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1 **Title: Inflammation without pain: immune-derived opioids hold the key**

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1 **Abstract.**

2 Visceral pain is commonly associated with acute or remitting inflammatory bowel disease
3 (IBD). In marked contrast, chronic IBD is often painless, even in the presence of active
4 inflammation. This suggests that inflammation in itself is insufficient to sustain altered
5 nociceptive signaling and raises the possibility that there is an endogenous analgesic system in
6 effect in chronic disease. A new study by Basso *et al.* published in this issue of
7 *Neurogastroenterology & Motility* provides additional support for an immune-mediated
8 mechanism that suppresses visceral hypersensitivity. The authors examined visceral pain in the
9 IL-10-piroxicam model of chronic colitis, which differs from other experimental IBD models
10 in that it involves immune suppression. During active inflammation, responses by these mice
11 to graded increases in colorectal distension were equivalent to healthy controls, consistent with
12 normal afferent signaling. However, treatment with a peripherally-restricted opioid receptor
13 antagonist resulted in marked visceral hypersensitivity to the same stimuli. This effect was
14 attributed to the production of endogenous opioids by colitogenic CD4⁺ T cells present in the
15 mucosa. This minireview provides a brief overview of analgesia by immune-derived opioids
16 under inflammatory conditions and highlights how the work of Basso *et al.* contributes to this
17 area of research. Potential pharmacological approaches to harness or mimic this system are
18 provided. These strategies may prove to be an effective means through which targeted and
19 sustained relief of IBD pain may be achieved.

1 | Introduction

Pain with inflammation, inflammation without pain. Hypersensitivity of afferent signaling to colorectal distension is associated with clinical IBD, is proportional to inflammatory damage, and is believed to underlie visceral pain¹. This is replicated in commonly used models of experimental colitis, allowing detailed interrogation of the mechanisms that initiate, amplify, and sustain aberrant nociceptive signaling¹. However, an unexpected finding is that pain does not always accompany overt inflammation, as evidenced by the absence of visceral pain in a subset of patients with chronic IBD^{2,3}. A recent study by Zeitz and colleagues found that in a cohort of 1263 patients with IBD (UC or CD), 29% experienced no associated general pain during the course of the disease⁴. These clinical observations can be replicated experimentally. Architectural damage, erosion and immune cell infiltration at the site of inflammation are comparable in tissues from mouse models of acute and chronic intestinal inflammation. However, acute colitis is associated with greater sensitivity to colorectal distension⁵. This raises questions about the pain signaling pathways in these subsets of patients and whether the nature of this signaling is determined by the differential release of endogenous analgesic and algesic mediators within the tissue. Given the roles of innate and adaptive immune mechanisms in the development of IBD, varied pain symptomatology may result from differing neuro-immune interactions, although direct evidence of this is still lacking.

Immune-derived opioids suppress pain signaling. Insight into why visceral hypersensitivity and reported pain may be absent in IBD comes from earlier studies of somatic inflammatory pain. These studies described the involvement of immune-derived opioids in the suppression of pain and inflammation, as well their targeted release at sites of inflammation^{6,7}. Tissue damage leads to hyperexcitability of peripheral afferents and to associated central sensitization. A naturally occurring analgesic mechanism operates both in the periphery and centrally, where endogenous opioid peptides inhibit nociceptive neurons through activation of opioid receptors. This neuroimmune interaction was initially described in rodent models of somatic pain⁷ and has been further validated in clinical studies of pain associated with knee surgery⁸. Subsequent studies demonstrated that opioidergic analgesia also occurs in neuropathic pain⁹. This discovery represented a major conceptual advancement away from models in which immune mechanisms only drive algesic and inflammatory changes.

