Can inflammation relieve pain?

Immune cells secreting the opioid peptide β-endorphin undergo selectin-mediated migration to peripheral sites of injury where they promote analgesia (pages 1425–1428).

FRANK PORRECA, JOSEPHINE LAI & T. PHILIP MALAN, JR.

NEW RESEARCH FINDINGS continue to advance our understanding of the central and peripheral nervous system pathways that regulate the neural detection of pain (nociception) in the post-injury state. In this issue of *Nature Medicine*, Machelska *et al*.[1] now show that cells of the immune system are key players in promoting peripheral analgesia after tissue injury. They show that immunocytes producing the opioid peptide β-endorphin migrate preferentially to sites of inflammation (in this case, the inflamed hind paw of the rat) where they release β-endorphin, which binds to opioid receptors on sensory nerve terminals and blocks the pain response. The immunocyte migration is selectin-dependent because treatment with fucoidin (which binds to selectins) blocks immunocyte migration through the vascular endothelium into the inflamed tissue and abolishes analgesia. This physiological mechanism of peripheral analgesia may form the basis of a new class of drugs that would be analgesic/anti-hyperalgesic as well as inhibitors of neurogenic inflammation.

There are many mechanisms through which the central nervous system facilitates and maintains the pain response after peripheral injury. It has long been appreciated that the central nervous system has evolved mechanisms to transmit high intensity pain stimuli after tissue damage. The presence in central nociceptive nerve terminals of peptide neurotransmitters, such as substance P, combined with the release of the excitatory amino acid glutamate, results in temporal summation. This alters the response characteristics of the second order cells in the spinal dorsal horn to sustained C-fiber input (a process called 'wind-up'[2]), and the post-translational modification of excitatory amino acid receptors resulting from such sustained input results in a state of 'central sensitization'[3]. Furthermore, the release of substance P and activation of its receptors increases the likelihood of depolarization of the post-synaptic cell and the enhancement of glutamate and glutamate (NMDA) receptor activity. The presence of presynaptic NMDA receptors on the central nerve terminals is also likely to promote further release of glutamate[4], resulting in an increase in synaptic strength[5]. Other examples of central pain facilitation mechanisms include the presence at supraspinal sites of so-called 'on cells' that appear to act as the focal point of a descending pain facilitation system[6], the purpose of which remains unclear. Such mechanisms have evolved, apparently, to increase survival through the rapid and sensitive detection of harmful stimuli.

Perhaps not surprisingly, however, the central nervous system has also developed mechanisms that ameliorate the transmission of nociceptive signals in response to chronic pain states such as those following injury. In addition to central pain facilitation mechanisms, a relatively well-characterized descending analgesic circuit that modulates nociceptive information has been identified[6]. Tonic activity of inhibitory neurotransmitters, including GABA, glycine and endogenous opioids clearly contribute to the negative modulation of the pain signal[7]. That the nociceptors detecting pain are pseudounipolar cells ensures the facilitation of pain through efferent activity of afferent neurons (the axon reflex of Lewis), which is partly responsible for the sensitization of peripheral nociceptors[8]. These local events activate peripheral nociceptors but also sensitize adjacent nociceptor nerve terminals, thus allowing for a diffuse pain response to injury. Moreover, it is well established that injury results in the release of an inflammatory 'soup' from injured cells and blood vessels, which in turn promotes inflammation and further nociception[9]. An additional complexity of this process is the invasion of the injured tissue by immune cells, which release cytokines that promote inflammation and further facilitate the transduction and transmission of the pain signal[10].

Although we understand many aspects of central pain modulation, our knowledge of possible endogenous mechanisms that negatively modulate nociceptive transmission in the periphery is less advanced. Potentially important mechanisms appear to have evolved that act locally to terminate the consequences of the injury state and associated pain. Thus, it has been hypothesized that inflammatory consequences of peripheral injury are modulated by the so-called 'Autocoid Local Inflammatory Antagonism' (ALIA) mechanism. This is mediated in part by the release from mast cells of palmitoylethanolamides that are thought to be endogenous agonists of the cannabinoid CB2 receptor (which mediates analgesia in the peripheral nervous system)[11].

Now, the report by Machelska and colleagues[1] identifies a new and exciting mechanism for the control of local nociception. They propose that selectins mediate the invasion of injured tissue by immunocytes that secrete β-endorphin. Blockade of selectins by fucoidin prevents immunocyte localization in the inflamed hind paw of rat and reduces local β-endorphin content. The presence of cells containing β-endorphin in the inflamed site, together with the release of this opioid peptide in response to the systemic stress that accompanies severe pain, provides a mechanism for dampening the transmission of the pain signal by activation of opioid receptors expressed on the peripheral nociceptor terminals.

