Chapter

Immune Cell Activation: Stimulation, Costimulation, and Regulation of Cellular Activation

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Abstract

Opiate receptor (uOR) is expressed in central nervous system, gastrointestinal tract, male and female reproductive tissues, and immune cells. Morphine, a ligand for opioid receptor family, is known to activate the hypothalamic-pituitary-adrenal axis and release immunosuppressive glucocorticoids. Herein we present that minor changes, in the form of nonsynonymous single nucleotide polymorphisms, in μOR have cumulative impact on receptor-mediated signaling and functions of specific cell type(s). Significant reduction was seen in cells in M and S phases with coactivation of immune receptors with μOR. Flow cytometry-based experiments established a reduction in B and T lymphocytes, NK cells, and macrophages. Differences in types of immune cells were found to be significant to reduce immune response(s) mounted by GG(mutant allele)-bearing individuals. This is the first report on cross-talk between LPS-binding and μOR, explaining the reduction in the number of T and B cells after chronic opiate use and also the association of this impact on immunocytes with functional SNP, rs1799972/118G allele of OPRM1 gene as an explanation for the immune suppression in opiate users. Initially present lower cell titers can be further lowered by exogenous opiates and account for immunosuppression seen in chronic opiate users or after long-term treatment with opiate drugs for chronic pain.

Keywords: immune response activation, costimulation, cellular activation, opioid receptors

1. Immune system: a brief introduction

The immune system is a complex and highly developed system, yet its mission is simple: to seek and kill intruders. It is the body's defense system against infectious organisms and other invaders. The purpose of the immune system is to keep infectious microorganisms, such as bacteria, viruses, and fungi, out of the body, and to destroy any infectious microorganisms that do invade the body. Through a series of steps called the immune response, the immune system protects us against invading organisms. It is a network of cells, tissues, and organs working together to protect the body. The most important cell types involved in immune response are white blood cells, which come in two basic types that combine to seek out and destroy disease-causing organisms. Leukocytes are produced or stored in different locations in the body, like the thymus, spleen, bone marrow lymph nodes, and special deposits of lymphoid tissue (as in the gastrointestinal

Cell type	Location and function
Leukocytes	Derived from myeloid or lymphoid lineage, these are the main cells in immune system, which provide either innate or specific adaptive immunity. Myeloid cells include phagocytic, motile neutrophils, monocytes, and macrophages, providing the first-line defense against pathogens. Other myeloid cells involved in defense against parasites & in genesis of allergic reactions include eosinophils, basophils, & mast cells. Lymphocytes regulate the action of other leukocytes & generate specific responses to prevent chronic/recurrent infections [1]
B cells	In mammals, B cells mature in the bone marrow where as in birds, B cells mature in the bursa of Fabricius, a lymphoid organ first discovered by Chang and Glick [2]. They develop into antibody-secreting plasma cells. B cells express B cell receptors (BCRs) on their cell membrane that allows them to bind to specific antigen, against which it initiates a specific antibody response.
T cells	Originate in the bone marrow and mature in the thymus giving rise to helper, regulatory, cytotoxic T cells, or memory T cells. From the thymus, they migrate to peripheral tissues, blood, & lymphatic system. On stimulation, they secrete chemical messengers called cytokines, which stimulate the differentiation of B cells into antibody-producing cells also called plasma cells. Cytotoxic T cells in the presence of various cytokines bind to and kill infected and/or cancer cells.
T helper cells	Subset of T cells, found throughout the body, with especially high titers in lymphoid organs (lymph nodes and spleen), as well as the liver, lung, blood, and the intestinal tract. T helper TH or CD4+ T cells coordinate and regulate immunological responses. T_H cells mediate responses by secreting lymphokines that act on other cell types involved in mounting an immune response.
T cytotoxic cells	Subset of T cells, found throughout the body, with especially high titers in lymphoid organs (lymph nodes & spleen), as well as the liver, lung, blood, and the intestinal tract. T cytotoxic Tc or CD8+ T cells are involved in directly killing virus-infected cells, transplanted cells, and sometimes, eukaryotic parasites and tumor cells. CD8+ T cells have been shown to play a role in downregulating the immune response.
Natural Killer cells	NK cells are similar to Tc cells. They directly kill certain tumors such as melanomas, lymphomas and virus-infected cells- and clear herpes and cytomegalovirus-infected cells. In contrast to Tc cells, NK cells kill their target cells more effectively without the need for recognition of antigen in association with MHC molecules and are activated by secretions from T_H cells.
Macrophages	These are phagocytic cells and function as antigen-presenting cells (APCs) as they ingest foreign materials and present these to other members of the immune system such as T cells and B cells. Besides being the initiators of an immune response, they also act as immune modulators by secreting cytokines and can also be stimulated by lymphokines, to exhibit increased levels of phagocytosis.

Cell type	Location and function
Dendritic cells	Originate in the bone marrow and form another class of APCs. These are found in the lymphoid organs such as the thymus, lymph nodes, and spleen along with the bloodstream and other tissues. They function to capture and process antigens in lymphoid organs at the time of initiation of an immune response.
Neutrophils	These cells, which ingest, kill, and digest pathogens, are the most highly adherent and motile, phagocytic leukocytes; the first cell types to be recruited to acute inflammatory sites. Their functions are dependent on adherence molecule CD11b/CD18, or biochemical pathways, such as the respiratory burst associated with cytochrome b558.
Eosinophils	Defend against parasites and participate in hypersensitivity reactions. Their cytotoxicity is mediated through cytoplasmic granules containing eosinophilic basic and cationic proteins.
Basophils	Along with their tissue counterparts, mast cells produce cytokines required for defense against parasites and allergic inflammation. They display surface membrane receptors for IgE antibodies and possess cytoplasmic granules containing heparin and histamine. When cell-bound IgE antibodies are cross linked by antigens, the eosinophils degranulate releasing low-molecular weight vasoactive mediators (e.g., histamine), which mediate their biological effects.
Monocytes/macrophages	These are involved in phagocytosis and intracellular killing of microorganisms. Macrophages are differentiated monocytes, residing in the reticuloendothelial systems and act as antigen-presenting cells presenting processed peptides to T cells. They are recruited to inflammatory sites and further activated by exposure to certain cytokines, which potentiate their biologic functions.

Table 1.
Major cell types of adaptive immunity.

tract). These cells circulate through the body between the organs and nodes via lymphatic and blood vessels, and work in a coordinated manner to monitor the body for foreign invasions. A potent and active immune system is vital for staying healthy. The immune system differentiates between invaders and the body's own cells—when immune system is not able to differentiate between self and nonself, a reaction against "self" cells and molecules causes autoimmune disease. The immune system will remain active by getting enough sleep, exercise, and good nutrition.

Immune response leads to inflammation. The goal of inflammation is to get rid of the stimulus—both the disease-causing pathogens and/or neoplastic tissue. Significant steps involved in inflammation include recruitment of immune cells, interactions of these cells in the affected tissue and activation pattern of the interacting cells. The immune system continuously looks for pathogens, xenobiotics, and other nonself signals. Thus, the cell types of the immune system are incredibly dynamic and capable of upregulating processes required for handling these insults

on a time scale of minutes to hours. Several cell types in this system are capable of activation to secrete cytokines, rapidly proliferate, or otherwise communicate to surrounding cells that there is a pathogen to consider. Upon clearance of the pathogen, the cell population must contract in a controlled manner. Furthermore, in some cell populations (e.g., T cells), a subset of cells is retained as long-lived memory cells to protect and prime the system for future insults. Researchers are increasingly focused on early events in immune cell activation, where the response to an inflammatory signal can be tuned to impact overall cell function. In areas such as immuno-oncology, increased activation is connected to improve cell expansion whereas in the field of immunosuppression, the converse is desired. Clinical successes in targeting the immune system for treating cancer have generated a resurgence of effort to harness the immune system more routinely for therapeutic intervention (**Table 1**).

2. Mechanism of activation of adapted immunity

Adaptive immune responses carried out by lymphocytes are of two broad classes:

- antibody responses carried out by B cells
- cell-mediated immune responses carried out by T cells

During an immune response, the B cells are activated to secrete immunoglobulins, which circulate in blood, permeate to other body fluids, and bind specifically to foreign antigen that stimulated their production in the first place. This binding inactivates viruses and microbial toxins by blocking their interaction with the host cells. Antibody binding also marks invading pathogens for destruction by phagocytic cells of the innate immune system [3].

