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Overview

A Review of the Effects of Pain and Analgesia on Immune System Function and Inflammation: Relevance for Preclinical Studies

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One of the most significant challenges facing investigators, laboratory animal veterinarians, and IACUCs, is how to balance appropriate analgesic use, animal welfare, and analgesic impact on experimental results. This is particularly true for in vivo studies on immune system function and inflammatory disease. Often times the effects of analgesic drugs on a particular immune function or model are incomplete or don't exist. Further complicating the picture is evidence of the very tight integration and bidirectional functionality between the immune system and branches of the nervous system involved in nociception and pain. These relationships have advanced the concept of understanding pain as a protective neuroimmune function and recognizing pathologic pain as a neuroimmune disease. This review strives to summarize extant literature on the effects of pain and analgesia on immune system function and inflammation in the context of preclinical in vivo studies. The authors hope this work will help to guide selection of analgesics for preclinical studies of inflammatory disease and immune system function.

Abbreviations and acronyms: CB,Endocannabinoid receptor; CD,Crohn disease; CFA, Complete Freund adjuvant; CGRP,Calcitonin gene-related peptide; COX,Cyclooxygenase; CTL, Cytotoxic T-Lymphocytes; DAMP,Damage-associated molecular pattern molecules; DRG,Dorsal root ganglion; DSS, Dextran sodium sulphate; ECS,Endocannabinoid system; IBD, Inflammatory bowel disease; IFA,Incomplete Freund adjuvant; Las, Local anesthetics; PAMP,Pathogen-associated molecular pattern molecules; PGE_2, Prostaglandin E2; P2Y, ATP purine receptor Y; P2X, ATP purine receptor X; TNBS, 2,4,6-Trinitrobenzene sulphonic acid; TRP, Transient receptor potential ion channels; TRPV, Transient Receptor Potential Vanilloid; TG,Trigeminal ganglion; UC,Ulcerative colitis

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The immune system is comprised of 2 arms, innate and adaptive immunity, which function in concert to protect an organism from pathogens and toxins. The immune system also plays an integral role in the process of tissue repair after injury.[58,127] The process by which the immune system responds to pathogens, toxins, and tissue injury and initiates tissue repair is known as inflammation. Inflammation and its association with pain have been recognized since it was first described by the Roman, Aulus Celsus.[177] More recently, it has been elucidated that the immune and nervous systems interact to mediate and modulate central and peripheral nociceptive processes that influence acute and chronic pain.[69,95,190,217] There is a dizzying array of cells, receptors, enzymes, cytokines, peptides, and neurotransmitters that constitute the inflammatory process and neuroimmune interactions related to pain. To further complicate the picture, drugs that are used to control pain, modulate immune function and the immune system can produce endogenous analgesics.[129,200] The goal of this article is to improve the reader's understanding of the relationship between pain, analgesia, and immune function in the context of preclinical in vivo studies. We hope this article will serve as a guide for laboratory animal professionals, IACUCs, and investigators in the selection of appropriate analgesics for preclinical studies of inflammatory disease and immune system function.

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Neuroimmune Interactions

With regards to pain and analgesia, it is critical to understand the complex interactions between the immune and nervous systems. It has been postulated that a well-regulated neuroimmune response to infection, noxious stimuli, and tissue injury represents a cohesive system for host defense and tissue healing.[36] Thus, conceptual siloing of immune and nervous system responses to pain is no longer appropriate.

Neurogenic Inflammation

Multiple lines of evidence indicate that nociceptive neurons can initiate and modulate inflammation. Considering the speed at which they respond to any form of insult, (traumatic, thermal, chemical) and their broad tissue distribution, nociceptive neurons are uniquely poised to function as monitors and rapid initiators of a neuroimmune response.[36] When triggered by noxious stimuli or alarmins (ATP, uric acid, hydroxynonenals) from damaged tissue, receptors primarily in the Transient Receptor

