# N/OFQ-NOP System in Peripheral and Central Immunomodulation

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## Contents



**Abstract**

Classical opioids (μ: mu, MOP; δ: delta, DOP and κ: kappa, KOP) variably affect immune function; they are immune depressants and there is good clinical evidence in the periphery. In addition, there is evidence for a central role in the control of a number of neuropathologies, e.g., neuropathic pain. Nociceptin/Orphanin FQ (N/OFQ) is the endogenous ligand for the N/OFQ peptide receptor, NOP; peripheral and central activation can modulate immune function. In the periphery, NOP activation generally depresses immune function, but unlike classical opioids this is in part driven by NOP located on circulating immune cells. Peripheral activation has important implications in pathologies like asthma and sepsis. NOP is expressed on central neurones and glia where activation can modulate glial function. Microglia, as resident central 'macrophages', increase/infiltrate in pain and following trauma; these changes can be reduced by N/OFQ. Moreover, the

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interaction with other glial cell types such as the ubiquitous astrocytes and their known cross talk with microglia open a wealth of possibilities for central immunomodulation. At the whole animal level, clinical ligands with wide central and peripheral distribution have the potential to modulate immune function, and defining the precise nature of that interaction is important in mitigating or even harnessing the adverse effect profile of these important drugs.

**Keywords**
Astrocytes · Gliosis · Immune function · Lymphocytes · Microglia · N/OFQ receptor (NOP) · Neuropathic pain · Nociceptin/Orphanin FQ · Sepsis

## 1 Introduction

Classical opioids (μ: mu, MOP; δ: delta DOP and κ: kappa, KOP) are immunomodulatory; this has been known for decades. Indeed, Hussey and Katz reported in 1950 that opioid addicts were more prone to infection and this was unlikely due to the injection itself (Hussey and Katz 1950). The site of this immunomodulation can be peripheral or central with the precise targets (especially peripheral) being disputed and highly controversial. Prescribing physicians are advised to consider and discuss immune modulation in chronic use decisions. Since its first de-orphanisation N/OFQ and NOP [non-classical opioid receptor (Lambert 2008)] have also been ascribed a role in immunomodulation, and in this chapter we review their roles at peripheral and central sites.

## 2 Peripheral Immune Actions

### 2.1 Classical Opioids

Opioid receptor expression on immune cells is still highly controversial. It is widely accepted that opioids have immunomodulatory properties, for example inhibition of T-cell activity or inhibition of B-cell antibody production (Manfredi et al. 1993; Morgan 1996). However, there is significant debate as to whether this action occurs through direct or indirect mechanisms. Evidence is strongly divided regarding the detection of classical opioid receptor (MOP, DOP and KOP) expression on immune cell types (Caldiroli et al. 1999; Bidlack 2000; Cadet et al. 2001; Al-Hashimi et al. 2013, 2016; Kadhim et al. 2018b). Some have posited that the action of morphine in immune responses is via the toll-like receptors (TLR), which have been shown to possess a morphine binding domain (Madden et al. 2001; Hutchinson et al. 2012).