Cell-mediated reactions depend on direct interactions between T lymphocytes and cells bearing the antigen that the T cells recognize. T cells are specialized to recognize foreign antigens as peptide fragments bound to proteins of the major histocompatibility complex (MHC). The cytotoxic T cells recognize any infected cells with the help of viral antigens displayed on the surface of the infected cells [1]. Other T lymphocytes that activate the cells they recognize are marked by the expression of the cell-surface molecule CD4 on helper T cells. The CD4 T lymphocytes can be divided into two subsets, which carry out different functions by defending the body particularly from bacterial infections. Bacteria phagocytosed by macrophages are destroyed in the lysosomes, which contain several enzymes and antimicrobial substances. The intracellular bacteria, as in case of tuberculosis (Mtb), survive, because the vesicles they occupy do not fuse with the lysosomes. These infections are modified by a subset of CD4 T cells, namely TH1 cells, which activate macrophages, induce fusion of lysosomes and phagocytic vesicles containing the bacteria, and at the same time stimulate other antibacterial mechanisms of the phagocyte. CD4+ T cells play critical role during Mtb infection by mediating protection, contributing to inflammation, and regulating immune response. Th1 and Th17 cells are the main effector CD4+ T cells during Mtb. Th1 cells release cytokines and chemokines that attract phagocytes to the site of infection and impart protection from Mtb by secreting IFN-γ and activating antimycobacterial action in macrophages.

T cells not only destroy intracellular pathogens by killing infected cells and by activating macrophages, but they also have a central role in the destruction of extracellular pathogens by activating B cells. This is the specialized role of the second subset of CD4 T cells called TH2 cells with special properties that can activate naive B lymphocytes. Most antigens require an accompanying signal from helper T cells before they can stimulate B cells to proliferate and differentiate into cells secreting antibody. Cytotoxic T cells and TH1 cells interact with antigens produced by pathogens that have infected the target cell or that have been ingested by it. Helper T cells, in contrast, recognize and interact with B cells that have bound and internalized foreign antigen by means of their surface immunoglobulin.

Antigen-specific activation of effector T cells is aided by coreceptors that distinguish between the two classes of MHC molecule—CD8 coreceptor bearing cytotoxic cells that binds MHC class I molecules, whereas TH1 and TH2 cells express the CD4 coreceptor with specificity for MHC class II molecules. The maturation of T cells into either CD8 or CD4 T cells reflects the type of T-cell receptor specificity that occurs during development, and the selection of T cells that can receive survival signals from self-MHC molecules. On recognizing their targets, the three types of T cell are stimulated to release different sets of effector molecules namely, cytokines, which play crucial role in the clonal expansion of lymphocytes as well as in the innate immune responses.

T cells are thus crucially important for both humoral and cell-mediated responses of adaptive immunity. The adaptive immune response seems to have engrafted specific antigen recognition by highly diversified receptors onto innate defense systems, which have a central role in the effector actions of both B and T lymphocytes. The vital role of adaptive immunity in fighting infection is illustrated by the immunodeficiency and/or autoimmune diseases and the problems caused by pathogens that succeed in evading or subverting an adaptive immune response. The antigen-specific suppression of adaptive immune responses is the goal of treatment for important human diseases involving inappropriate activation of lymphocytes, whereas the specific stimulation of an adaptive immune response is the basis of successful vaccination for several childhood infections.

3. Opioid receptor and immune function

As early as 1987, Jankovic and Maric [4] showed that the neuropeptides—methionine-enkephalin, and leucine-enkephalin, exhibit a protective action against anaphylactic shock in rats sensitized to ovalbumin. Subsequent studies have shown that enkephalins can act both as suppressors and potentiators of immune response in a dose-dependent manner. Animal studies, where nutritional status, environmental influences, history of drug abuse, and genetic variability can be controlled more easily, have shown that morphine treatment results in significant immune deficits. Chronic morphine use has been shown to result in severe immunosuppression, posing as a significant risk factor for opportunistic infection [5], and this finding is also supported by epidemiological studies that show an increased prevalence of opportunistic infections in opiate users [6]. Chronic morphine has been shown to effect early reactions of innate immunity and later responses of adaptive immunity against microbes [7]. In addition, morphine has also been shown to affect the brain-immune axis by an IL-1β-dependent pathway [8]. Various studies support the idea that chronic morphine exposure in vivo attenuates lymphocyte proliferation [9], NK cell cytotoxicity [10], antibody and serum hemolysin formation [11], and phagocytic properties of peripheral mononuclear leukocytes [12]. Morphine exposure has also been shown to increase mortality of infected mice [12–14]. Novick et al. [15] showed that long-term abuse of opiates results in impaired NK cell activity and altered CD4+

and CD8+ T cell numbers. In animal models, parenteral use of opiates was shown to inhibit mitogenic and effector cell responses in both B and T cells [9, 16].

3.1 Opioid receptors in various immune cell types and their functional implications

Molecular biology studies have shown that immune cells differentially express opioid receptors (OR), and morphine affects their development, differentiation, and function [17]. Binding sites and protein expression for delta (δ) and kappa (κ) subclasses of G protein-coupled ORs [18, 19], in addition to gene expression of δ, κ, and μ subclasses [20, 21], have been described in leukocytes. Chuang et al. [20] reported the presence of mRNA for the μOR in human T- and B-cell lines, CD4+ T cells, monocytes, macrophages and granulocytes. Retinoid receptor activation increases the expression of the μOR in U937 cells, a mononuclear cell line [22]. Mu (μ-), Kappa (κ-), and Delta (δ-) opioids have been shown to possess chemoattractant activity and induce the chemotaxis of both monocytes and neutrophils [23–28]. Simpkins et al. [24] and Van Epps and Saland [25] showed that opiates, acting through δ and μ subclasses of OR expressed on human monocytes and neutrophils, are capable of inhibiting subsequent migratory responses to chemokines, and that this process of heterologous desensitization/trans-deactivation is associated with phosphorylation of chemokine receptors. Grimm et al. [26] showed that phagocytes respond chemotactically, with a chemotaxis index 2- to 2.5-fold higher than controls, to met-enkephalin and morphine, and this chemotaxis was inhibited by the OR antagonist naloxone. Liu et al. [27] demonstrated that pretreatment with opioids, including morphine, heroin, met-enkephalin, the selective μ-agonist DAMGO, or the selective δ-agonist [D-Pen2, D-Pen5] enkephalin (DPDPE), leads to the inhibition of the chemotactic response of leukocytes to complement-derived chemotactic factors. They also affect the chemokines macrophage inflammatory protein (MIP-1α)/CCL3, RANTES/CCL5, monocyte chemotactic protein-1 (MCP-1)/CCL2, and IL-8/CXCL8 [28]. Many investigators choose to study the effects of morphine on immune function because morphine has clinical applications and shows good affinity for all three types of ORs. However, use of morphine as the opioid of choice has limited the ability to delineate, which type of OR mediates the given immunological response/s due to binding to all receptor types.

Existence of a low-affinity, naloxone-insensitive morphine binding site on human peripheral blood macrophages has also been reported [29]. Opioid alkaloids, such as morphine and the endogenous peptides, including β-endorphin and dynorphin, directly modulate the function of lymphocytes and other cells involved in host defense and immunity, ORs preferentially bind to the (−)-enantiomer of most opioid alkaloids, for example, ORs will bind the antagonist (−)-naloxone but not (+)-naloxone [30].

3.2 Immunosuppression mediated by opiates

The role of opiate drugs in suppressing a variety of immunological endpoints such as proliferation, functions and responses of both T and B cells, and attenuation of the cytokine system has been studied extensively [31, 32]. Opiate drug administration has also been reported to suppress movement and number of white blood cells [33, 34]. Heroin use has been documented to depress E-rosette formation indicating clinical immune suppression [35]. Long-term use of opiate drugs has been reported to depress T cell reactivity and cause a loss of T helper (T_H) cells [36, 37], reduces T helper/T cytotoxic cell ratios, and decreases T helper cell function [38–41]. Use of opiate drugs produces atrophy of lymphoid organs, decreases lymphoid content, and alters antigen-specific antibody production [42, 43]. Opiate

addiction induces immunonutritional deficiencies [44, 45] and impairs immunoglobulin synthesis and function [46]. Naik et al. [47] showed a decrease of IgA levels and increase of IgG levels in Indian opiate users as compared to nonusers. Opioids bind directly on immune cells and modulate the function of these cells and also bind to classical ORs in the CNS, causing the release of catecholamines and/or steroids, which in turn also affect the immune cells. At the same time, morphine is known to activate the hypothalamic-pituitary-adrenal axis and release glucocorticoids, which are immunosuppressive in their own capacity [48].