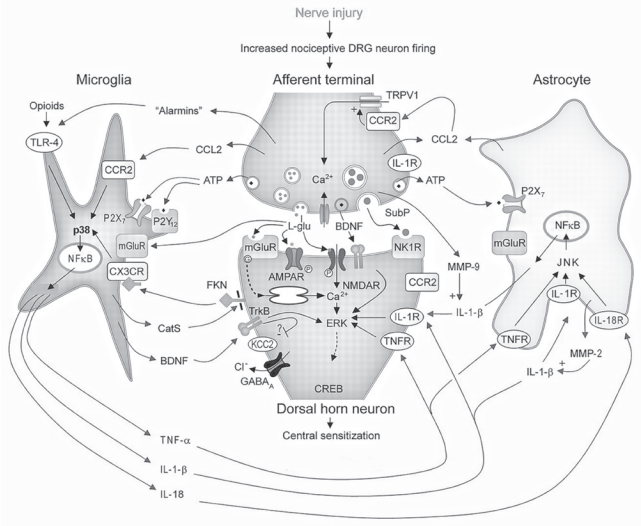

Figure 1. Interactions between nociceptive neurons and microglial cells after neuronal damage or activation by alarmins are depicted. l-glutamate (l-glu), substance P (SubP), adenosine triphosphate (ATP), brain-derived neurotrophic factor (BDNF), cysteine-cysteine chemokine ligand CCL2 neurokinin-1 receptors (NK-1R), extracellular signal-regulated kinase (ERK), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPAR), cyclic adenosine monophosphate response element binding protein (CREB). ATP purinergic receptors, (P2X7, P2Y12 and P2Y13R), mitogen-activated kinase (p38), c-jun-N terminal kinase (JNK), nuclear factor kappa B (NFκB), Interleukin-1β (IL1β) and its receptor, (IL-1R) tumor necrosis factor α (TNFα) and its receptor (TNFR), chloride (Cl–) transporter (KCC2), gamma aminobutyric acid A receptor (GABAA), chemokine ligand 2 (CCL2), chemokine receptor 2,3 (CCR2, CCR3), Cathepsin S (CatS), fractalkine (FKN, also termed CX3C-chemokine ligand 1), chemokine receptor 1 (CX3CR), Matrix metalloprotease 2, 9 (MMP2 MMP9), toll-like receptor 4 (TLR4).
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Potential ion channels (TRP) and ATP Purine Receptor X (P2×) and Y (P2Y) family activate nociceptors which release neuropetides that initiate a response referred to as neurogenic inflammation.[36,95] Specifically, tachykinins (substance P and neurokinin A) and calcitonin gene-related peptide (CGRP), released from nociceptive neurons, act on vascular endothelium and smooth muscle cells, causing vasodilation and increased endothelial permeability.[16,116,143,174,179,185] Activated nociceptive neurons also release neuropeptides and cytokines which attract and activate innate and adaptive immune cells.[65,85,86,104,187,219] Over a course of days, the inflammatory response recruits monocytes, which differentiate into macrophages. Over time, macrophages undergo phenotype changes from inflammatory/host defense (M1) to antiinflammatory/wound healing (M2) cells as the wound microenvironment changes.[58,127] Thus, nociceptor activation can induce the factors that cause the classic signs of inflammation: rubor, tumor, calor, and dolor, and may contribute to wound healing.

Bidirectional Interactions

Interactions between the immune system and nervous system are bidirectional and not all nociceptor driven. Nociceptive neurons express and respond to a receptor profile similar to that of leukocytes. These include receptors for cytokines, eicosanoids (prostaglandins), Toll-like receptors, and ATP purine receptors P2× and P2Y.[76,147,209,214] Leukocytes express TRP receptors, macrophages, express high levels of Transient Receptor Potential Vanilloid (TRPV2), and mast cells express TRPV1. To further complicate the picture, both leukocytes and nociceptors express μ, δ, and κ opioid receptors, and T-lymphocytes, granulocytes and monocytes-macrophages can release endogenous opioid peptides.[129,133,154,200]

The pattern of aforementioned receptor expression suggests that the immune system can modulate nociception, and the nervous system can modulate inflammation. Nociceptors can also be directly activated by infectious agents, damage-associated molecular pattern molecules (DAMPs) and pathogen-associated molecular pattern molecules (PAMPs) through Toll-like receptors.[35,59,146,214] It has been well established that inflammatory mediators activate nociceptors (causing pain) and can initiate neural plasticity in nociceptive pathways. This results in peripheral and central nociceptor sensitization.[15,43,199] Clinically, peripheral and central nociceptor sensitization manifests as allodynia, hyperesthesia, and hyperalgesia.[208]

Neuroimmune activation of nociceptive neurons occurs in the PNS and CNS. In response to injury or alarmin stimulation, leukocytes, endothelial cells, and neurons in the dorsal root ganglion (DRG), trigeminal ganglia (TG) and the dorsal horn of the spinal cord release eicosanoids, growth factors, kinins, and cytokines.[120,121,173,223] This milieu of biochemicals binds to receptors on nociceptive neurons, which are coupled to TRPV receptors and ion channels, resulting in increased neuron activity and sensitization.[110,111,164,199,206,208]

Microglial Activation

Activation and proliferation of microglial cells is thought to be a central feature of the neuroimmune interface both in physiologic and pathologic pain, and a primary element in the development of central sensitization and potentially, chronic pain (Figure 1).[216] Microglia are the predominant cell type in the CNS, and reside at a critical interface; the synapse between 1st and 2nd order nociceptive neurons in the spinal dorsal horn. The activation of microglia represents a key feature of the neuroimmune interface, since activation can occur through neuronal, immune and pathogen mediated pathways.[49,96,217] In addition, sex differences in glial activation have been reported, which may contribute to the established sexual dimorphism of pain.[49,134]

Microglia are activated by ATP, CC-chemokine ligand 2 and 21, CX_3CL1 (fracktalkine), and neuregulin 1, released from 1st order neurons during high threshold activation or injury.[148,212] Of particular note is that the receptor for CX_3CL1, CX_3CR1, is only expressed on microglia and may represent a unique neuroimmune interface.[212] Pathogens, DAMPs and PAMPs can directly activate microglial cells through binding to Toll-like receptors.[72,184] Cytokines released from leukocytes both activate microglial cells and directly contribute to nociceptive hyperactivity. When activated, microglia release proinflammatory cytokines, reactive oxygen species, brain-derived neurotrophic factor, and integrins. This biochemical barrage results in enhanced excitability in 2nd order neurons, increased release of substance P, glutamate and excitatory amino acids from primary afferent neurons, astrocyte activation, inhibition of inhibitory interneurons and recruitment of T-cells.[216]

Endocannabinoid System

The Endocannabinoid system (ECS), an endogenous "on-demand" messaging system comprised of lipophilic ligands,

their receptors, and synthetic proteins, represents another neuroimmune interface.[167] The ECS has widespread and varied physiologic functions throughout the body including neuroimmune modulatory effects. The 2 principle endocannabinoid receptors (CB) are CB_1 and CB_2. CB_1 receptors are found primarily in presynaptic neurons and are abundant in peripheral and central nociceptive pathways.[1,82,83,102] CB_2 receptors are expressed at lower levels in neurons and principally reside in peripheral tissues and leukocytes, including microglia.[162] While CB_1 expression appears constitutive in the CNS, CB_2 is highly induced by inflammation and tissue injury.[20,21,40,41,125] However, it remains unclear if the increase in CB_2 is due to increased expression in resident leukocytes or due to infiltration from CB_2 expressing monocytes. In response to high levels of activity, the primary endocannabinoid ligands 2-arachidonoylglycerol and arachidonoylethanolamide are synthesized from membrane phospholipids in postsynaptic neurons.[4,45,222] Glial cell production of endocannabinoids has also been demonstrated in vitro, and is postulated to occur during neuronal injury.[32,52] Ligand binding to CB_{1r} results in antinociception by activation of descending antinociceptive pathways and inhibition of nociceptive neurotransmission and supraspinal processing.[1,82,102,139,153] Cannabinoids exert broad antiinflammatory effects on peripheral leukocytes and glial cells, including reduced proinflammatory cytokine release, increased antiinflammatory cytokine release, decreased cell migration and activation, and inducing apoptosis.[30,167]