An equivalent mechanism was identified in the gastrointestinal tract by Verma-Gandhu *et al.* (2006)¹⁰ using mouse models of chronic colitis. They reported that immune-deficient SCID mice exhibited greater sensitivity to colorectal distension relative to immunocompetent mice. This was normalized by T cell transfer, supporting the role of the immune system in regulating visceral afferent activity. Subsequent studies demonstrated that acute DSS colitis is associated with visceral pain, whereas mice with more prolonged or chronic colitis exhibit similar responses to colorectal distension to that of healthy controls^{5,11,12}. In chronic colitis, these reduced visceral pain scores were associated with the accumulation of antigen primed CD4⁺ T lymphocytes at inflamed sites⁵. This hypothesis is further supported by electrophysiology studies, where exposure of dorsal root ganglion (DRG) neurons to supernatants derived from colon tissue of mice with chronic DSS colitis have suppressed neuronal excitability, consistent with analgesia¹¹. These effects were mediated via a naloxone-sensitive mechanism, further supporting the importance of immune-derived opioids in the control of visceral hypersensitivity¹¹. An equivalent mechanism is likely to operate in IBD. Guerrero-Alba *et al.* (2016)¹³ reported **inhibition** of neuronal excitability following incubation

of mouse DRG neurons with colonic tissue supernatants from patients with chronic ulcerative colitis.

2| How does Basso *et al.* advance this concept?

In their study, Basso *et al.* examined experimental colitis in IL-10 deficient mice, a model which replicates similar defects in IL-10 signaling and expression associated with early onset IBD and IBD susceptibility in humans¹⁴. It suggests that this analgesic mechanism would still operate in IBD patients with loss of function mutations to IL-10. This model differs from those more commonly used to study visceral hypersensitivity, including TNBS and DSS colitis¹, as it involves suppression, rather than hyperactivation of the immune response. They demonstrated that there were equivalent visceromotor reflex responses to colorectal distension of the healthy and inflamed colon. An endogenous analgesic mechanism in mice with colitis was revealed following dosing with the peripherally-restricted non-selective opioid receptor antagonist naloxone methiodide. This group has previously described an equivalent mechanism using an adoptive T-cell transfer model of chronic colitis in SCID mice¹². In a follow-up paper, the authors then identified colitogenic T cells as the source of opioid release and demonstrated that transfer of T cells from mice deficient in proenkephalin resulted in greater inflammatory damage and to visceral hypersensitivity¹⁵.

3| Can this endogenous analgesic mechanism be enhanced to target pain at the source?

This study by Basso *et al.* is largely confirmatory, providing evidence for an equivalent analgesic mechanism in a different experimental model. The next steps for this field are to define means to harness this endogenous-opioid system and thereby develop novel therapeutic strategies for the treatment of pain in acute inflammation or IBD.

Limitations impacting the long-term use of orthosteric opioid receptor agonists, such as morphine, for pain-relief are well-documented. Central modulation of opioid receptors is associated with significant and life-threatening side-effects including respiratory depression, dependence and tolerance. Opioids can also exert their analgesic actions through a peripheral mechanism¹⁶. However, prolonged administration of opioids can lead to intractable constipation through direct actions on the enteric nervous system¹⁷, which can impact patient compliance. Therapies that augment the endogenous opioidergic tone therefore represent an attractive strategy as they largely avoid these adverse effects. Furthermore, approaches that suppress afferent signaling may have the added benefit that they reduce or prevent associated central plasticity associated with chronic pain. Several features make immune-derived opioid analgesia an attractive target for therapeutic modulation. There is evidence for upregulated opioid receptor expression, trafficking to afferent terminals and G protein-coupling in nociceptive neurons, and for reduced opioid tolerance¹⁸. As outlined above, production of opioids is elevated in colitis where they play a pivotal role in visceral pain. Moreover, this mechanism is also likely to influence nociception and pain in human IBD. There are a range of pharmacological approaches that could be taken to enhance analgesia arising from immune-derived opioids (summarized in Figure 1). These include:

1. Enkephalinase inhibition. Inhibition of enkephalin-degrading enzymes, such as neprilysin (NEP) and aminopeptidase N, will effectively increase the duration and magnitude of analgesia by preventing the degradation of locally-released opioid peptides. Proof of concept is provided by evidence that enkephalinase inhibition reduces somatic and visceral pain in rodents^{19,20}.