3.3 Impact of functional polymorphism in OPRM1 gene on cell function

Several studies suggest that immune cells contain μORs along with existence of morphine binding sites differing from classical μORs, and measurements of the mRNAs that encode the neuronal types of OR show low levels of receptor mRNA in immune cells [49]. μOR is known to depict a total of 43 variants within coding and noncoding regions of the *OPRM1* gene, and 52 different haplotypes were predicted in the subgroup of African Americans. These haplotypes were classified by similarity clustering into functionally related categories, and one of these was significantly more frequent in substance-dependent individuals, viz. [−1793T-A, −1699insT, −1320A-G, −111C-T, +17C-T (+118A-G)], which was associated with substance dependence [50]. Studies evaluating the effects of 118A > G SNP on the intracellular signaling cascades resulting from μ-OR activation have shown conflicting results. Both DAMGO and morphine were twofold more potent in inhibiting calcium channel currents in sympathetic neurons transfected with the 118G allele than in neurons expressing the wild-type receptors [51]. However, Kroslak et al. [52] showed in HEK293 and AV-12 cells that stable expression of the 118G variant was associated with decreased agonist-mediated cyclic adenosine monophosphate (cAMP) signaling for morphine, methadone, and DAMGO, but not for β-endorphin. These results suggest that cellular environment may influence the phenotype associated with the variant receptor. Deb et al. [53], using murine neuroblastoma Neuro 2 A cells stably transfected with cDNA containing 118G variant, studied the effect on PKA, ERK, and CREB activation and documented no upregulation of PKA activity but a differential response of ERK phosphorylation in comparison to 118A variant, following chronic morphine treatment. Zhang et al. [54] analyzed 87 human brain tissue samples derived from autopsies and performed in vitro experiments on Chinese hamster ovary (CHO) cells, to show that the amount of mRNA transcribed from the 118G allele was twofold lower than the mRNA derived from the 118A allele. The levels of variant protein were ten-fold lower compared with those of the wild-type receptor. They also showed that after transfection into CHO cells with a cDNA representing only the coding region of *OPRM1* and inhibition of transcription with actinomycin D, the mRNA turnover was same for 118A and 118G variants. An *in silico* study by Pang et al. [55] showed that the substitution of the A with G at position 118 of the *OPRM1* gene abolishes three transcription factor binding sites, while creating a novel exon splice enhancer as well as p53 and a zinc finger protein binding sites, predicting a direct effect of 118A > G on gene expression and on the processing of heterogeneous nuclear RNA into mature mRNA. Huang et al. [56] described the role of the 118A > G SNP in posttranslational mechanisms suggesting that N-glycosylation may affect receptor expression, since it plays an important role in correct folding of receptors in the endoplasmic reticulum and, hence, their sorting from the endoplasmic reticulum to the plasma membrane. Huang et al. [56] also showed that the variant receptor had lower relative molecular mass than the wild-type one, which may be explained by a differential glycosylation status between the two receptors. Pulse-chain (or chase) experiments revealed that the

half-life of the mature form of the variant receptor (~12 h) was shorter than that of the wild-type receptor (~28 h) showing its effect on protein stability. Thus, several lines of evidence suggest that the 118G variant may affect *OPRM1* gene expression in addition to mRNA translation, posttranslational processing, or turnover of the μ-opioid receptor protein, which can all effect signaling pathway/s.

3.4 Epigenetics of OPRM1 gene and its impact on cell function

Human genome has about 45,000-C-phosphate-G-(CpG) islands, many in the promoter regions of genes. The CpG islands are located upstream of the transcription start site to within the first exon [57]. Nielsen et al. [58] and Chorbov et al. [59] reported that in DNA obtained from peripheral lymphocytes, two of 16 CpG sites in a region of *OPRM1* gene promoter had significantly higher methylation in former heroin addicts than in controls. These two CpG sites are located in binding sites for the potential Sp1 transcription factor. Oertel et al. [60] showed that substitution of an A with a G at gene position +118 introduces a new CpG-methylation site at position +117, which leads to enhanced methylation of *OPRM1* gene resulting in decreased expression. Using m-fold software, Johnson et al. [61] showed that 118G variant demonstrated an altered folding that could affect mRNA stability. The epigenetic mechanism reported by Oertel et al. [60] impedes μ-OR upregulation in brain tissue, and they concluded that while in wild-type subjects, a reduced signaling efficiency associated with chronic heroin exposure was compensated for by an increased receptor density; this upregulation was absent in carriers of the 118G receptor variant due to diminished *OPRM1* mRNA transcription. The *OPRM1* 118A > G SNP variant not only reduces μ-OR signaling efficiency, but by a genetic-epigenetic interaction, also reduces OR expression and therefore, depletes the opioid system of a compensatory reaction to chronic exposure, providing evidence that a change in the genotype can cause a change in the epigenotype with major functional consequences.

3.5 Receptor-receptor interactions

Oligomerization is a general characteristic of cell membrane receptors that is shared by G protein-coupled receptors (GPCRs). GPCRs do not exist in isolation and interact with components of the bilayer, such as lipids and sterols, as well as with other GPCRs to form dimers and higher order oligomers, which are of functional significance as this affects the ligand binding and signaling properties of GPCRs [62, 63]. Recent studies of these complexes, both in vivo and in purified reconstituted forms, unequivocally support this contention for GPCRs [64]. A large number of direct binding assays indicating negative or positive cooperativity suggest clustering of GPCRs [65]. Mansoor et al. [66] reported that GPCRs can come together in the presence of lipids. Oligomerization and the two monomers comprising a GPCR dimer could be nonequivalent, thereby allowing more refined regulation of GPCR activity [66–68]. Thus, GPCR dimerization can be the result of the receptors forming heterodimers as well as homodimers [69], with many dimers displaying modified pharmacology [70], altered responsiveness to viral entry through GPCRs [71], or attenuated signaling [72]. Therefore, it is apparent that the oligomeric potential of GPCRs allows further diversification of their repertoire as a result of more complex ligand-receptor relationships than envisioned for monomeric receptors due to a more complex ligand-receptor relationship [64]. Although monomeric GPCRs can activate G proteins, the pentameric structure constituted by one GPCR homodimer and one heterotrimeric G protein may constitute the functional unit, and oligomeric entities can be viewed as multiples of dimers [73].

3.6 Interactions of opioid receptors

Fluorescence correlation spectroscopy (FCS) studies suggest that μ-opioid receptors exist primarily as dimers that oligomerize with δ-opioid receptors into tetramers [74]. High-resolution crystallographic structures of the μ-opioid by Manglik et al. [75] showed that they exist as parallel dimers and/or tetramers. Some TM domains have been observed more often than others. TM5 and TM6 residues constituted the main interfaces for the μ-opioid receptor crystallized dimers, with extensive contacts throughout the length of these TM helices in μ-opioid receptor dimers. The μ-opioid dimers also showed a second, less prominent symmetric interface, involving TM1, TM2, and helix 8 (H8; the helix adjacent to TM7 running along the internal membrane surface [75]). For GPCRs, the majority of crystal structures that are currently available refer to antagonist-bound (inactive) structures. The inferred dimeric interfaces may, therefore, depend on specific conformational states. Furthermore, the TM5-TM6 interface inferred by the crystal structure of μ-opioid dimers could preclude either monomer from properly coupling to G protein, because the agonist-induced receptor-G protein interaction depends on rearrangements of TM5 and TM6 within the seven-helical domain bundle, suggesting that different receptor conformations stabilized with different ligands may also promote different dimeric interfaces [75]. Huang et al. [76] suggested that the comparison of the differences in the interfaces observed from the crystallized structures of the antagonist-bound μ-opioid and chemokine CXCR4 receptors and the ligand-free β1-adrenoceptor suggest that the TM5 interface can partner in the interaction with TM4 or TM6, depending on the conformation of the receptor.