Effect of Pain on Immune System Function

Clearly, the nervous and immune systems are inexorably linked. However, separating the effect of pain resulting from tissue injury and the direct effect of tissue damage on immune function can be problematic. In addition, the effects of chronic pain on immune system function are significantly different than the effects of acute pain, and often involves a chronic inflammatory stimulus. Experimental procedures that employ noxious stimuli which do not (or should not) cause tissue damage have been shown to suppress selective immune function. For example, foot shock has been shown to suppress NK cell activity and mitogen induced cell proliferation. [172,183,193] Suppression of antigen stimulated IgG production and a reduced in vitro proliferative response to alloantigens (as assessed by mixed lymphocyte reaction) has been demonstrated in a tail-shock model.[63,112] These studies suggest the possibility that pain or aversion (stress) induce the release of immunosuppressive hormones that modulate immune function in these models.

Surgery

Surgical procedures have well documented and marked effects on immune system function in humans, including increased susceptibility to infection, delayed wound healing, and enhanced tumor growth and spread of metastatic cancer.[44,77,135] Similar data exists in animal models. Reduced NK and B-cell and T- cell activity and enhanced tumor growth have been demonstrated in rat and mouse surgical model.[7,19,159,168,186,204,205] Impairment of macrophage function, including reduced phagocytosis of pathogens, microbicidal activity and H_2O_2 release and seemingly paradoxical increased TNFα release has been shown after surgical procedures in rodents.[92,142,149,189] Macrophage dysfunction shows a phasic response over time in surgical models and decreased antigen presentation can last for a week.[142] T-cell dysfunction characterized, by decreased production of IL2, IFNγ, and loss of T cell receptor – ζ occurs after laparotomy in mice, and may be due to T-cell suppression by myeloid $CD11b^+$/$Gr\text{-}1^+$ cells that infiltrate the spleen after surgery.[132] Serum levels of the proinflammatory cytokines IL6 and IL1β transiently increase after laparotomy, and the potent angiogenic cytokine Vascular Endothelial Growth Factor, implicated in enhanced tumor growth, increases significantly around 6 to 12 d after surgery.[168] Seven days after surgical trauma and hemorrhage, there is a shift in splenic T-helper cytokine profiles from Th1 (decreased IFNγ, IL2) to Th2 (increased IL4, IL5, IL6, and IL10) in mouse.[130] Shifts in Th1 to Th2 phenotypes are associated with increased susceptibility to viral, bacterial and helminth infections and the development of sepsis.[87,105] In addition, the effects of the surgical trauma and hemorrhage protocol on immune function are more pronounced in 18 to 20 mo old animals compared with 6 to 8 wk old.[98]

The mechanisms underlying immunosuppression in surgery and trauma models are complex and involve, pain, activation of the Hypothalamic-Pituitary-Axis, sympathetic nervous system activation, tissue trauma, and the effects of anesthesia and analgesia.[81,105,107] Despite evidence that analgesics can inhibit immune function, a significant body of research suggests that robust pain management in humans reduces surgically related immunosuppression.[3,9,17,18,97,192,225, 227] Although not as extensive as the human literature, similar findings have been made in rodents, which suggest that surgical pain management improves immune function and reduces tumor spread.[14,74,165,166,192]

Analgesic Modulation of Immune Function. ***Opioids.*** Opioids are some of the most common and potent analgesics used in laboratory animal medicine and in vivo research. Considerable effort has gone into elucidating the effects of opioids on immune function, however, the exact mechanism by which opioids modulate immune function has not been clearly elucidated. Postulated mechanisms for opioid modulation of immune function include alterations in the Hypothalamic-Pituitary-Axis, mu-opioid receptor activation, drug binding to nonopioid receptors on leukocytes, modulation of autonomic tone, drug structure, or a combination of effects.[5,25] In general, opioids can be classified as drugs with mild to moderate effects on immune function (buprenorphine, hydromorphone, oxycodone, tramadol, hydrocodone, oxycodone) or marked effects on immune function (codeine, methadone, morphine, fentanyl, remifentanil).[5] Because the effects of opioids on immune function vary by drug and species, this discussion will examine the immune modulatory profile of each drug individually.

Buprenorphine. Arguably, buprenorphine is the most commonly used opioid in laboratory animal medicine. It appears that buprenorphine has the least effect on immune function, compared to other opiods, although not inert in this respect. When used as an analgesic in the guinea pig Sereny test (0.05 mg/kg BID for the duration of the test) buprenorphine had no effect on *Shigella* antigen induced or vaccine induced antibody responses or severity ratings.[73] When infused to healthy dogs for 24 h, buprenorphine (1.7 µg/kg/h) had no effect on leukocyte stimulated cytokine production, apoptosis, neutrophil phagocytosis, or oxidative burst. Similar effects were noted for morphine.[151]

Pain induced by immunization with complete Freund adjuvant (CFA) and incomplete Freund adjuvant (IFA) in mice was reduced by buprenorphine (0.1 mg/kg BID X 72 h) and did not impair vaccine induced IgG titers.[108] Infusion of buprenorphine in mouse for up to 7 d at 300 µg/day had no effect on NK cell activity and splenocyte lymphoproliferation, γ interferon release or IL2 production.[140] In the mouse intracranial lymphocytic choriomeningitis virus model, infusion of buprenorphine (0.15 mg/kg/d) reduced pain scores and had no effect on the numbers

of splenic $CD8^+$,$CD4^+$, NK1.1, and $CD19^+$ cells or cytotoxic T-cell responses to viral epitopes.[155] CNS Infiltration of leukocytes and virus-specific cytotoxic T cells in response to infection was also not affected.[155] Administration of buprenorphine to mice at 2 mg/kg SID for 7 d had no effect on IgG and IgM titers in responses to sheep red blood cells, and increased the number of antibody producing cells.[60] In the same study, using a contact hypersensitivity model, a process dependent on Th-1 lymphocytes and macrophage function, buprenorphine and oxycodone were shown to suppress reactions during the induction and effector phase.[60] Nitric oxide release from macrophages was suppressed, and no significant effects on cytokine release from either unstimulated or LPS stimulated macrophages was noted.[60] Although not reported as statistically significant, macrophage surface markers were also reduced by buprenorphine treatment.[60]