## 2.2 N/OFQ-NOP

Conversely, there is significant evidence for expression of NOP receptors on immune cell subtypes. Several studies have identified the presence of ppN/OFQ and NOP mRNA (the precursor to N/OFQ) in polymorphonuclear cells, B cells, T cells and monocytes and mast cells (Peluso et al. 1998; Arjomand et al. 2002; Williams et al. 2008a; Singh et al. 2013; Al-Hashimi et al. 2016). Interestingly, screening of phytohemagglutinin (PHA)-activated human lymphocytes identified AT7-5EU cDNA, which encodes NOP, with divergent coding of a non-translated 5′ region in comparison to neuronal tissue. This message is encoded into B and T cell NOP mRNA, and suggests tissue-specific expression of the NOP receptor. Furthermore, these experiments indicated a tenfold increase in NOP mRNA expression after induction with PHA, implying NOP has an important role in immune function (Wick et al. 1995). Further studies have demonstrated similar levels of NOP mRNA in both immune cells and neuronal tissue (Peluso et al. 1998). Expression of functional NOP receptor has been identified in numerous continuous cell lines generated from immune cells. Using [$^{125}$I]-N/OFQ, Horn and colleagues identified surface expression of NOP on Raji cells, a human B cell lymphoma line (Hom et al. 1999). NOP was further identified in CEM and MOLT-4 T cell leukemic lines and the monocyte lymphoma cell line U-937 using [$^{3}$H]-N/OFQ to identify binding sites (Peluso et al. 1998). The addition of phorbol-12-myristate-13-acetate (PMA) to Mono Mac 6 cells, a monocyte leukemic cell line, led to increases in ppN/OFQ mRNA via the inhibition of mitogen-activated protein kinase signal transduction pathways (Zhang et al. 2016). Identification of NOP expression on primary immune cells has been challenging due to poorly selective antibodies for the NOP receptor and acquiring the necessary yield of protein to undertake a radioligand binding assay. Recently, a fluorescent marker for NOP, N/OFQ$_{ATTO594}$, has been used to identify NOP receptor expression on human polymorphonuclear cells taken from healthy volunteers (Bird et al. 2018). Interestingly, not all polymorphonuclear cells expressed the NOP receptor protein; this is a cautionary note when assuming mRNA will always translate into protein.

Immune cells have also been shown to express N/OFQ. Human CD19+ B cells were amongst the first to be identified as expressing a novel N/OFQ mRNA transcript resulting in a truncated N/OFQ precursor lacking the signal peptide. Following mitogen-activation, N/OFQ mRNA transcripts, similar to that found in neuronal tissue, was upregulated in all lymphocytes (Arjomand et al. 2002). The mRNA transcript for ppN/OFQ has also been found in polymorphonuclear cells, which include neutrophils, eosinophils and granulocytes (Williams et al. 2008a). Furthermore, neutrophils stimulated with N-formyl-methionine-leucine-phenylalanine (FMLP) have been shown to release N/OFQ (Fiset et al. 2003).

The presence of both N/OFQ and NOP in immune cells would strongly indicate a role in immunological function for this ligand-receptor pairing. An area where this pairing may have significant effect is in the trafficking of immune cells, with N/OFQ

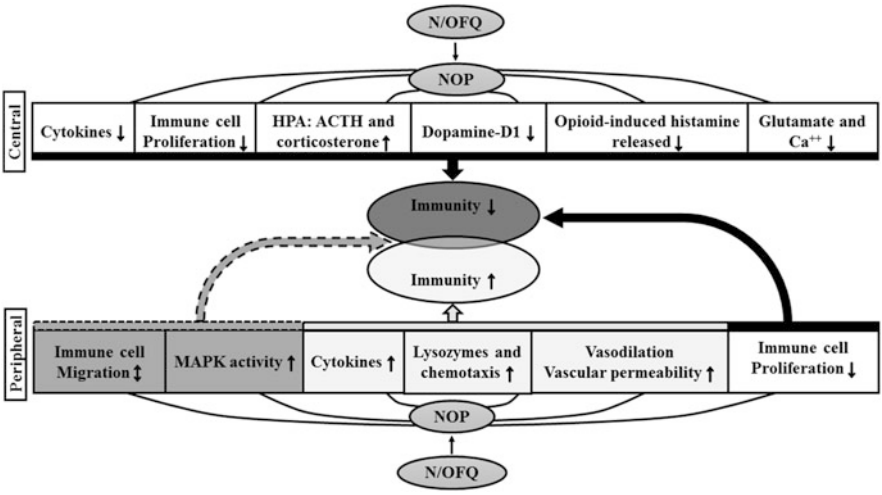


**Fig. 1** Mechanisms by which N/OFQ can affect the immune system. Different central (upper panel) and peripheral (lower panel) 'targets' can inhibit (black arrows), activate (light grey arrow) or both inhibit/activate immune function (grey dotted arrow)