Presumably, drugs that promote the selective recruitment of opioid-positive immune cells to the site of injury via selectin-mediated adhesion may enhance these peripheral analgesic effects. In addition, it is possible that blocking adhesion molecules may prevent the accumulation of opioid-containing cells and might serve to promote local pain control. Although the findings are exciting, several points need to be addressed before any clinical potential becomes clear. First, Machelska *et al*. demonstrate that fucoidin treatment only partially blocks the accumulation of opioid-containing cells in inflamed sites and the anti-hyperalgesia induced by corticotrophin releasing factor (CRF; an important promoter of the response to stress). This indicates that other facilitatory or independent mechanisms are involved in recruiting these immune cells. Thus, it is not completely clear how effective selectins would be as targets for

drugs promoting the recruitment of opioid-producing cells. Second, the inhibition of selectin binding by fucoidin occurs without any change in the baseline pain sensitivity response. Fucoidin treatment only blocks analgesia induced by either CRF or the physiological response to systemic stress (in this case, the cold water swim test).

These findings are consistent with the induction of local anti-hyperalgesic effects by immune-cell release of β-endorphins. Although it is possible that in some cases the inflammatory and nociceptive response to injury is sufficient to promote a systemic stress response capable of causing opioid release, in other cases the immune cell-mediated analgesic activity may require the co-administration of agents such as CRF receptor agonists. Thus, analgesics that promote the adhesive interactions of opioid-positive immune cells alone may be inadequate. An essential, and as-yet undetermined, point is whether selectin-mediated immunocyte invasion relieves other types of pain besides that caused by inflammation.

Although there is an obvious need to address these and other issues, the observations of Machelska and co-workers provide exciting and provocative evidence for the existence of additional local pain regulatory mechanisms that integrate immune, opioid and nociceptor function. Such new information is sure to provide fertile ground for exploration of novel approaches to pain control, including the development of analgesics that target the immune system and lack the side effects of current pain killers.

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Departments of Pharmacology and Anesthesiology
University of Arizona Health Sciences Center
Tucson, Arizona 85724, USA
email: frankp@u.arizona.edu

Malaria: A 21st century solution for an ancient disease

The sequencing of the *Plasmodium falciparum* genome should reveal new parasite protein targets for drug and vaccine development.

DYANN WIRTH

DESPITE MASSIVE EFFORTS to eradicate malaria in the 1950s and early 1960s, today there are more humans infected with malaria than at any other time in history. More than 500 million people are infected with malaria worldwide, and one fourth of the world's population is at risk of infection. Furthermore, at least 2.5 million children die each year of malaria, most of them in Africa. Those children surviving chronic infection suffer a combination of anemia and immune suppression that leaves them vulnerable to other fatal illnesses. Drug resistance in the parasite is now widespread, further compromising prevention and treatment strategies. Malaria has confounded some of the best minds of this century. A hundred years after the discovery that mosquitoes transmit malaria, we still do not know enough about the disease to defeat it permanently[1]. However, a principal milestone in the field of malaria research has now been reached with the recent *Science* paper by Gardner *et al*.[2] that presents the full-length sequence of one of the 14 chromosomes of *Plasmodium falciparum*, the organism that causes the most deadly form of human malaria. This sequencing achievement is the first for an organism as complex as *P. falciparum* and is particularly notable because it was feared that the intensely AT-rich *Plasmodium* genome would prove intractable to even the most sophisticated sequencing technology.

The Malaria Genome Sequencing Project is a cooperative effort between three sequencing centers—The Institute for Genomic Research (TIGR), the Sanger Center and the Stanford Genome Center—and the malaria research community with the goal of sequencing the entire genome of *P. falciparum*. It is sponsored by the National Institutes of Health, the Burroughs Wellcome Fund, the U.S. Department of Defense and the Wellcome Trust, and seeks to define every gene in the organism and, armed with this information, to identify new targets for drug and vaccine development. The sequence of chromosome 2, reported by the TIGR and Department of Defense groups[2], is proof that it will be possible to achieve this goal.

The *P. falciparum* genome presents the sequencing 'gurus' with a number of technical hurdles to overcome. Most prominent of these is its remarkably high A+T content (80.2 percent in chromosome 2). The sequencing of chromosome 2 required the investigators to adapt standard cloning techniques, modify computer programs that assemble the sequence, and develop new methods to finish the sequence analysis. The entire genome is approximately 30 Mb in length and is arranged in 14 individual chromosomes that vary in length between wild-type isolates.

Chromosome 2 is just under 1 Mb in length and contains 209 predicted open reading frames. By comparing gene sequences in the parasite with those of other organisms, the investigators were able to identify homologues for about 40 percent of the genes, with the remaining 60 percent falling into the class of unidentified open reading frames. This percentage of unidentified open reading frames is substantially higher than that reported in other organisms. The authors suggest that this may be due, in part, to the unusually high percentage of