Buprenorphine can have strain and species dependent effects. In Lewis rat, buprenorphine reduced NK cell activity and suppressed mitogen stimulated proliferation and γ-interferon release from splenic lymphocytes in a dose-dependent fashion.[33] Suppression of immune function was noted after single doses of buprenorphine either 0.1 and 1.0 mg/kg, although not at 0.01 mg/kg. The immunosupressive effects of buprenorphine were inhibited by administration of naltrexone, suggesting mu-receptor modulation of immune function in this study.[33] Conversely, in Fischer rats, 2 doses of buprenorphine (0.1 mg/kg) given 5 h apart, were shown to preserve NK cell function in a surgical model[64] and 0.66 nmol injected once into the midbrain had no effect on splenic NK cell, T cell, and macrophage function.[68]

The advent of sustained release formulations of buprenorphine invites questions as to the potential effects of such preparations on immune function. Evidence is emerging that sustained release buprenorphine has a different immunomodulatory fingerprint and may be less immunomodulatory than buprenorphine HCl.[6,78]

Morphine and Fentanyl. Morphine and fentanyl have well documented immunosuppressant effects in humans. Owing to their infrequent use as analgesics, the effects of morphine and fentanyl on immune function in laboratory animals is not as well established. It is clear; however, that morphine and fentanyl have different immunomodulatory profiles, despite their antinociceptive action being primarily through mu receptor binding. In the mouse, fentanyl infusion (12.5 mg/h) over 7 d resulted in significant depression of NK cell activity, lymphoproliferation and IL2 and IFNγ release at day 1 and 3 of treatment.[140] At day 7, immunotolerance appeared to develop, and no significant changes in the aforementioned dependent measures were noted.[140] Several studies in mouse have documented the suppressive effects of morphine and fentanyl on macrophage dependent humoral responses, stimulation of reactive oxygen intermediate production, and the alteration of immune responses in a contact hypersensitivity model.[60,61] Morphine and fentanyl inhibit LPS induced TNFα release after single doses.[146] Repeated treatment every 8 h induces immunotolerance to morphine and sensitization to fentanyl after 6 to 8 doses.[150] Single doses of morphine (0.1 to 10 mg/kg) had antiinflammatory effects in a murine incision model.[38] However the relevance of all these findings to clinical analgesia is questionable.

Tramadol. Although not commonly used, tramadol appears to have antinociceptive effects in rodents and dog.[122,152,182,198,230] Tramadol is considered a drug with minimal immunosuppressive activity[11,122,182,198,230] although it can have profound antiinflammatory action and in some models be an immunostimulant.[23,181,230]

Local Anesthetics

Local anesthetics (LAs) are extremely effective and are important drugs for pain prevention and management protocols. All LAs work through the same basic mechanism, by inhibiting voltage gated sodium channels in nociceptive neurons, blocking depolarization and thus, neurotransmission. Thus, LAs would be expected to exert an antiinflammatory effect by preventing the release of proinflammatory molecules that occurs when nociceptive neurons depolarize. Because a component of the pathophysiology of inflammatory pain is upregulation of sodium channels in nociceptive neurons, in this context, LAs inhibition of nociceptive neuron depolarization should prevent peripheral and central sensitization induced by inflammatory mediators.[8] Most studies on LAs use lidocaine as the prototypical drug and occasionally bupivacaine, and assume comparable effects across all LAs. Leukocytes, excepting neutrophils, express voltage gated sodium channels, some of which may be important in microglia and macrophage function.[42] The extent to which the direct inhibition of Na channels on leukocytes, interactions with other receptors such as G-protein-coupled receptors, and the indirect inhibition of inflammatory mediator release contributes to the immunomodulatory effects of LAs is not known. Another noteworthy phenomenon is that the antiinflammatory effects of LAs in vitro require supra-clinical drug concentrations and that in vivo effects occur at clinically relevant doses. LAs have been shown to modulate PMNs, macrophages, and cytokine release in a variety of models.[82] PMN and macrophage functions (including chemotaxis, adherence, production of toxic oxygen species, phagocytosis, and cytokine release) are inhibited by LAs.[10,12,13,70,84,191] Lidocaine has been shown to inhibit cell proliferation, cytokine production, and mitogen-activated protein kinase activation in T cells and upregulate regulatory T-cells that promote an antiinflammatory t-cell phenotype.[84,94,101,118] One study in mouse showed that release of antiinflammatory cytokine IL10 may be enhanced by lidocaine.[211] Questions remain as to how long the immunomodulatory effects of LAs persist after drug administration is complete. To date, there do not appear to be any studies that have addressed this question.

Nonsteriodal Antiinflammatory Drug (NSAID). NSAID are arguably the most commonly used class of analgesic drugs in veterinary medicine and their use is prevalent in laboratory animal medicine. All NSAID work through the same primary mechanism; the inhibition of prostaglandin synthesis by inhibition of Cyclooxygenase (COX) isoenzymes 1 and or 2. NSAID anti-inflammatory and toxic effects, mediated by inhibition of prostaglandin synthesis, is exceptionally well documented in both human and veterinary literature. However, the analgesic effects of NSAID do not seem to rely on how selective an NSAID is for COX1 or 2. Recently, a host of prostaglandin and COX independent anti-inflammatory and analgesic effects have been proposed for NSAID. These effects vary by drug, but include antioxidant activity, inhibition of Nuclear Factor-κ B, inhibition of 5-lipoxygenase, prostaglandin receptor antagonism, antibradykinin actions and inhibition of fatty acid amide hydrolase, cytokine release, cell adhesion, and metabolism of arachidonic acid. [27,46,48,56,79,89,93,115,157,188] Since virtually every cell in the body constitutively expresses COX1, and COX2 can be markedly induced by inflammatory mediators, inhibition of COX has been ascribed to anti-inflammatory action in a staggering number of human and animal models. In addition, a wide range of behavioral actions have been associated with NSAID inhibition of COX.[34,119,128,160] Data have been compiled on NSAID classified by chemical structure, COX selectivity, and putative mechanism of action. Any data on COX inhibitors must be carefully evaluated,

since COX selectivity is almost always based on *in vitro* determinations using human cells, varies depending on the type of assay employed, and may not translate from human to animal cells or from one species to another.[47,114,115,117,176] Thus the impact of any given NSAID on immune function in a particular animal species cannot be accurately extrapolated from other NSAID or human data.