having significant effects on cell migration, both positive and negative. A significant example of the positive effects of N/OFQ was measured using monocytes taken from healthy volunteers. The monocytes were exposed to either FMLP or N/OFQ and chemotaxis measured (Trombella et al. 2005). FMLP caused robust migration of monocytes which was matched by N/OFQ, which displayed a high potency ($pEC_{50}$ 11.15) in producing migration. Confirmation of action through the NOP receptor was obtained through pharmacological characterisation using several NOP selective agonists, the inability of naloxone to block the function of N/OFQ at monocytes and through antagonism of migration via the NOP antagonist UFP-101 (Trombella et al. 2005). Neutrophil chemotaxis is also positively affected by the addition of N/OFQ. N/OFQ induced chemotaxis with maximal effect at 100 pM in ex vivo migration studies, and these findings were matched in mouse in vivo models whereby N/OFQ increased neutrophil migration into ad-hoc air pouches (Serhan et al. 2001). Conversely, both lung mast cells and eosinophils have been shown to be negatively affected by N/OFQ in regards to migration (Singh et al. 2016). Both human mast cell line-1 (HMC-1) and primary human lung mast cell migration produced by stem cell factor (SCF) were significantly inhibited by the addition of N/OFQ. Clearly there is a cell and tissue specific migratory response to NOP activation. In addition, and as reviewed by Thomas et al. (2014), N/OFQ induces vasodilation and increases the vascular permeability, actions that play a central role in immune response modulation (Fig. 1).

## 2.3 N/OFQ-NOP in Disease

The presence of NOP and/or N/OFQ in the immune system, as well as its ability to affect immune cell movement and function, identify a potential mediator of disease-related activity in immunity. NOP and N/OFQ activity has been demonstrated to show potential roles in several immune based diseases. Both NOP and N/OFQ have been implicated in the pathogenesis of colitis, an inflammatory bowel disease (Kato et al. 2005). NOP knockout mice demonstrated significant reduction in symptoms following treatment with dextran sulphate sodium (DSS), which is capable of producing acute colitis. In further studies, administration of SB612,111 (a high affinity NOP antagonist) to DSS-induced colitis also reduced symptoms of colitis as well as a reduction of the cytokines interferon-γ (IFN-γ), interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α). These cytokines are all known mediators of colitis (Alt et al. 2012). Increased levels of N/OFQ have also been detected in the synovial fluid of patients suffering with rheumatoid arthritis (Fiset et al. 2003). The increased level of N/OFQ was believed to be related to the high concentration of polymorphonuclear cells usually found in synovial fluid of patients suffering with this disease.

As previously noted, both lung eosinophils and mast cells express the NOP receptor. This is particularly relevant to asthma. Asthma is the result of obstruction of airflow (airway constriction, immune infiltration and remodelling) leading to difficulty in breathing (Haldar et al. 2008; Lotvall et al. 2011; Gough et al. 2015). Initial studies indicated that activation of NOP, via N/OFQ, led to inhibition of airway contraction and the release of the inflammatory peptide, substance P (Shah et al. 1998). This initial evidence for NOP receptor function in airway constriction was verified by work in ex vivo human bronchial tissue (Basso et al. 2005). Electric field stimulation produced contractions in the tissue, which was inhibited by N/OFQ in a concentration-dependent manner. Furthermore, the actions of N/OFQ could be blocked by the NOP antagonist, UFP-101, indicating action through the NOP receptor. In a more recent work, tissues from both healthy volunteers and asthmatic patients were screened for the presence of NOP and N/OFQ via PCR. In these studies, N/OFQ was identified in lung eosinophils and, in asthmatic patients, levels were found to be increased in sputum (Singh et al. 2016). In parallel experiments, N/OFQ was found to inhibit migration of immune cells through NOP receptor activation, as well as increasing wound healing in isolated human airway smooth muscle (HASM) cells. Using the same cells, it was found that N/OFQ led to relaxation of HASM cells in spasmogen-stimulated gel contraction experiments, a finding mirrored in Ovalbumin-sensitised mice. These findings suggest that NOP agonists could be potential therapeutic agents for asthma, with a spasmolytic and immune depressor profile.

Sepsis is the result of the immune system producing an overwhelming and potentially life-threatening response to an infection. Treatment options are limited to antibiotics, fluids and supportive care. Translation from the laboratory to the clinic has been poor and there is a real need for novel therapeutics. The mechanisms by which sepsis occurs are poorly understood, but NOP and N/OFQ have been

implicated in this disease. Initial evidence for the role of N/OFQ-NOP in sepsis was found using rat models subjected to caecal ligation and puncture to induce sepsis. In these models, addition of N/OFQ to caecal ligation and puncture led to increased mortality, whereas addition of UFP-101 increased survival rates through inhibition of cell migration and modulation of pro-inflammatory cytokines and chemokines (Carvalho et al. 2008).