In δ-μ-OR heteromers, it was shown that binding and signaling by morphine or μ receptor agonists were potentiated by δ-OR antagonists, and reciprocally, binding and signaling by δ-OR agonists were potentiated by μ receptor selective antagonists [77, 78]. Studies carried out with the δ-opioid-cannabinoid CB1 receptor heteromer have also revealed allosteric modulations of cannabinoid CB1 receptor ligands on δ-OR ligand binding properties [79, 80]. They also showed that in recombinant systems expressing both receptors, as well as endogenous tissues, binding and consequently signaling by δ-OR could be potentiated by a low, nonsignaling dose of cannabinoid CB1 receptor agonist or a selective antagonist. In the δ-μ-OR heteromers, Gupta et al. [81] showed that morphine-induction increases heteromer abundance. Similarly, the δ-opioid-CB1 receptor heteromer increases in the brain after peripherally elicited neuropathic pain [79]. Studies by Zheng et al. [82] have revealed a complex interplay among cholesterol, palmitate, receptor dimerization, and G protein activation. They showed that reducing cholesterol levels or preventing palmitoylation of the μOR reduced receptor dimerization and Gα association. Additionally, preventing palmitoylation reduced the association of μOR with cholesterol, suggesting a functional complex of receptor, palmitate, and cholesterol. The authors also demonstrate that mutagenesis of the palmitoylated cysteine residue in μOR has no effect on ligand binding but decreased signaling efficiency, probably by impairing GPCR-G protein association. The same mutant had significantly reduced dimerization, and it was proposed that this was responsible for the reduced G protein coupling. Zhang et al. [83] demonstrated that the palmitate-free mutant associated more weakly with cholesterol. A model of the μOR dimer in which cholesterol and palmitate pack together to facilitate receptor dimerization reveals that cholesterol interactions contribute approximately 25% of the total interaction energy at the homodimer interface [82].

3.7 Receptor dimerization and immune cell stimulation and functioning

Chemokines are chemotactic cytokines that mediate their effects on leukocytes through a number of G protein-coupled, seven transmembrane-spanning (STM) receptors [84]. Specificity is provided by patterns of receptor and G protein expression, ligand potency, and levels of receptor desensitization. Interactions among receptors are mediated through a process known as receptor cross regulation, or heterologous desensitization [85]. Ali et al. [86] showed that thrombin receptor activation causes phosphorylation of several chemoattractant receptors, including the IL-8 receptor CXCR1, the C5a receptor, and the receptor for platelet-activating factor 3. Ben-Baruch et al. [87] have shown that homologous desensitization through phosphorylation of the IL-8 receptor CXCR2 occurs in response to its native ligands IL-8 and neutrophil-activating peptide (NAP)-2. Grimm et al. [28] showed that opiates, acting through δ and μ subclasses of opioid receptors expressed on human monocytes and neutrophils, are capable of inhibiting subsequent migratory responses to chemokines, and that this process of heterologous desensitization or trans-deactivation is associated with phosphorylation of chemokine receptors. Szabo et al. [88] showed that the chemotactic activities of both μ- and δ-OR are desensitized following activation of the chemokine receptors CCR5, CCR2, CCR7, and CXCR4 but not of CXCR1 or CXCR2 receptors. The inhibition of CCL3 and CCL5 responses following opioid pretreatment is consistent with the desensitization of either CCR1 or CCR5 or both. This receptor cross talk results in heterologous desensitization and phosphorylation of some of the chemokine receptors, which subsequently contribute to the immunosuppressive effects of the opioids.

4. Immune receptors and their function

Cells of the immune system intercommunicate by ligand-receptor interactions between cells and/or via secreted molecules called cytokines. Cytokines produced by lymphocytes are termed lymphokines (i.e., interleukins & interferon-γ), and those produced by monocytes and macrophages are termed monokines [89]. The

	T cells	B cells	NK cells	Monocytes/ macrophages
Antigen recognition	+	+	–	–
Antigen presentation	–	+	–	+
Antibody production	–	+	–	–
Cellular immunity	+	–	–	+
Immune regulation	+	–	+	+
Phagocytosis	–	–	–	+
Cytotoxicity	+	–	+	+
Receptor	TcR	IgM & IgD	CD16	CD11b
Other surface markers	CD3, CD4, CD8	CD19–21	CD56	CD14
Mononuclear cells in blood (%)	~75	~10	10	5

Table 2.
Major features and functions of mononuclear leukocytes.

main receptors in the immune system are pattern recognition receptors (PRRs), Toll-like receptors (TLRs), killer activated and killer inhibitor receptors (KARs and KIRs), complement receptors, Fc receptors, and B & T cell receptors. Many are

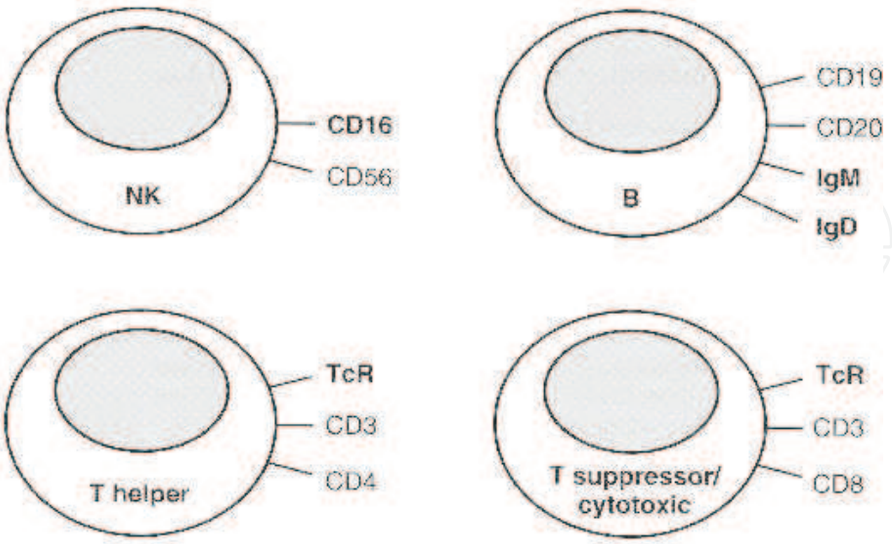

Figure 1.
Principal surface markers of lymphocyte populations.

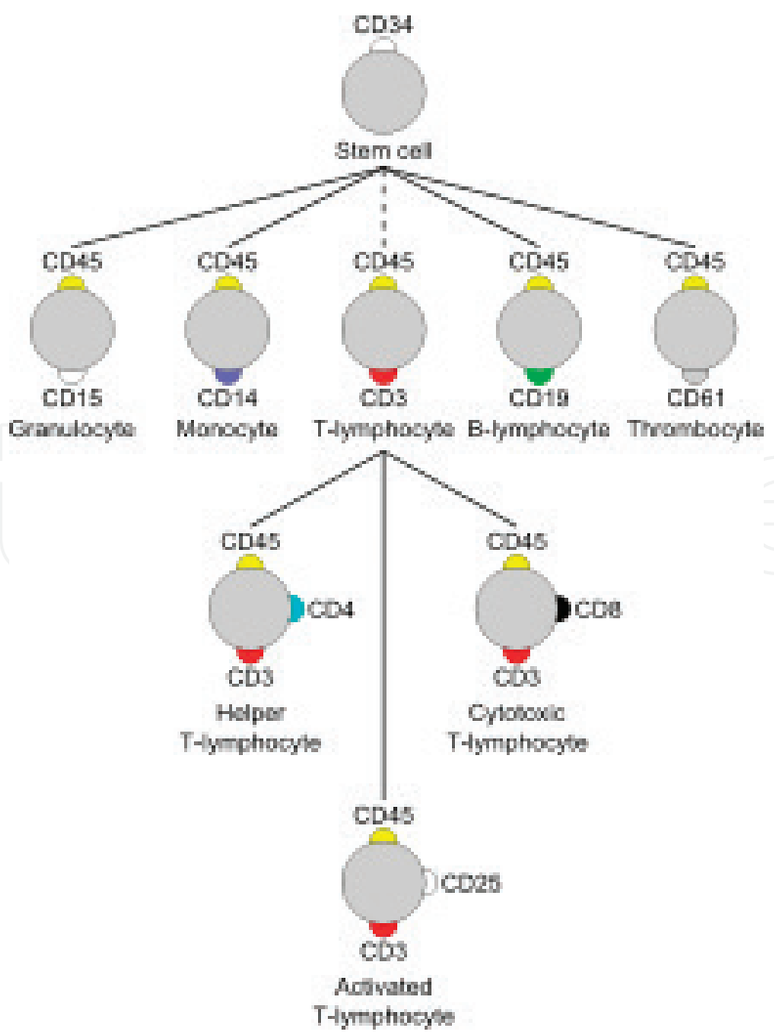

Figure 2.
Diagram showing CD markers on various immune cell types.

phagocytic receptors that stimulate ingestion of the pathogens they recognize. Some are chemotactic receptors, such as the f-Met-Leu-Phe receptor, which binds the N-formylated peptides produced by bacteria and guide neutrophils to sites of infection. A third function, which may be mediated by some of the phagocytic receptors as well as by specialized signaling receptors, is to induce effector molecules that contribute to induce innate immune responses and molecules that influence the initiation and nature of any subsequent adaptive immune response [1]. Various immune cell functions regulated by receptors on immunocytes are summarized in **Table 3** and **Figure 3**.