Although macrophages and neutrophils are thought to be the principle target leukocytes for NSAID actions, T cells and NK cells may also be impacted by NSAID. In neurodegenerative diseases with an inflammatory component, such as Alzheimer, the immune function of neurons, microglia, astrocytes, and endothelial cells can all be altered by NSAID.[123] In T cells, NSAID inhibition of COX1 interferes with T cell receptor dependent activation of p38 MAP-kinase, which blocks upregulation of COX2.[163] Both isoforms of COX and their metabolites play a significant role in the differentiation of $CD4^+$ T cells to Th1, Th2, and Th17 phenotypes. In general, COX and their eicosanoid products suppress Th1 differentiation, and augment Th2 and Th17 phenotypes and function.[117] In this fashion, NSAIDs may profoundly alter immune function, impacting a wide variety of models and processes that depend on $CD4^+$ T-cell differentiation.

The effects of NSAID on immune function varies by compound and species. The following section will discuss the effect of the most commonly used NSAID drugs (carprofen, ketoprofen, meloxicam), on immune indices.

Meloxicam. In a mouse vaccination study using complete CFA, meloxicam was shown to reduce CFA associated pain without altering primary or secondary antibody responses.[108] In 2 separate mouse models of infectious disease, meloxicam was shown to markedly reduced release of PGE_2, TNFα, IFNγ, IL4, IL10 and increase IL2 release from splenocytes.[144,145] Normalization of lymphoproliferation, and reduced parasitemia and mortality were noted in response to meloxicam in the *T. cruzi* study.[145] Meloxicam has also been shown to inhibit Nuclear Factor-κ B activation in LPS stimulated mouse macrophages.[89] Conversely, in a rabbit model of antigen induced arthritis, meloxicam was shown to decrease PGE_2, leukocyte infiltration and release of IL8 and had no effect on monocyte chemotactic peptide-1.[124] Meloxicam had no effect on LPS stimulated serum IL6 release and augmented TNF release in Guinea pig.[180] To date, no data has been published on immune modulation by the sustained release formulation of meloxicam.

Carprofen. Similar to results for meloxicam in mouse,[105] carprofen had no significant effect on CFA enhanced polyclonal antibody production in rabbit.[62] Carprofen reduced inflammatory cell infiltrates, thrombus weight, vein wall thickness, and serum IL6 in a mouse model of venous thrombosis.[80] TNFα activity was reduced by carprofen in a rat subcutaneous pouch model of inflammation[109] In a mouse model of traumatic brain injury, carprofen was shown to be neuroprotective and reduced brain levels of IL6 and IL1.[208]

Ketoprofen. Although several studies on the effects of ketoprofen on immune endpoints in rat have been reported, the use of this drug in rat is likely contraindicated due to its potential for gastrointestinal toxicity[194] and availability of other, less toxic options. In mice, ketoprofen has been shown to have profound effects on clinical endpoints, reducing cytokine release, and suppressing lymphocyte proportions of Th1 and Th17 cells in a collagen-induced arthritis model.[37] In several mouse models, ketoprofen has been shown to increase TNFα levels which appears to be an effect of the S-isomer of the drug.[66,67,141,158] In pig, ketoprofen can inhibit LPS stimulated cytokine release in vitro, although not in vivo, despite inhibiting PGE_2 under both conditions.[224]

Model Specific Effects of Analgesia

Rodents are commonly used for studies of immunology, inflammation, and infectious disease. A partial list includes vaccine development, antibody production, inflammation induced with CFA or carrageenan, and models of inflammatory bowel disease and arthritis. The majority, if not all, of these studies are completed in rodents without analgesics despite being associated with significant levels of pain. A limited number of infectious disease models have assessed the effects of analgesia on immune endpoints and disease severity or mortality. The following section will discuss the effects of analgesia on immune function and in specific models.

Vaccines and Monoclonal Antibody Production. The administration of vaccines is not generally associated with pain; however, the administration of infectious agents or neoplastic cells that the vaccines are targeting may be associated with significant pain. This is especially true with the recent focus on the use of vaccines and immunotherapies to treat various cancers. Unfortunately, very few studies have attempted to look at the effects of analgesics on vaccine efficacy (see Figure 2). Kolstad and colleagues demonstrated that acetaminophen, meloxicam, and buprenorphine decreased signs of pain in male C57BL/6J mice, but did not decrease the antibody response to immunization with antigen in either CFA or IFA.[108] However, in conflict with this, Filipczak and colleagues showed that the timing of administration and the type of opioid administered affects the cell- and humoral- mediate immune response in CBA mice, with oxycodone having the weakest immunomodulatory properties in mice.[60,61] Another group, who recognized that analgesics are never withheld from cancer patients, specifically studied the effects of physiologically relevant doses of analgesics on an antitumor vaccine. This study found that morphine administered alone suppressed the antitumor effect of the antigen-specific DNA vaccine, but when coadministered with ketorolac, analgesia was provided to female C57BL/6 mice without compromising the antigen-specific immunity and antitumor effect of the naked DNA vaccine.[203]