Both ppN/OFQ and NOP mRNA levels were decreased in peripheral blood taken from healthy volunteers exposed to varying concentrations of LPS. Furthermore, cytokines, such as TNF-α, IL-1β, IL-10 and IFN-γ, also demonstrated the ability to decrease ppN/OFQ and NOP mRNA levels in healthy volunteer blood (Zhang et al. 2013). While it was initially posited that this was a negative feedback loop downregulating N/OFQ and NOP expression, further data have demonstrated an increase in protein levels. In a small cohort of patients diagnosed with sepsis, plasma N/OFQ concentrations were measured; levels were higher in patients who died (3 pg $mL^{-1}$) compared to survivors (1 pg $mL^{-1}$) (Williams et al. 2008b). An inverse relationship was discovered with regards to ppN/OFQ mRNA in septic patients, with ppN/OFQ levels showing significant reduction when compared to healthy volunteers. Furthermore, this study demonstrated a correlation between increased levels of the septic inflammatory marker, procalcitonin and decreased levels of ppN/OFQ (Stamer et al. 2011). A larger prospective study was undertaken assessing 82 septic patients who were sex and age matched to healthy volunteers. Plasma N/OFQ was measured on the first 2 days after admission to the intensive care unit, with a follow-up sample taken in the recovery period. Radioimmunoassay and PCR data demonstrated an increase in plasma N/OFQ concentrations in Days 1 and 2 compared to recovery. Conversely, mRNA levels of ppN/OFQ and NOP decreased compared to healthy volunteers (Thompson et al. 2013).

## 3 Central Immune Actions

### 3.1 CNS Can Propagate an Immune Response Through Several Mechanisms

Despite a long history, the idea that the CNS is an immune-privileged organ is disappearing; the brain can mount immune responses and fight invading organisms (Galea et al. 2007). The meningeal lymphatic vasculature can transport cells and molecules resulting in cross-talk between the peripheral and central immune systems (Raper et al. 2016). According to clinically relevant studies, CNS innate immunity can be activated against pathogenic invasion (Carare et al. 2014). Beside microglia, resident central macrophages, meningeal macrophages and dendritic cells (namely in dura, arachnoid and pia mater, choroid plexus and perivascular spaces) can produce significant *protective* actions (Herz et al. 2017).

Several cellular components are involved in regulation of central immune response. Microglia are the central immune responders; they have specialised functions with higher reactivity and mobility than other cell populations in the

CNS and respond to antigens and neuronal damage. When activated they can release proinflammatory mediators and undergo morphological changes (from round and small cell body with long processes to amoeboid with shorter processes) (Inoue and Tsuda 2018). In addition, they migrate to the site of injury, proliferate, perform phagocytic activity and change their protein expression profile (mainly express complement receptors and major histocompatibility complex proteins). Fully activated microglia resemble other macrophages (Hanisch and Kettenmann 2007; Davoust et al. 2008; Colton and Wilcock 2010).

Astrocytes are the most abundant cell population in the CNS and the term 'astrocytes' or 'astroglia' is attributed to their star-like shape with diverse processes and morphology depending on anatomical location (Raff et al. 1983; Bailey and Shipley 1993). Their processes cover synapses, contact nodes of Ranvier and form gap junctions between the processes of neighbouring astrocytes. Astrocytes are multifunctional elements participating in local blood flow regulation (Attwell et al. 2010), supplying neuronal nutrients and controlling brain haemostasis (Mulligan and MacVicar 2004; Magistretti 2006; Araque and Navarrete 2010). They form the majority of the blood–brain barrier and control its endothelial elements (Giaume et al. 2007). They can be precursors and are involved in neurogenesis and gliogenesis (Kettenmann and Verkhratsky 2008) along with detection process and guiding the growth of axons and development of certain neuroblasts when neuronal repair is required (Powell and Geller 1999; Araque and Navarrete 2010). Due to their high number of connection sites, astrocytes have high integration capacity and important roles in the regulation of neuronal activity (Smith 2010). They have a role to play in a number of central pathologies (Bundgaard and Abbott 2008). While neurons are able to propagate action potentials, astrocytes are not, and their excitability occurs through increasing the intracellular concentration of calcium ($[Ca^{2+}]_i$) and release of glutamate, purines, Gamma-aminobutyric acid and D-serine. These transmitters might be responsible for astrocyte–astrocyte communication and/or astrocyte–neuron cross-talk (Nedergaard et al. 2003; Seifert et al. 2006). In addition, these gliotransmitters control the dynamics of the synaptic cleft (Cornell-Bell et al. 1990; Volterra and Meldolesi 2005).