5. CD14 receptor coactivation with μOR: effect on cell division and NF-κB phosphorylation

LPS-mediated lymphocyte activation and effect of costimulation with opioid receptor agonists were studied by treating the cells with the μOR agonist DAMGO for time intervals of 5, 30, or 240 min. A549, a cell line having both CD14 and μOR, was used to study the effect on cell proliferation and NFκB phosphorylation. Treatment of A549 revealed that DAMGO was able to mitigate the LPS-mediated induction of phosphorylated NFκB after cotreatment for 4 h (**Figure 2**). Similarly, DAMGO was also able to suppress the cell proliferation by LPS, significantly reducing the percentage of cells in M-phase as well as in S-phase of the cell cycle (**Tables 2** and **3**).

Treatment	Cell population in M phase (%)	Paired T-test	Cell population in S-phase (%)	Paired T-test
LPS	6.07[1,2]		15.7[4, 5]	
DAMGO	4.83[1,3]	P < 0.0001[1]	10.5[4, 6]	p = 0.029[4]
LPS + DAMGO	3.89[2,3]	P < 0.0001[2], p = 0.0018[3]	7.99[5, 6]	p = 0.0001[5], p = 0.002[6]

[1]*NT vs. LPS.*

[2]*NT vs. DAMGO.*

[3]*NT vs. LPS + DAMGO.*

[4]*LPS vs. DAMGO.*

[5]*LPS vs. LPS + DAMGO.*

[6]*DAMGO vs. LPS + DAMGO.*

Table 3.
Effect of LPS and DAMGO on cell proliferation at 2 h after treatment.

6. Effect of μOR activation with a selective KOR agonist and μOR antagonist

The effect of coactivation of two different opioid receptors, U50488, a selective KOR agonist was used for lymphocyte activation, and effect of costimulation of μOR was achieved by using DAMGO, an opioid receptor agonist. Cells were treated with μOR agonists DAMGO for varying time intervals, and effect on cell proliferation and NF-κB phosphorylation was studied. Results using HepG2 revealed that U50488 (an agonist for KOR) was able to mitigate the effect of DAMGO, an agonist for μOR, and a significant reduction in the percentage of cells in both M-phase and S-phase of the cell cycle was observed at 2 and 24 h of treatment. U50488 alone also showed a larger percentage of cells in G0 phase both at 2 and 24 h in comparison with DAMGO, and the mitigation of U50488 induced effects indicating cross talk of receptors (**Figure 3**).

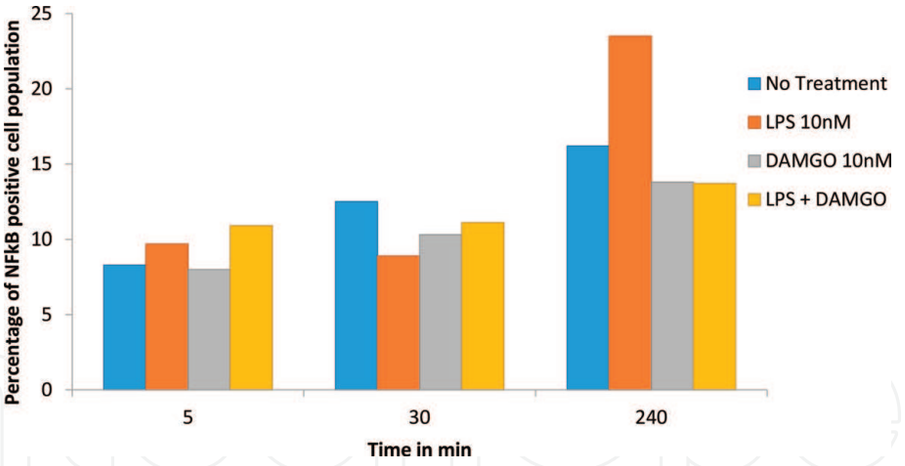

Figure 3.
Effect of cotreatment with agonist of OPRM1 (DAMGO) and agonist of CD-14 receptor (LPS) on NF-κB phosphorylation.

Treatment	Cell population in M phase (%)	Paired T-test	Cell population in S phase (%)	Paired T-test
DAMGO	26.66[1,2]		9.40[4,5]	
U50488	14.15[1,3]	$P < 0.0001$[1]	14.15[4,6]	$p < 0.001$[4]
U50488+ DAMGO	17.86[2,3]	$P < 0.0001$[2], $p < 0.0001$	8.07[5,6]	$p < 0.0001$[5] $p = 0.0002$[6]

1.[4]DAMGO vs U50488.
2.[5]DAMGO vs U50488 + DAMGO.
3.[6]U50488 vs U50488+ DAMGO.

Table 4.
Effect of U50488 and DAMGO on cell proliferation at 2 h after treatment.

Our studies show that U50488, agonist for κOR, was able to mitigate the effect of DAMGO, an agonist for MOR and a significant reduction in the percentage of cells in both M-phase and S-phase of cell cycle was observed at 2 and 24 h of treatment (**Table 4**).

7. Effect of μOR coactivation on cell proliferation

Receptor-receptor interactions and di/oligomerization are established pathways for altering response to any given ligand. As both homo- and heterodimers of μOR have been reported, it is speculated that dimerization of μOR may have a role in regulating receptor signaling. This intermolecular cross talk within receptor oligomers often results in allosterism between the different binding pockets of the individual monomers. Negative binding cooperativity has been observed for both GPCR homo- and heteromers using equilibrium binding and/or radio ligand dissociation [90].

Assays with TLR and μOR coactivation showed that agonist activation of μOR using DAMGO causes a significant lowering of LPS-induced cell division in cells coexpressing μOR and TLR. This has direct bearing on pathogen clearance as TLRs are present and expressed in sentinel cells such as macrophages and dendritic cells, which recognize structurally conserved molecules derived from microbes. These large phagocytes are found in essentially all tissues where they patrol for potential pathogens by amoeboid movement. These cells play a critical role in nonspecific

defense (innate immunity) and also help initiate specific defense mechanisms (adaptive immunity) by recruiting other immune cells such as lymphocytes. Where a pathogen is involved, monocytes are commonly preceded by neutrophils, which release a range of toxic agents designed to kill extracellular pathogens. The macrophages then clear out both the dead pathogens and the dead neutrophils. The process of recruitment of neutrophils and macrophages involves the activation of resident macrophages. Activated resident macrophages respond to local stimuli by producing cytokines that make the endothelial cell surface more sticky (through induction of increased expression of cell adhesion molecules such as P-selectin) and chemokines, that promote and direct migration of inflammatory cells. Macrophages treated with LPS express high levels of cyclin D2 [91]. The CD14 and TLR signaling funnels into the activation of NF-κB, AP-1 and IRF3 pathways via the ERK1/2 and MEK1/2 channel, which is also activated by μOR signaling. Both signaling pathways funnel into the MAPK cascade and hence effect the activation of NFκB. Preliminary data from our study using both A549 and HepG2 cells show a significant decrease in LPS-induced cell division in the simultaneous presence of ligands for μOR. Cell cycle arrest was observed primarily in the S and the M phase of cell cycle with a significant decrease under conditions of costimulation with both the receptor agonists. Cell cycle arrest observed as a result of receptor coactivation needs to be further explored as it can have important therapeutic implications as on one hand a particular signaling pathway or end point is associated with a therapeutic response, such as analgesic effect of opioids, while on the other hand it is associated with unwanted side effects, namely cell cycle suppression on exposure to LPS and opioids together.

Receptor heteromers, with their allosteric properties, give rise to a new kind of pharmacological target. The ability of one of the protomers to act as an allosteric modulator of the second protomer in the receptor heteromer gives the possibility of finding selective ligands for the protomer acting as conduit of the allosteric modulation [73]. As opioid drugs are used in more than one clinical condition understanding opioid receptor interactions with other GPCRs on the surface of different cell types can prove to be very beneficial in managing adverse, unwanted effects of these important therapeutic molecules. The immunomodulatory effects of morphine have been characterized both in animal and human studies and was found to decrease several functions of both natural and acquired immunity, interfering with important intracellular pathways involved in immune regulation [92]. Opiates namely morphine, heroin, fentanyl and methadone all induce immune-suppression and affect both innate and adaptive immunity defining a role of μOR in these functions.