While vaccines may not be painful, monoclonal antibody production can be associated with significant amounts of pain and distress.[170,178] *In vivo* growth of hybridoma cells, resulting in accumulation of ascites fluid, has been reported to be a source of pain and distress, as has the injection of adjuvants and antibodies used to induce ascites.[202] The effects of morphine on antibody production has been evaluated in a number of studies, and results suggest that it may suppress antibody production in a strain, but not sex dependent manner.[28] More specifically, morphine consistently suppressed the primary antibody response in C3HeB/FeJ, C3H/HeJ, and C57BL/6 but not CxBk/ByJ or Balb/cByJ mice.[28] In addition, C57BL/6J bg^J/bg^J mice, which tend to be less sensitive than other strains to analgesic effects of morphine, were shown to have a decreased capacity to respond to antigenic challenge when implanted with morphine pellets.[29]

In contrast to morphine, clinically relevant doses of meloxicam, buprenorphine, or a combination of both, did not affect antibody production in male BALB/c mice injected with pristane followed by hybridoma cells for antibody production, compared with saline controls.[136]

Due to the variety of immunomodulatory effects seen in vaccine and antibody production studies, caution should be used with any analgesic agent. Partial μ-agonists (for example

Model	Species	Drug	Effect on Pain	Effect on Model	Reference
IFA/CFA immunization	Mouse	Acetaminophen (300 mg/kg PO, water)	↓ signs of pain	No effect	108
		Meloxicam (2 mg/kg SC SID)	↓ signs of pain	No effect	
		Buprenorphine (0.1 mg/kg SC BID)	↓ signs of pain	No effect	
Ascites/macrophage response	Mouse	Morphine (20 mg/kg IP BID)	No comment	↓ HI and CMI	61
		Fentanyl (10 mg/kg IP BID)	No comment	↓ HI and CMI	
		Methadone (30 mg/kg IP SID)	No comment	↓ HI and CMI	
Ascites/macrophage response	Mouse	Buprenorphine (2 mg/kg IP SID)	No comment	↓ CMI, ↑ HI	60
		Oxycodone (20 mg/kg IP BID)	No comment	↓ CMI, no effect on HI	
Vaccine challenge	Mouse	Morphine (5 or 20 mg/kg/day IP)	Delayed tail flick	↓CMI ↑ tumor growth	203
		Ketorolac (2 or 5 mg/kg/day IP)	—	No effect	
		Morphine + Ketorolac	Delayed tail flick	Not tested	
Ascites	Mouse	Meloxicam (2 mg/kg SC SID)	No comment	No effect	136
		Buprenorphine (0.1 mg/kg SC BID)	No comment	No effect	
		Meloxicam + Buprenorphine	No comment	No effect	
Ascites	Mouse	Morphine (75 mg SC pellet)	No comment	↓ antibody production	28,29

Figure 2. Summary of vaccine and antibody production models in which analgesic effects were evaluated. HI – humeral immunity, CMI – cell mediated immunity, SID – once a day, BID – twice a day, PO – by mouth, SC – subcutaneous, IP - intraperitoneal

buprenorphine) or combinations of NSAID and partial μ-agonist can likely be used, but pilot studies may be necessary to identify any potential confounding effects of drug administration.

Inflammation models. Inflammation and associated pain is a primary component of many disease and injury conditions. Inflammatory pain can result from thermal, chemical, or mechanical injuries via nociceptors in the neural system. Mice and rats are used in a variety of different inflammation models that mimic the human condition, most commonly without any analgesia despite the knowledge that these conditions are associated with significant pain in humans. Figure 3 summarizes the effects that analgesics have been reported to have in models of inflammation.

Complete Freund Adjuvant and Carrageenan. An inflammatory state can be created by injecting chemical agents, such as CFA or carrageenan. Plantar intradermal injections of CFA have been used to study the effects of COX isoenzymes and is also a good model for studying novel analgesics for rheumatoid arthritis.[152] Both ketorolac and celecoxib, administered intrathecally, transiently increased expression of inducible COX2 in the spinal cord of male Sprague–Dawley rats with adjuvant induced inflammation and relieved thermal hyperalgesia through blockade of COX.[88] In CFA-induced unilateral paw inflammation in a rodent model, μ and κ agonists decrease the severity of inflammation.[201] Similarly, carrageenan injection induces granuloma formation which has been used to evaluate general antiinflammatory agents. Butorphanol decreased paw inflammation following carrageenan injections, with or without concurrent administration of indomethacin in Sprague–Dawley rats,[210] and acetaminophen reduced inflammatory hyperalgesia without affecting inflammation and central hyperalgesia in male Sprague–Dawley rats.[22] It appears that both NSAID and opioids can have strong inflammation-modulating effects in these models and that their use is best avoided to avoid confounding analysis of the inflammatory response.

Rheumatoid Arthritis. Rheumatoid arthritis (RA) is a painful, chronic, autoimmune disease. Rodent models of rheumatoid arthritis are similarly painful, and significant refinement of these models to improve rodent welfare is necessary. NSAIDs are the mainstay therapy for pain relief in human RA patients, and opioids are rarely used. Although NSAID may provide appropriate analgesia for rodent subjects in models of RA, they can also markedly confound experimental results, by significantly modulating the inflammatory response and decreasing disease severity.[2,54,75,91,228] Opioids have shown variable effects on model endpoints that depend on the animal stock or strain used, type of opioid administered route of administration, and method of arthritis induction.[50,71,213,215] A full discussion of the various effects of both NSAID and opioid analgesic agents can be found in the review by Peterson and colleagues.[171] Because of the mixed response to conventional analgesics, pilot studies should be performed to evaluate the confounding effects of any analgesic and nonpharmacological measures are strongly recommended to enhance animal comfort and welfare.

Inflammatory Bowel Disease (IBD). IBD is a complex inflammatory disease that is generally considered to include both ulcerative colitis (UC) and Crohn disease (CD). Inflammatory lesions are generally limited to the large intestines and rectum in UC, but can occur in any part of the gastrointestinal tract in CD.[175] Regardless of the type of IBD, the condition is generally associated with significant abdominal pain, and requires management with an analgesic regimen in humans. Current work in mice shows that activation of the polymodal ion channel TRPV1 is also associated with chronic abdominal pain in the dextran sodium sulphate model (DSS) of ulcerative colitis.[110] Unfortunately, translational rodent models frequently ignore the pain component of the disease process and analgesics are not commonly provided.

