Bacteria's painful truth

By C. Simone Fishburn, Senior Editor

In a surprise finding likely to alter how neurologists, immunologists and microbiologists view infection-associated pain, a group at **Boston Children's Hospital** has shown that bacteria can directly trigger action potentials in pain fibers, leading to the release of neuropeptides that suppress the inflammatory response.[1] The findings point to a role for sensory neurons as immune modulators and could provide new bacterial targets for pain.

Until now, the immune system has been considered the primary instigator of pain from bacterial infections. Activation of immune cells triggers the release of inflammatory mediators such as cytokines, growth factors and prostaglandins, which are thought to cause pain by activating receptors on nerve terminals.

More recently, the recognition of bacterial patterns by toll-like receptors (TLRs) on sensory neurons has been pegged as an additional contributing mechanism, although the full picture remains unclear.[2]

Clifford Woolf and colleagues set out to shed some light on the mechanism by identifying specific inflammatory mediators responsible for acute bacterial pain. Unexpectedly, the team discovered that the pain and inflammatory responses were not coordinated and that the traditional model might not be accurate.

Woolf is director of the F.M. Kirby Neurobiology Center at Boston Children's Hospital and a professor of neurology and neurobiology at **Harvard Medical School**.

"The first major clue something interesting was going on was that the pain occurred out of sync with the immune response," Woolf told *SciBX*.

To identify pain-promoting cytokines active in the acute stage of bacterial infection, the group injected mice with a community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) strain linked to wound infections. The group then tracked the timing of pain response, mobilization of inflammatory markers and bacterial load.

The pain response peaked at six hours postinfection, but the mobilization of immune markers such as neutrophils and inflammatory cytokines only started to occur at that time point and reached maximal levels 24–72 hours after infection.

Bacterial concentrations were the sole marker whose time course corresponded with pain, suggesting the organisms themselves may play a role in generating the hypersensitivity response.

To test this, the team applied live or heat-inactivated bacteria directly to dorsal root ganglia neurons, looking for differences that might reveal elements in live bacterial infections that cause pain.

In both cases the researchers detected increases in calcium flux and nerve firing, confirming that bacteria can directly activate sensory nerve fibers (nociceptors).

However, the live and heat-treated bacteria activated different subpopulations of nociceptors, suggesting that distinct heat-stable and heat-sensitive factors might be involved.

Several bacterial strains reproduced the results of CA-MRSA following heat inactivation, including *Streptococcus pneumonia*, *Helicobacter pylori* and *Pseudomonas aeruginosa*, providing a clue that the heat-stable component might be a common secreted bacterial factor.

The team focused on bacterial N-formylated peptides because they are heat-stable compounds that were recently associated with olfactory sensation.[3] The group determined that *N*-formyl-methionyl-isoleucyl-phenylalanine-leucine (FMIFL) was the heat-resistant factor from *S. aureus* and established that FMIFL interacts with formyl peptide receptor 1 (FPR1) on mechanical nociceptors.

In live dorsal root ganglion cultures, the heat-sensitive bacterial factor that triggered bacterial pain was identified as the pore-forming toxin α-hemolysin (αHL). Although pore-forming toxins have been widely studied as bacterial virulence factors, the new findings are the first demonstration of a role in sensory pain.

The team showed that αHL binds to nerve fibers via the membrane anchor ADAM10, creating pores in the neuronal membrane that cause an influx of calcium and trigger action potential firing.

These action potentials travel along the nerve both toward and away from the spinal cord. Signals that travel toward the spinal cord enter the CNS and send pain messages to the brain, whereas those traveling in the opposite (antidromal) direction stimulate the release of neuropeptides at peripheral nerve terminals (*see* **Figure 1, "A dual role for nociceptors"**).