Moreover, prolonged administration of enkephalinase inhibitors is not associated with antinociceptive tolerance²¹. This approach is predicted to be safe based on the use of the clinically-approved neprilysin inhibitor Racecadotril (acetorphan) for the treatment of diarrhea. There is some suggestion that this could be effective for treatment of visceral hypersensitivity in humans. Patients with acute diarrhea reported greater and more sustained reduction in abdominal pain following Racecadotril treatment relative to vehicle²² or loperamide²³. However, the complex effects of opioids on gastrointestinal function complicates interpretation of this observation. Whether this reduction was due to an antispasmodic or dampening effect on GI motility, reduced distension due to hypersecretion, or an action on visceral afferents cannot be determined²⁴. NEP inhibitors also prevent breakdown of pronociceptive and proinflammatory neuropeptides, such as substance P and bradykinin, which could potentially result in exacerbation of symptoms²⁵. Naturally occurring enkephalinase inhibitors with pain-relieving actions have been identified^{26,27}. The effects of opiorphin and sialorphin on the visceromotor response to colorectal distension were recently assessed, with only sialorphin having a significant inhibitory action²⁰. It is possible that greater efficacy may be achieved in inflammation-induced models of visceral afferent hypersensitivity or with improved methods of delivery.

2. Allosteric modulation. Positive allosteric modulators (PAMs) bind to G protein-coupled receptors (GPCRs) at topographically distinct sites to orthosteric ligands, leading to potentiation of receptor signaling^{28,29}. PAMs of the mu and delta opioid receptor subtypes have been characterized³⁰. These provide a pharmacological mechanism through which endogenous signaling may be selectively enhanced when and where this occurs, thereby retaining the spatiotemporal dynamics required for normal physiological processes. Importantly, these PAMs may provide specific modulation without intrinsic agonist activity, potentially limiting side-effects commonly associated with orthosteric opioid receptor agonists. If latent visceral pain in IBD is revealed as immune-derived opioid release is diminished, it may be possible for PAMs to compensate for this reduction in input through amplification of opioid receptor signaling at afferent terminals.

3. pH-sensitive opioid drugs. Strategies that favor or target delivery of opioid drugs to peripheral sites of inflammation are predicted to lead to analgesia without adverse effects. As outlined above, tissue acidosis is associated with inflammation and with pain. Computational modeling of ligand binding to the mu opioid receptor was recently used to develop NFEPP, a fluorinated fentanyl derivative with greatest activity at low pH³¹. Assessment in an inflammatory pain model exhibited effective analgesia with minimal on target side-effects including respiratory depression and delayed colonic transit. Importantly, this study demonstrated that beneficial aspects of the endogenous analgesic mechanism could be achieved through use of small molecules. NFEPP was subsequently examined in the acid writhing model of abdominal pain³². Although efficacious via a peripheral mechanism, analgesic activity has not been assessed in an inflammation-induced visceral pain model, such as colitis or pancreatitis.

4| Conclusion.

There is compelling evidence to support the existence of endogenous immune-derived analgesic mechanisms in inflammatory disease. The next significant challenge is to effectively harness this as a therapeutic strategy to selectively enhance and prolong this targeted activity.

1 There are several promising approaches currently being investigated, with great potential to
2 alleviate the debilitating visceral pain associated with IBD.

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Fig 1. Endogenous pain relief in the inflamed colon and pharmacological strategies to harness this mechanism. Chronic inflammation is associated with the peripheral release of opioids from mucosal CD4⁺ T cells. These opioids activate mu and delta opioid receptors expressed on sensory afferent terminals, activation of which leads to a suppression of action potential firing and to reduced sensitivity^{33,34}. This inhibits release of proinflammatory and algescic neuropeptides in the periphery and at the dorsal horn of the spinal cord, reducing neurogenic inflammation and nociception, respectively. **1.** Block of enkephalin-degrading enzymes effectively increases the amount and duration of their action. **2.** pH-sensitive opioid receptor agonists or nanoparticulates favor receptor activation in the acidic microenvironment of the inflamed colon. **3.** Positive allosteric modulators of opioid receptors enhance their responsiveness to endogenous opioids.

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