Cellular changes associated with microglial or astroglial activation (gliosis; microgliosis and astrogliosis, respectively) have been reported in models of inflammation and chronic pain (Beggs and Salter 2006; Ji and Suter 2007; Inoue and Tsuda 2018; Kohno et al. 2018). Regardless of the order, the sequence and the intensity of glial activation (due to infection, chronic or neuropathic pain and/or opioid tolerance), astrocytes and microglia have been found to be involved in the pathogenesis of the immunomodulation (e.g., in neuropathic pain) in terms of initiation and progress (Raghavendra et al. 2003; Tanga et al. 2004; Ledeboer et al. 2005; Hald et al. 2009). Following activation, glial cells produce and release pain mediators such as nitric oxide and prostaglandins (Watkins and Maier 2000) and proinflammatory cytokines such as IL-1 and TNF-α (Watkins et al. 2001; Marchand et al. 2005; Charo and Ransohoff 2006; Scholz and Woolf 2007).

Oligodendrocytes are well-known myelin producing cells, providing neurone 'insulation' and a propagated action potential. In addition, these cells are sensitive

to the release of neurotransmitters and neural activity (Bakiri et al. 2009). They play an important role in the pathogenesis of different neurological diseases such as multiple sclerosis. They can release and/or respond to proinflammatory cytokines in response to brain injury (Jurewicz et al. 2005; Ramesh et al. 2012). On the other hand, and despite their specialised function, neurons have been found to release or respond to cytokines in different immunomodulatory conditions (Oh et al. 2001; Zhang et al. 2005).

In summary, complex interplay between neurons, immune cells, and glial cells are responsible for normal regulation and also initiation and maintenance of a number of neuropathologies of which neuropathic pain is an example.

## 3.2 The Effect of N/OFQ on the Central 'Immune System'

NOP is expressed centrally by neurons in the brain and spinal cord (Pettersson et al. 2002). In addition, a range of glial cells (astrocytes, oligodendrocytes and microglia) have been found to express NOP receptor (Eschenroeder et al. 2012; Kadhim et al. 2018a). N/OFQ is also produced and released by N/OFQ releasing neurons as well as by a wide range of glial cells (Buzas et al. 1998; Buzas 2002; Eschenroeder et al. 2012; Bedini et al. 2017). N/OFQ-NOP therefore has the potential to modulate glial function.

N/OFQ has been found to play an important role in central immunomodulation but the underlying mechanisms remain to be fully understood. Several possible mechanisms have been proposed (Fig. 1). Proinflammatory cytokines, the main immune modulating molecules, are likely modulated by N/OFQ. Intrathecal administration of N/OFQ induced antagonist-reversed down-regulation of cytokine mRNA transcripts. It has been found that pain processing is accompanied by astrocyte activation, which is characterised by an elevated level of proinflammatory cytokines (Lai et al. 2018). Hence, the antinociceptive effect of N/OFQ might be related to its ability to inhibit cytokine expression and/or release in the CNS (Fu et al. 2007; Finley et al. 2008). In addition, infiltration of peripheral immune cells is an important event in the pathophysiology of immunomodulation and pain (Boddeke 2001). Zhao et al. (2002) reported that increased numbers of microglia induced by trauma were reduced by central administration of N/OFQ. N/OFQ-induced immunomodulation may be as a result of inhibition of the proliferation and migration of infiltrating and resident immune cells (note: in the periphery N/OFQ can both promote and inhibit migration). Furthermore, in the hypothalamic-pituitary-adrenal (HPA) axis, adrenocorticotrophic hormone (ACTH) is well known as a site of immunomodulation and there is controversial evidence with classical opioids (Al-Hashimi et al. 2013). N/OFQ has been found to activate HPA axis and increase the levels of ACTH (Devine et al. 2001).