To determine the role of these neuropeptides, Woolf's team created knockout mice lacking the NaV1.8 (PN3; SCN10A)-lineage

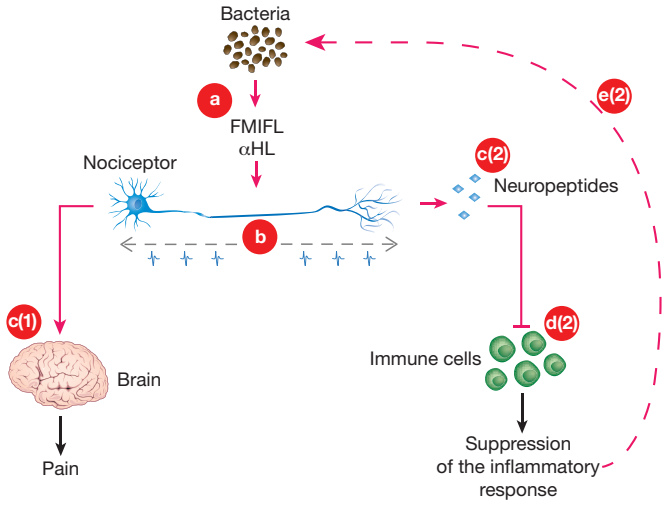

Figure 1. A dual role for nociceptors. Bacteria release factors, such as *N*-formyl-methionyl-isoleucyl-phenylalanine-leucine (FMIFL) and α-hemolysin (αHL), that directly stimulate nerve fibers [a] and produce action potentials in two directions [b]. In one direction, action potentials travel toward the spinal cord and send pain messages to the brain [c(1)]. In the other direction, they travel toward the periphery, causing the release of neuropeptides [c(2)]. The neuropeptides inhibit the activation of immune cells [d(2)], decreasing their ability to clear the bacteria [e(2)] and showing an immune-suppressive role for nociceptors that accompanies their role in pain transmission.

nociceptors, the main neurons involved in the bacterial pain response. Unexpectedly, this resulted in lymph node enlargement and an increase in tissue swelling and greater numbers of neutrophils and monocytes at the sites of infection compared with no knockout.

That finding led the researchers to conclude that the nociceptors themselves, via the neuropeptides, suppress immune activation.

Through a series of microarray analyses and *in vitro* experiments, the researchers identified three neuropeptides—calcitonin gene-related peptide (CGRP), galanin (GAL) and somatostatin—that are highly expressed in sensory neurons and prevent macrophages from releasing inflammatory cytokines.

Together, the data from the extensive study generated two key findings. The first is that pathogens can directly activate nociceptors. The second is that this activation causes the release of factors that modulate the immune response.

Data were published in *Nature*.

"As a body of work this is a paradigm shift in how bacteria cause pain," said Michael Gold, a professor of anesthesiology at the **University of Pittsburgh School of Medicine**. "Traditionally, it has been all about recruitment and activation of immune cells, leading to them releasing various factors that act on nerve cells. Now they are showing the bacteria to be the main modulators."

Victor Nizet agreed and told *SciBX* that the finding that nociceptors serve an immune function at the same time as mediating pain extends beyond neurology. "It's almost like discovering a new immune cell, the nociceptor," he said.

Nizet is a professor of pediatrics and pharmacy at the **University of California, San Diego** and specializes in molecular microbiology and innate immunity.

He added, "In addition to skin cells and other epithelial cells that produce molecules that control and kill bacteria, you see that the nociceptor now also participates in the immune response. Understanding this mechanism could lead the way for preventing adverse consequences."

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Painful remedies

The therapeutic targets identified by the paper are the bacterial proteins FMIFL and αHL or their points of entry, FPR1 and ADAM10. However, based on ongoing research, Woolf believes that these may represent only two of several bacterial protein mediators of pain. Thus, he thinks hitting these targets may have only a limited analgesic effect.

Nonetheless, according to Nizet, the findings provide two new handles for attacking bacteria such as MRSA.

The first is the bacterial virulence factors, in this case αHL, which can be targeted to render the pathogen harmless. Nizet said that this approach has been exploited for antivirals but underutilized for antibacterials, which have relied heavily on traditional antibiotics that kill or inactivate the organisms.

The second handle is the nociceptor, which has immunomodulatory properties. Blocking the release of neuropeptides could remove the brake on the immune system, thus enhancing the local immune response and enabling the body to clear the bacteria, he told *SciBX*.

David Yeomans, an associate professor of anesthesiology and perioperative and pain medicine and director of pain research at **Stanford University**, was more cautious about the translational potential of the findings.

Yeomans said that the study is mechanistically groundbreaking but creating analgesics that block pain transmission in nociceptors now appears to carry the risk that it could remove a necessary brake on the immune system and result in local inflammation.

"Until now not a whole lot of attention has been paid by the pain people or the anti-infective people to each other's fields, but this may make them communicate more," he told *SciBX*.

The findings have not been patented.

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