8. Association of OPRM1 functional alleles with immune cell function

Sharad et al. [93] used a genetic approach to correlate a functional *OPRM1* gene polymorphism with known action of opiates on immunity and undertook a prospective study to understand the relationship of the 118G variation with the amount of exogenous opiates consumed and correlated the immunosuppressive effects of exogenous opiates with the *OPRM1* allele type. They studied the immune status of opiate users by measuring serum Ig (IgG and IgA) levels, in association with specific *OPRM1* genotype, and confirmed that the mean circulating levels of Ig were significantly lower in opiate users when compared with levels in cohort controls. Among opiate-dependent subjects, individuals with AA genotype were found to have the lowest levels of circulating immunoglobulins, both IgG and IgA ($p = 0.0001$), while the AG genotype carrying individuals had a higher level of both immunoglobulins. The homozygous GG genotype was in between the AA and AG genotypes. Alternatively, in opiate naïve subjects, the AA individuals showed the highest titers of

circulating IgG, and the GG individuals showed the lowest with AG having intermediate values [94]. The immunosuppressive effects documented in opiate naïve individuals can be attributed to altered regulation of PKA and pERK1/2 due to the levels of endogenous opioid. In addition to the absence of G genotype in the immortalized cell lines and based on the cell culture data showing cell cycle arrest observed in the present study (in A549 and HepG2 cell lines), we hypothesize that coactivation of μOR in presence of 118G allele leads to a suppression/arrest of cell division.

To test this hypothesis, another pilot study was carried out in which healthy opiate naïve volunteers were enrolled and the cell count for circulating lymphocyte subsets was studied as a measure of immune competence. Genotypic association studies showed a correlation between the immune cell numbers. Total lymphocyte count showed a significant lowering in cell numbers in 118G-allele-bearing individuals when compared to 118A-bearing individuals. However, cell numbers in all individuals remained within the documented normal range of 500–4000 cell/ml. The GG allele individuals showed significantly lower cell count, averaging 490, which differed markedly from cell numbers observed in AA-bearing individuals with mean numbers of 1976 cells/ml, ($p = 0.008$) and a correlation factor, r^2 of 0.79 between the genotype and average cell numbers. Our data show a significant lowering in all immunocytes, namely leucocyte populations (CD45+ve cells), B lymphocytes (CD10+ve cells), T lymphocytes (CD3, CD4, and CD8+ve cells), NK cells (CD56+ve cells), activated monocytes (CD 11b+ve cells), and mesenchymal progenitors in GG-bearing individuals when compared to AA-allele-bearing individuals but not always in comparison with those bearing the AG allele. This baseline lowering of cell numbers in GG-bearing individuals supports the hypothesis that GG genotype suppresses cell division, and since mounting of a successful immune response and/or overstimulation of immune system, as in case of patients with autoimmune disorders, depends on activation of both innate and adaptive immune responses, the 118G-bearing individuals would be prone to immune suppression due to lack of amplification by selective cell division, a critical step in elimination of the pathogen or an autoimmune response to an antigen.

9. Conclusion and future perspectives

In conclusion, there is a significant correlation between the circulating number of lymphocytes T_H and Tc, B cells and NK cells, and the μOR allele present, and this difference can be further increased in the presence of exogenous opioids either during clinical treatment or substance dependence, as the 118G allele affects the process of cell division arresting cells at the S or M phase of the cell cycle, or by modulating the action of cell division–linked secretion of stimulating cytokines/chemokine known to induce clone-specific and cell type–specific proliferation, because of the propensity of opioid receptor to heterodimerize and to selectively bias the subsequent ligand engagement/s with the dimerized/oligomerized receptors. This "subliminal immune suppression" in G allele–bearing individuals can have far reaching impact on onset of diseases such as cancer and obesity (both have an element inflammation) and vaccination for infectious diseases and even dreaded diseases as cancer. This immune suppression will certainly lower the individual's risk for autoimmune disorders such as rheumatoid arthritis, lupus, etc. The relationship between the μOR-mediated cell signaling and impact of stimulation of MOR as partner receptors, which influences binding of the second ligand in immunocytes and thereby the outcome on immune cells function in mounting and regulating the immune response/s, needs more detailed molecular exploration.

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References

[1] Janeway CA Jr, Travers P, Walport M, et al. Receptors of the innate immune system. In: Immuno-biology: The Immune System in Health and Disease. 5th ed. New York: Garland Science; 2001. Available from: http://www.ncbi.nlm.nih.gov/books /NBK27129/

[2] Cooper MD, Peterson RD, Good RA. Delineation of the thymic and bursal lymphoid systems in the chicken. Nature. 1965;**205**:143-146

[3] Alberts B, Johnson A, Lewis J, et al. Chapter 24: The adaptive immune system. In: Molecular Biology of the Cell. 4th ed. New York: Garland Science; 2002. Available from: http://www.ncbi.nlm.nih.gov/books/NBK21070/

[4] Janković BD, Marić D. Enkephalins and immunity. I: In vivo suppression and potentiation of humoral immune response. Annals of the New York Academy of Sciences. 1987;**496**:115-125

[5] Wang J, Roderick Barke A, Sabita R. Transcriptional and epigenetic regulation of interleukin-2 Gene in activated T cells by morphine. The Journal of Biological Chemistry. 2007;**282**:7164-7171

[6] Vaswani M, Desai NG. HIV infection and high-risk behaviors in opioid dependent patients: The Indian context. Addictive Behaviors. 2004;**29**(8):1699-1705

[7] Sabita R, Jana N, Santanu B, Richard C, Subhas D, Raini D, et al. Opioid drug abuse and modulation of immune function: Consequences in the susceptibility to opportunistic infections. Journal of NeuroImmune Pharmacology. 2011;**6**(4):442-465

[8] Chang SL, Moldow RL, House SD, Zadina JE. Morphine affects the brain-immune axis by modulating an interleukin-1 beta dependent pathway. Advances in Experimental Medicine and Biology. 1996;**402**:35-42

[9] Bryant HU, Bernton EW, Holaday JW. Immunosuppressive effects of chronic morphine treatment in mice. Life Sciences. 1987;**41**(14):1731-1738

[10] Lefkowitz SS, Chiang CY. Effects of certain abused drugs on hemolysin forming cells. Life Sciences. 1975;**17**(12):1763-1767

[11] Güngör M et al. Effect of chronic administration of morphine on primary immune response in mice. Experientia. 1980;**36**(11):1309-1310

[12] Tubaro E, Borelli G, Croce C, Cavallo G, Santiangeli C. Effect of morphine on resistance to infection. The Journal of Infectious Diseases. 1983;**148**(4):656-666

[13] Chao CC, Sharp BM, Pomeroy C, Filice GA, Peterson PK. Lethality of morphine in mice infected with *Toxoplasma gondii*. The Journal of Pharmacology and Experimental Therapeutics. 1990;**252**(2):605-609

[14] Fecho K, Manning EL, Maixner W, Schmitt CP. Effects of carrageenan and morphine on acute inflammation and pain in Lewis and Fischer rats. Brain, Behavior, and Immunity. 2007;**21**(1):68-78

[15] Novick DM, Ochshorn M, Ghali V, Croxson TS, Mercer WD, Chiorazzi N, et al. Natural killer cell activity and lymphocyte subsets in parenteral heroin abusers and long-term methadone maintenance patients. The Journal of Pharmacology and Experimental Therapeutics. 1989;**250**(2):606-610

[16] Taub DD, Eisenstein TK, Geller EB, Adler MW, Rogers TJ. Immunomodulatory activity of mu- and kappa-selective opioid agonists. Proceedings of the National

Academy of Sciences of the United States of America. 1991;**88**(2):360-364

[17] Roy JD, Massicotte L, Sassine MP, Seal RF, Roy A. A comparison of intrathecal morphine/fentanyl and patient-controlled analgesia with patient-controlled analgesia alone for analgesia after liver resection. Anesthesia and Analgesia. 2006;**103**(4):990-994

[18] Carr DJ, Kim CH, de Costa B, Jacobson AE, Rice KC, Blalock JE. Evidence for a delta-class opioid receptor on cells of the immune system. Cellular Immunology. 1988;**116**(1):44-51

[19] Carr DJ, DeCosta BR, Kim CH, Jacobson AE, Guarcello V, Rice KC, et al. Opioid receptors on cells of the immune system: Evidence for delta- and kappa-classes. The Journal of Endocrinology. 1989;**122**(1):161-168

[20] Chuang TK, Killam KF, Chuang LF, Kung HF, Sheng WS, Chao CC, et al. Mu opioid receptor gene expression in immune cells. Biochemical and Biophysical Research Communications. 1995;**216**(3):922-930

