RE, Heijnen CJ. Differential effects of β-endorphin on cAMP levels in human peripheral blood mononuclear cells. Brain Behav Immun **4**:171–179, 1990.
132. Hough CJ, Halperin JI, Mazorow DL. Yeandle SL, Millar DB. β-endorphin modulates T-cell intracellular calcium flux and c-myc expression via a potassium channel. J Neuroimmunol **27**:163–171, 1990.
133. Carr DJJ, Blalock JE. Neuroendocrine characteristics of the immune system. EOS-J Immunol Immunopharmacol **9**:195–199, 1989.
134. Kay NE, Morley JE, Allen JI. Interaction between endogenous opioids and IL-2 on PHA-stimulated human lymphocytes. Immunology **70**:485–491, 1990.
135. Hadjipetrou-Kourounakis L, Karagounis E, Rekka E, Kounounakis P. Immunosuppression by a novel analgesic-opioid agonist. Scand J Immunol **29**:449–458, 1989.
136. Wiedermann CJ. Interleukin-1 interaction with neuroregulatory systems: Selective enhancement by recombinant human and mouse interleukin-1 of in vitro opioid peptide receptor binding in rat brain. J Neurosci Res **22**:172–180, 1989.
137. Stefano GB, Smith EM, Hughes TK. Opioid induction of immunoreactive interleukin-1 in *Mytilus edulis* and human immunocytes: an interleukin-1-like substance in intervebrate neural tissue. J Neuroimmunol **32**:29–34, 1991.