Moreover, several neurotransmitters involved in the regulation of immune function are affected by N/OFQ-NOP system; these include dopamine, histamine, noradrenaline and glutamate. Dopamine is an immunomodulatory neurotransmitter and inhibition of its release can reduce immune activity (Tsao et al. 1997; Basu and Dasgupta 2000; Nakano et al. 2009). There is an extensive literature base

demonstrating that dopamine release is inhibited by N/OFQ (Murphy et al. 1996; Murphy and Maidment 1999; Marti et al. 2004, 2005). Histamine release is an important event involved in the propagation of immune response; morphine-induced central histamine release is also affected by N/OFQ (Eriksson et al. 2000). Along with important roles in the pain pathway, noradrenaline is also an immunomodulator, and its release is inhibited by N/OFQ (Kappel et al. 1998). Given that glutamate and calcium signalling can be important players in immune activation (Watkins et al. 2001; Mattson and Chan 2003), N/OFQ-induced inhibition of glutamate (Nicol et al. 1996; Meis and Pape 2001; Kallupi et al. 2014; Meyer et al. 2017) and LPS-induced calcium signalling (Bedini et al. 2017) possibly affect the pattern of immune activation. The majority of these data are from work in neurones but as we note the brain is so much more than neurones. It can be concluded that the activation of NOP by N/OFQ can participate in central immunomodulation via multiple pathways; if there is disease specificity then this might open some new therapeutic options.

## 3.3 The Effect of Immunomodulation on the N/OFQ and NOP Receptor

The majority of the text above has covered immunomodulatory effect of NOP, but immune modulation can affect NOP and N/OFQ (the reverse) in the same ways as seen in the periphery in pathologies such as sepsis. As noted in Table 1, the expression profile, integrity and the activity of NOP and N/OFQ can be affected

**Table 1** The effect of different immunomodulatory conditions on the expression and activity of central NOP receptor and/or N/OFQ

| Cell/tissue type-species | Proinflammatory mediator/process | Effect on NOP | Effect on N/OFQ | Study |
|---|---|---|---|---|
| Primary rat astrocytes | LPS, IL-1β and TNF-α | – | ↑ (mRNA) | Buzas (2002) |
| Human U87 astrocytes | LPS | ↓ (mRNA and protein) | ↑ (mRNA and protein) | Bedini et al. (2017) |
| Rat | PTSD | – | ↑ (protein) | Zhang et al. (2012) |
| Rat | Traumatic brain injury | ↔ | ↑ | Witta et al. (2003) |
| Rat cortical neurons | Leukaemia inhibitory factor | – | ↑ (mRNA) | Minami et al. (2001) |
| Mice DRG neurons | LPS | – | ↑ (protein) | Acosta and Davies (2008) |
| Rat (in vivo) Rat primary microglia | Chronic constriction injury | ↑ NOP activity | ↑ | Popiolek-Barczyk et al. (2014) |
| Rat amygdala complex | Ethanol | – | Epigenetic modulation of ppN/OFQ | D'Addario et al. (2013) |

by a wide range of immunomodulatory conditions. These include bacterial products such as LPS, proinflammatory cytokines, ethanol traumatic brain injury, spinal cord injury in cultured neurons cultured glial cells or in whole animals.

## 4 Conclusions

Since its early description as a peptide receptor system involved in the modulation of pain processing, a plethora of biological functions, pathological indications and, importantly, therapeutic opportunities have been described. We know that classical opioids can modulate immune function and that immune pathologies can modulate opioid receptor, peptide and drug responsiveness. Here we have discussed both peripheral and central immune modulation by N/OFQ-NOP where there are similarities and differences in the brain and periphery. With the generally improved side effect profile for NOP activation with N/OFQ, and novel ligands such as cebranopadol close to the clinic, understanding the clinical consequences of the immune modulatory effects described above will be an area of research focus.

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