[21] Chao CC, Gekker G, Hu S, Sheng WS, Shark KB, Bu DF, et al. Kappa opioid receptors in human microglia downregulate human immunodeficiency virus 1 expression. Proceedings of the National Academy of Sciences of the United States of America. 1996;**93**(15):8051-8056

[22] Royal W 3rd, Leander MV, Bissonnette R. Retinoid-induced mu opioid receptor expression by phyto-hemagglutinin-stimulated U937 cells. Journal of Neurovirology. 2005;**11**(2):157-165

[23] Wetzel MA, Steele AD, Eisenstein TK, Adler MW, Henderson EE, Rogers TJ. μ-Opioid induction of monocyte chemoattractant protein-1, RANTES, and IFN-γ-inducible protein-10 expression in human peripheral blood mononuclear cells. The Journal of Immunology. 2000;**165**(11):6519-6524

[24] Simpkins CO, Dickey CA, Fink MP. Human neutrophil migration is enhanced by beta-endorphin. Life Sciences. 1984;**34**(23):2251-2255

[25] Van Epps DE, Saland L. Beta-endorphin and met-enkephalin stimulate human peripheral blood mononuclear cell chemotaxis. Journal of Immunology. 1984;**132**(6):3046-3053

[26] Grimm MC, Ben-Baruch A, Taub DD, Howard OMZ, Resau JH, Wang JM, et al. Opiates Trans-deactivate Chemokine Receptors: δ and μ Opiate Receptor–mediated Heterologous Desensitization. The Journal of Experimental Medicine. 1998;**188**(2):317-325

[27] Liu Y, Blackbourn DJ, Chuang LF, Killam KF Jr, Chuang RY. Effects of in vivo and in vitro administration of morphine sulfate upon rhesus macaque polymorphonuclear cell phagocytosis and chemotaxis. The Journal of Pharmacology and Experimental Therapeutics. 1992;**263**(2):533-539

[28] Ruff MR, Wahl SM, Mergenhagen S, Pert CB. Opiate receptor-mediated chemotaxis of human monocytes. Neuropeptides. 1985;**5**(4-6):363-366

[29] Makman MH, Dvorkin B, Stefano G. Murine macrophage cell lines contain mu 3-opiate receptors. European Journal of Pharmacology. 1995;**273**(3):R5-R6

[30] Bowdle TA. Adverse effects of opioid agonists and agonists-antagonists in anesthesia. Drug Safety. 1998;**19**:173-189

[31] Brown SM, Stimmel B, Taub RN, Kochwa S, Rosenfield RE. Immunologic dysfunction in heroin addicts. Archives of Internal Medicine. 1974;**134**(6):1001-1006

[32] Noel RJ. Opiates, immune system, acquired immunodeficiency syndrome, and nonhuman primate model. Journal of Neurovirology. 2008;**4**:279-285

[33] Miyagi AU et al. Opioids suppress chemokine-mediated migration of monkey neutrophils and monocytes—An instant response. Immunopharmacology. 2000;**47**:53-62

[34] Perez-Castrillon JL, Pérez-Arellano JL, García-Palomo JD, Jiménez-López A, De Castro SL. Opioids depress in vitro human monocyte chemotaxis. Immunopharmacology. 1992;**23**(1):57-61

[35] Donahoe RM. Neuro-immuno-modulation by opiates: Relationship to HIV infection and AIDS. Advances in Neuroimmunology. 1993;**3**:31-46

[36] McDonough RJ, Madden JJ, Falek A, Shafer DA, Pline M, Gordon D, et al. 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. Journal of Immunology. 1980;**125**(6):2539-2543

[37] DesJarlais DC, Friedman SR, Marmor M. Development of AIDS, HIV seroconversion, and potential cofactors for T4 cell loss in a cohort of I.V. drug users. AIDS. 1987;**1**:105-111

[38] Culver KW, Ammann AJ, Partridge JC, Wong DF, Wara DW, Cowan MJ. Lymphocyte abnormalities in infants born to drug-abusing mothers. The Journal of Pediatrics. 1987;**111**(2):230-235

[39] Mientjers GH, Miedema F, van Ameijden EJ, van den Hoek AA, Schellekens PT, Roos MT, et al. Frequent injecting impairs lymphocyte reactivity in HIV-positive and HIV-negative drug users. AIDS. 1991;**5**(1):35-41

[40] Rouveix B. Opiates and immune function: Consequences on infectious diseases with special reference to AIDS. Thérapie. 1992;**47**:503-512

[41] Thomas PT, Bhargava HN, House RV. Immunomodulatory effects of in vitro exposure to morphine and its metabolites. Pharmacology. 1995;**50**(1):51-62

[42] Varela P, Marcos A, Santacruz I, Ripoll S, Requejo AM. Human immunodeficiency virus infection and nutritional status in female drug addicts undergoing detoxification: Anthropometric and immunologic assessments. The American Journal of Clinical Nutrition. 1997;**66**(2):504S-508S

[43] Rho YM. Infections as fatal complications of narcotism. New York State Journal of Medicine. 1972;**72**:823-830

[44] Islam M, Frye RF, Richards TJ, Sbeitan I, Donnelly SS, Glue P, et al. Differential effect of IFNalpha-2b on the cytochrome P450 enzyme system: A potential basis of IFN toxicity and its modulation by other drugs. Clinical Cancer Research. 2000;**8**(8):2480-2507

[45] Islam N, Kanost AR, Teixeira L, Johnson J, Hejal R, Aung H, et al. Role of cellular activation and tumor necrosis factor-alpha in the early expression of mycobacterium tuberculosis 85B mRNA in human alveolar macrophages. The Journal of Infectious Diseases. 2004;**190**(20):341-351

[46] Islam SK, Hossain KJ, Kamal M, Ahsan M. Serum immunoglobulins and white blood cells status of drug addicts: Influence of illicit drugs and sex habit. Addiction Biology. 2004;**9**(1):27-33

[47] Naik S, Vaswani M, Desai NG. Humoral immune function in non-parenteral heroin dependence: Indian data. Alcoholism. 2001;**37**(1):25-34

[48] Roudebush RE et al. Dissociation of immunosuppression by chlorpromazine and trifluoperazine from

pharmacologic activities as dopamine antagonists. International Journal of Immunopathology and Pharmacology. 1991;**13**(7):961-968

[49] Bidlack JM. Detection and function of opioid receptors on cells from the immune system. Clinical and Diagnostic Laboratory Immunology. 2000;**7**(5):719-723

[50] Hoehe MR, Köpke K, Wendel B, Rohde K, Flachmeier C, Kidd KK, et al. Sequence variability and candidate gene analysis in complex disease: Association of mu opioid receptor gene variation with substance dependence. Human Molecular Genetics. 2000;**9**:2895-2908

[51] Margas W, Zubkoff I, Schuler HG, Janicki PK, Ruiz-Velasco V. Modulation of Ca^{2+} channels by heterologously expressed wild-type and mutant human micro-opioid receptors (hMORs) containing the A118G single nucleotide polymorphism. Journal of Neurophysiology. 2007;**97**(2):1058-1067

[52] Kroslak T, Laforge KS, Gianotti RJ, Ho A, Nielsen DA, Kreek MJ. The single nucleotide polymorphism A118G alters functional properties of the human mu opioid receptor. Journal of Neurochemistry. 2007;**103**(1):77-87

[53] Deb I, Chakraborty J, Gangopadhyay PK, Choudhury SR, Das S. Single-nucleotide polymorphism (A118G) in exon 1 of OPRM1 gene causes alteration in downstream signaling by mu-opioid receptor and may contribute to the genetic risk for addiction. Journal of Neurochemistry. 2010;**112**(2):486-496

[54] Zhang Y, Wang D, Johnson AD, Papp AC, Sadée W. Allelic expression imbalance of human mu opioid receptor (OPRM1) caused by variant A118G. The Journal of Biological Chemistry. 2005;**280**:32618-32624

[55] Pang GS, Wang J, Wang Z, Goh C, Lee CG. The G allele of SNP E1/A118G at the mu-opioid receptor gene locus shows genomic evidence of recent positive selection. Pharmacogenomics. 2009;**10**(7):1101-1109

[56] Huang P, Chen C, Mague SD, Blendy JA, Liu-Chen LY. A common single nucleotide polymorphism A118G of the μ-opioid receptor alters its N-glycosylation and protein stability. The Biochemical Journal. 2012;**441**(1):379-386

[57] Garden Gardiner and Frommer M&M. CpG islands in vertebrate genomes. Journal of Molecular Biology. 1987;**196**:261-282

[58] Nielsen DA, Vadim Y, Sara H, Colin J, Ann H, Jurg O, et al. Increased OPRM1 DNA methylation in lymphocytes of methadone-maintained former heroin addicts. Neuropsychopharmacology. 2009;**34**:867-873

[59] Chorbov VM, Todorov AA, Lynskey MT, Cicero TJ. Elevated levels of DNA methylation at the OPRM1 promoter in blood and sperm from male opioid addicts. Journal of Opioid Management. 2011;**7**(4):258-264

[60] Oertel BG, Doehring A, Roskam B, et al. Genetic-epigenetic interaction modulates μ-opioid receptor regulation. Human Molecular Genetics. 2012;**21**(21):4751-4760

[61] Johnson AD, Zhang Y, Papp AC, et al. Polymorphisms affecting gene transcription and mRNA processing in pharmacogenetic candidate genes: Detection through allelic expression imbalance in human target tissues. Pharmacogenetics and Genomics. 2008;**18**(9):781-791

[62] Goddard Alan D, Anthony W. Regulation of G protein-coupled receptors by palmitoylation and cholesterol. BMC Biology. 2012;**10**:27

[63] Maurice P, Kamal M, Jockers R. Asymmetry of GPCR oligomers supports their functional relevance.