Many different methods are commonly employed to induce experimental inflammatory bowel disease. These are associated with acute and chronic intestinal inflammation and they all recapitulate different aspects of IBD.[53,175,221,222] Pain is an essential feature of IBD and optimal treatment in animals can aid the translation to human medicine, where the challenge of intestinal pain is frequently met with opioids.[24,31] This is because IBD is characterized by periods of remission and reactivation, and NSAID consumption is considered a primary cause of disease reactivation.[57,106] Figure 4 summarizes the effects that analgesics have been reported to have in models of IBD.

In human medicine, it is not uncommon to also use nontraditional analgesic agents to manage the visceral pain associated with IBD.[31,197] This includes: antidepressants, peppermint oil (antispasmodic), 5-HT3 receptor antagonists, nonabsorbed antibiotics (such as, rifaximin), secretogogues, H1-receptor antagonists, Neurokinin-2 receptor antagonists, and GABAergic agents.[31,197] These agents remain largely untested in animals, but may provide alternative means of analgesia for the pain associated with experimental models of both UC and CD.

Model	Species	Drug	Effect on Pain	Effect on Model	Reference
CFA plantar injections	Rat	Celecoxib (20 or 100 ug IT once)	Delayed withdrawal	↑ spinal COX2 expression	88
		Ketorolac (5 or 25 ug IT once)	Delayed withdrawal	↑ spinal COX2 expression	
Carrageenan plantar injections	Rat	Indomethacin (1, 2.5 or 5 mg/kg PO once)	No comment	Dose-dependent ↓ paw edema	210
		Butorphanol (2 mg/kg SC once)	No comment	↓ paw edema	
		Indomethacin + Butorphanol	No comment	↓ paw edema	
Brewer's yeast plantar injections	Rat	Acetaminophen (25, 50, 100 mg/kg PO once)	No significant effect on tail flick	↓ inflammation	22
Adjuvant arthritis	Rat	Meloxicam (1.5 mg/kg PO SID)	No comment	↓diamine oxidase activity	228
				↑ myeloperoxidase activity	
				GI ulceration	
Adjuvant arthritis	Rat	Meloxicam (0.1 or 0.5 mg/kg PO SID)	No comment	↓ paw edema	2
				↓ oxidative stress	
Adjuvant arthritis	Rat	Meloxicam (0.06-0.5 mg/kg PO SID)	No comment	↓ paw swelling	54
				↓ bone and cartilage destruction	
		Piroxicam (0.15–1.35 mg/kg PO SID)	No comment	↓ paw swelling	
				↓ bone and cartilage destruction	
		Diclofenac (0.2–1.6 mg/kg PO SID)	No comment	↓ paw swelling	
		Tenidap (3.1–25 mg/kg PO SID)	No comment	↓ paw swelling	
Collagen-induced arthritis	Mouse	Celecoxib (30 mg/kg PO BID)	No comment	↓ paw swelling	91
				↑withdrawal latency (mechanical and thermal)	
Bacterial-induced arthritis	Rat	Buprenorphine (1 or 2 mg/kg PO BID)	Dose-dependent pain control	↓ paw swelling	213
				↓ bone destruction	
Adjuvant arthritis	Rat	Buprenorphine (0.01, 0.1 or 1 mg/kg SC SID)	No comment	↑ paw swelling	71
				↑ joint destruction	
Adjuvant arthritis	Rat	Morphine (SC, osmotic pump)	No comment	↑ paw swelling	50
				↑ bone demineralization	
				↑bone erosion	
Adjuvant arthritis	Rat	Morphine (10 or 60 mg/kg/day SC)	No comment	↓ arthritic changes	215
		Buprenorphine (0.6 mg/kg/day PO)	No comment	No effect	

Figure 3. Summary of inflammation models in which analgesic effects were evaluated. IT – intrathecal, SID – once a day, BID – twice a day, PO – by mouth, SC – subcutaneous, IP - intraperitoneal

Model	Species	Drug	Effect on Pain	Effect on Model	Reference
DSS colitis	Mouse	Rofecoxib (2.5–10 mg/kg PO, water)	No comment	↓ inflammation	137
DSS colitis	Rat	Indomethacin (1 mg/kg PO SID)	No comment	↑ inflammation	161
		Celecoxib (3 mg/kg PO SID)	No comment		
DSS colitis	Mouse	Buprenorphine (0.05 mg/kg SC BID)	↓ signs of pain	↓ inflammation	24
		Tramadol (20 mg/kg SC SID)	↓ signs of pain	No effect	
Acetic acid colitis	Rat	Methadone (5 or 10 mg/kg SC SID)	No comment	↓ inflammation	55
TNBS	Rat	Meloxicam (3 mg/kg PO SID)	No comment	↓inflammation	103
				Improved contractility	
TNBS	Rat	Diclofenac (10 mg/kg PO BID)	No comment	↑ colitis severity and mortality	26
		Indomethacin (5 mg/kg PO BID)	No comment	↑ colitis severity and mortality	
		Ketoprofen (10 mg/kg PO BID)	No comment	↑ colitis severity	
		Celecoxib (15 mg/kg PO BID)	No comment	No effect	
TNBS	Mouse	Buprenorphine (0.05 mg/kg SC BID)	↓ signs of pain	↓ inflammation, ↑ mortality	24
		Tramadol (20 mg/kg SC SID)	↓ signs of pain	No effect	
TNBS	Mouse	Buprenorphine analog (1 mg/kg IP once)	↓ signs of pain	↓ inflammation	229

Figure 4. Summary of IBD models in which analgesic effects were evaluated. SID – once a day, BID – twice a day, PO – by mouth, SC – subcutaneous, IP - intraperitoneal