Trends in Pharmacological Sciences. 2011;**32**(9):514-520

[64] Krzysztof P. Oligomeric forms of G protein-coupled receptors (GPCRs). Trends in Biochemical Sciences. 2010;**35**(11):595-600

[65] Park PS, Filipek S, Wells JW, Palczewski K. Oligomerization of G protein-coupled receptors: Past, present, and future. Biochemistry. 2004;**43**(50):15643-15656

[66] Mansoor SE, Palczewski K, Farrens DL. Rhodopsin self-associates in asolectin liposomes. Proceedings of the National Academy of Sciences of the United States of America. 2006;**103**(9):3060-3065

[67] Rovira X, Pin JP, Giraldo J. The asymmetric/symmetric activation of GPCR dimers as a possible mechanistic rationale for multiple signaling pathways. Trends in Pharmacological Sciences. 2010;**31**(1):15-21

[68] Arcemisbéhère L, Sen T, Boudier L, Balestre M-N, Gaibelet G, Detouillon E, et al. Leukotriene BLT2 receptor monomers activate the G (i2) GTP-binding protein more efficiently than dimers. The Journal of Biological Chemistry. 2010;**285**:6337-6347

[69] Milligan G. G protein-coupled receptor hetero-dimerization: Contribution to pharmacology and function. British Journal of Pharmacology. 2009;**158**(1):5-14

[70] Maggio R, Aloisi G, Silvano E, Rossi M, Millan MJ. Heterodimerization of dopamine receptors: New insights into functional and therapeutic significance. Parkinsonism & Related Disorders. 2009;**15**(S4):S2-S7

[71] Vischer HF, Nijmeijer S, Smit MJ, Leurs R. Viral hijacking of human receptors through heterodimerization. Biochemical and Biophysical Research Communications. 2008;**377**(1):93-97

[72] Zhang M, Feng X, Guan R, Hébert TE, Segaloff DL. A cell surface inactive mutant of the human lutropin receptor (hLHR) attenuates signaling of wild-type or constitutively active receptors via heterodimerization. Cellular Signalling. 2009;**21**(11):1663-1671

[73] Sergi F, Vicent C, Devi LA, Marta F, Ralf J, Lohse MJ, et al. G protein–coupled receptor oligomerization revisited: Functional and pharmacological perspectives. Pharmacological Reviews. 2014;**66**(2):413-434

[74] Golebiewska U, Johnston JM, Devi L, Filizola M, Scarlata S. Differential response to morphine of the oligomeric state of μ-opioid in the presence of δ-opioid receptors. Biochemistry. 2011;**50**(14):2829-2837

[75] Manglik A, Kruse AC, Kobilka TS, Thian FS, Mathiesen JM, Sunahara RK, et al. Crystal structure of the μ-opioid receptor bound to a morphine antagonist. Nature. 2012;**485**(7398):321-326

[76] Huang J, Chen S, Zhang JJ, Huang XY. Crystal structure of oligomeric β1-adrenergic G protein-coupled receptors in ligand-free basal state. Nature Structural & Molecular Biology. 2013;**20**(4):419-425

[77] Gomes I, Gupta A, Filipovska J, Szeto HH, Pintar JE, Devi LA. A role for heterodimerization of mu and delta opiate receptors in enhancing morphine analgesia. Proceedings of the National Academy of Sciences of the United States of America. 2004;**101**(14):5135-5139

[78] Gomes I, Ijzerman AP, Ye K, Maillet EL, Devi LA. G protein-coupled receptor heteromerization: A role in allosteric modulation of ligand binding. Molecular Pharmacology. 2011;**79**(6):1044-1052

[79] Bushlin I, Gupta A, Stockton SD Jr, Miller LK, Devi LA. Dimerization with

cannabinoid receptors allosterically modulates delta opioid receptor activity during neuropathic pain. PLoS One. 2012;**7**(12):e49789

[80] Rozenfeld R, Bushlin I, Gomes I, Tzavaras N, Gupta A, Neves S, et al. Receptor heteromerization expands the repertoire of cannabinoid signaling in rodent neurons. PLoS One. 2012;**7**(1):e29239

[81] Gupta A, Mulder J, Gomes I, Rozenfeld R, Bushlin I, Ong E, et al. Increased abundance of opioid receptor heteromers after chronic morphine administration. Science Signaling. 2010;**3**(131):ra54

[82] Zheng H, Pearsall EA, Hurst DP, Zhang Y, Chu J, Zhou Y, et al. Palmitoylation and membrane cholesterol stabilize μ-opioid receptor homodimerization and G protein coupling. BMC Cell Biology. 2012;**13**:6

[83] Zhang N, Oppenheim JJ. Crosstalk between chemokines and neuronal receptors bridges immune and nervous systems. Journal of Leukocyte Biology. 2005;**78**(6):1210-1214

[84] Murphy PM. Chemokine receptors: Structure, function and role in microbial pathogenesis. Cytokine & Growth Factor Reviews. 1996;**7**(1):47-64

[85] Snyderman R, Uhing RJ. Chemoattractant stimulus-response coupling. In: Gallin JI, Goldstein IM, Snyderman R, editors. Inflammation: Basic Principles and Clinical Correlates. 2nd ed. New York: Raven Press, Ltd.; 1992. pp. 421-439

[86] Ali H, Tomhave ED, Richardson RM, Haribabu B, Snyderman R. Thrombin primes responsiveness of selective chemoattractant receptors at a site distal to G protein activation. The Journal of Biological Chemistry. 1996;**271**(6):3200-3206

[87] Ben-Baruch A, Grimm M, Bengali K, Evans GA, Chertov O, Wang JM, et al. The differential ability of IL-8 and neutrophil-activating peptide-2 to induce attenuation of chemotaxis is mediated by their divergent capabilities to phosphorylate CXCR2 (IL-8 receptor B). Journal of Immunology. 1997;**158**(12):5927-5933

[88] Imre S, Xiao-Hong C, Li X, Adler MW, Howard OMZ, Oppenheim JJ, et al. Heterologous desensitization of opioid receptors by chemokines inhibits chemotaxis and enhances the perception of pain. Proceedings of the National Academy of Sciences of the United States of America. 2002;**99**(16):10276-10281

[89] Goldman AS, Prabhakar BS. Chapter 1: Immunology overview. In: Baron S, editor. Medical Microbiology. 4th ed. Galveston, TX: University of Texas Medical Branch at Galveston; 1996. http://www.ncbi.nlm.nih.gov/books/NBK7795/

[90] Springa JY, Urizar E, Costagliola S, Vassart G, Parmentier M. Allosteric properties of G protein-coupled receptor oligomers. Pharmacology & Therapeutics. 2007;**115**(3):410-418

[91] Vadiveloo PK. Macrophages—Proliferation, activation, and cell cycle proteins. Journal of Leukocyte Biology. 1999;**66**(4):579-582

[92] Sacerdote P. Opioid-induced immunosuppression. Current Opinion in Supportive and Palliative Care. 2008;**2**(1):14-18

[93] Sharad S, Gupta AK, Singh RA, Kapoor M, Kapur S. Correlation of circulatory immunoglobulin levels with mu opiate receptor allele. Indian Journal of Biochemistry & Biophysics. 2007;**44**(5):394-400

[94] Kapur S, Pal A, Sharad S. Role of Opioidergic System in Humoral Immune Response. Immunosuppression—Role in Health and Diseases. Rijeka, Croatia: InTech; 2012