Ulcerative Colitis. Dextran sodium sulphate (DSS) causes a progressive chemical injury to the intestinal epithelium, resulting in exposure of the lamina propria and submucosal compartment to luminal antigens and enteric bacteria, thereby triggering inflammation.[100] The effectiveness of DSS-induced UC depends on several factors, including dosage (typically 1% to 5% DSS), duration (acute or chronic), manufacturer or batch of DSS, strain of animals (C3H/HeJ and BALB/c mice strains are more susceptible), sex of animals (male mice are more susceptible), and microbiota of animals (for example germ free compared with SPF).[24,51,100,126,169] Several NSAID and opioids have been evaluated in both mice and rats in the DSS model for their effects on the inflammatory process. Rofecoxib decreased inflammation in male BABL/c mice,[137] whereas indomethacin and celecoxib both worsened the severity of inflammation in both sexes of Wistar rats.[161,195] Interestingly, although celecoxib administration exacerbated inflammation it protected from ulceration.[195] Buprenorphine was generally antiinflammatory in both BALB/c and CD1 mice, whereas tramadol did not affect inflammation, based on scoring of gut histology. Both treatment regimens appeared to provide adequate analgesia, and the authors recommend tramadol for future studies in either strain of mice.[24]

Oxazolone causes a superficial inflammatory acute colitis that is limited to the distal colon.[100, 126, 226] Animals demonstrate weight loss, diarrhea, ulcers, and loss of epithelial cells in the large intestines. Although rodents are anesthetized for intrarectal administration of Oxazolone, to the authors' knowledge, there have been no studies on the effects of analgesics, nor has analgesic use been documented in this model.[100,126,226]

Acetic acid administration causes a chemical injury to the mucosal epithelium that induces a transient phenotype mimicking UC.[53,126,131] The injury is characterized by ulceration of the distal colon and crypt abnormalities that begin to heal within days in mice and a few weeks in rats.[53,126,131] Few studies have evaluated the effects of analgesics in this model. In one study, specifically looking at the gastroprotectant effects of opioids, methadone improved macroscopic and microscopic disease scores of colitis in male Wistar rats previously treated with acetic acid.[55]

Crohn Disease. In the 2,4,6-Trinitrobenzene sulphonic acid (TNBS) model of Crohn disease, TNBS disrupts the epithelial layer of the colon and exposes the underlying lamina propria to bacterial components that lead to a severe transmural infiltrative colitis.[100] Colitis is associated with diarrhea, rectal prolapse, and weight loss. Several NSAID and opioids have been evaluated in both mice and rats in the TNBS model for their effects on the inflammatory process. Administration of rofecoxib reduced the colonic damage and inflammation in Wistar rats.[138] Administration of meloxicam to male Sprague–Dawley rats restored colonic contractility and decreased colonic inflammation.[103] Diclofenac, indomethacin, and ketoprofen all exacerbated colitis in male Wistar rats, but celecoxib had no significant effect.[26] In BALB/c mice, tramadol administration did not affect inflammation, but buprenorphine was antiinflammatory.[24] BU08070, a buprenorphine analog, produced a concentration-dependent decrease in inflammation and visceral pain-induced behaviors in male BALB/c mice.[229]

To keep murine models of UC and CD consistent with human treatments, opioids are generally recommended as therapeutics to decrease model associated discomfort and improve animal welfare. However, pilot studies are warranted to evaluate for potential confounding effects of opioid or NSAID analgesia in the specific model type and species. Based on the collective body of literature described herein, tramadol should be considered for IBD studies due to its clinical efficacy for relieving visceral pain and its lack of modulatory effects on inflammation.

Infectious Disease Models. Most extant studies on the effects of analgesics on immune function and disease in infectious disease models have used NSAID to explore the role of COX and prostaglandins in disease pathogenesis.[113,144,145,156,196,218] The one notable exception is a study on the effect of buprenorphine in a mouse model of intracranial lymphocytic choriomeningitis virus (LCMV) infection.[155] Intracranial LCMV in mouse is used to model CTL-mediated meningitis, and produces characteristic fatal meningitis 6 to 8 d post infection, which may be associated with significant pain and distress.[99] Mice intracranially infected with LCMV and treated with buprenorphine (0.05 mg/kg s.c) followed by osmotic pump delivery (0.15 mg/kg/day) for 1 wk, had markedly reduced pain scores and no clinical signs of pain.[155] Buprenorphine treatment had no effect on LCMV-induced CTL responses or LCMV induced brain infiltration by lymphocytes and virus specific CTLs.[155]

Conclusion

The balance between appropriate analgesic use for animal welfare, and analgesic impact on experimental results continues to present significant challenges to the research community.[171] Furthermore, relatively little is currently known about the role of gender in the interaction between analgesics and immune function. However, gender has a major influence on both the prevalence and severity of pain and sex related differences in neuroimmune interactions (in particular glial cell function) appears to underpin this phenomenon.[49,96,134] Thus in light of NIH directives, better understanding of gender-related differences in the effects of pain and analgesia on neuroimmune function in preclinical studies is critically important. In human medicine, archaic concepts such as "pain medication may mask clinical signs" and "nobody ever died from pain" have been refuted by years of research and clinical experience. It would be unethical and malpractice to withhold analgesics from human patients experiencing pain from cancer, autoimmune disease, infection or the innumerable other diseases which cause pain. In this context, the possibility should be considered that in some instances the translatability of animal models may be improved if analgesics are administered, not withheld, and used in a manner that more closely matches human treatment.[39,90] What is clear from this review is that many questions remain regarding the impact of analgesics on immune function and that there is no one drug that represents the "Magic Bullet" analgesic for all models. In many cases, the literature is incomplete, or does not exist, necessitating empirical choices or pilot studies to evaluate or optimize the use of analgesics for *in vivo* studies of immunology and inflammation. Responsibility for appropriate analgesic drug use in the absence of published data lies with the investigator, and is shared with laboratory animal veterinarians and IACUC members. Our hope is that research and development of new analgesic drugs and regimens will progress and help improve our ability to appropriately manage pain and minimally impact experimental results

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