$CD4^+$ T-Cell Modulation of Visceral Nociception in Mice

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Background & Aims: **Although inflammatory and immune cells are present in the gut in the absence of pathology, their presence does not result in sensitization of sensory nerves, implying the existence of a local antinociceptive influence. We hypothesized that a component of the immune system exerts an antinociceptive influence, thus enabling the gut to function in the absence of undue pain or discomfort.** ***Methods:*** **Visceromotor responses to colorectal distention were measured in mice with severe combined immune deficiency (SCID) and their wild-type controls.** ***Results:*** **SCID mice exhibited significantly lower pain thresholds. Transfer of $CD4^+$ T, but not B lymphocytes, normalized visceral pain in these mice. The restoration of normal visceral nociception following T-cell reconstitution in SCID mice was blocked by naloxone methiodide. Using an enzyme immunoassay and immunohistochemistry for β-endorphin, we showed that in vitro stimulation of T lymphocytes induced the synthesis and release of β-endorphin and that transfer of T cells into SCID mice increased the expression of β-endorphin in the enteric nervous system.** ***Conclusions:*** **These findings indicate that the immune system is a critical determinant of visceral nociception and that T lymphocytes provide an important opioid-mediated antinociceptive influence in the gut.**

The nervous system is involved in host defense and serves to provide early warning of imminent danger. The sensation of pain alerts the host to the threat of injury and results in the generation of avoidance or other protective responses. Although the induction of pain is a critical component of host defense, the persistence of pain is unhelpful in that it may interfere with wound healing[1] and may become chronic and debilitating to the host.

The primary role of the immune system is to protect the host through the identification, containment, and elimination of noxious agents. Initially, the role of the immune system is to orchestrate an inflammatory response to contain and neutralize pathogens and other injurious agents. In the longer term, the immune response is critical for the successful repair of the tissue.

Classically, the immune and nervous systems had been thought to work in isolation, but it is becoming increasingly evident that these systems work together in several biologically important contexts. For example, it has recently been shown that exposure to a bacterial toxin elicits a vagal reflex that down-regulates the inflammatory response.[2] Conversely, the immune system signals the brain during intestinal inflammation to modify feeding behavior through the elaboration of cytokines in the peripheral and central nervous systems.[3]

Products of the innate and adaptive immune responses contribute to the activation and sensitization of primary sensory neurons following tissue injury.[4] The sensitization of sensory nerves in the periphery may result in changes in neural function in the spinal cord and brain to produce a persistence of pain. However, in most instances, pain does not become chronic, implying the existence of a system that is capable of efficiently down-regulating pain at the site of the original injury.

Studies in the field of somatic pain have identified an antinociceptive role for lymphocytes using a model of inflammation-induced analgesia in the rat hind paw. Inflammation induced by Freund's complete adjuvant resulted in the release of opioids from lymphocytes and a subsequent increase in pain thresholds.[5] It should be emphasized that this antinociceptive role for lymphocytes was identified in a model of local tissue injury and inflammation; a role for lymphocytes in maintaining normal somatic pain thresholds has not been identified.

The gastrointestinal tract is constantly exposed to a large number of bacteria and has evolved a sophisticated mucosal immune system that avoids or limits penetration of the commensal intestinal bacteria. As a result, there is a significant inflammatory and immune cell

Abbreviations used in this paper: CRD, colorectal distention; LMMP, longitudinal muscle myenteric plexus; NLXM, naloxone methiodide; SCID, severe combined immune deficiency; VMR, visceromotor response.

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presence in the gut in the absence of pathogenic bacteria or other overt inflammatory stimuli. Moreover, recent work has shown that subtle changes in resident inflammatory or immune cell activity in the gut alter visceral pain thresholds.[6] Thus, although afferent nerves are readily receptive to the sensitizing effects of inflammatory or immune cell products, abdominal pain and discomfort only occur following overt injury in otherwise healthy subjects. This raises questions as to whether there is an antinociceptive influence in the gut that maintains visceral perception within an acceptable range in the face of a resident inflammatory and immune cell presence and whether the immune system itself is the source of this antinociceptive influence.

The present study investigated whether the immune system contributes to visceral nociception and specifically evaluated the role of lymphocytes. Our results provide the first demonstration that visceral nociception in the gut, in the absence of injury, is critically dependent on the integrity of the immune system and $CD4^+$ T cells in particular. This is based on the demonstration of hyperalgesia in mice with severe combined immune deficiency (SCID) and demonstration that nociception is normalized following reconstitution of SCID mice with $CD4^+$ T cells. Furthermore, our results identify opioids as the mediator of this immune-based peripheral antinociceptive mechanism.

Materials and Methods

Animal Housing and Handling

Male BALB/c and BALB/c SCID mice (6–8 weeks of age) were purchased from Harlan (Indianapolis, IN). Mice were kept under specific pathogen-free conditions at McMaster University Central Animal Care Facility (CAF). Upon arrival at our facility, mice were quarantined for 2 weeks before the start of experiments. Cages, bedding, and food were autoclaved as per standard procedure in McMaster University CAF. All experiments were approved by the McMaster University Animal Care Committee and the Canadian Council on Animal Care.

Lymphocyte Isolation and Mouse Reconstitution

Splenocytes from male BALB/c mice were isolated in Hank's balanced salt solution (HBSS) + 10% fetal bovine serum (FBS) + 1% antibiotic/antimycotic (A/A). Purified cells were resuspended in phosphate-buffered saline (PBS), and each mouse received 15×10^6 cells/200 μL via intraperitoneal (IP) injection. Cells were 95% viable as determined by trypan blue exclusion.

B cells were isolated using B220-selective magnetic beads (Dynal Biotech, Lake Success, NY). Isolated cells were resuspended in PBS, and each mouse received 5×10^6 cells/200 μL IP. For $CD4^+$ T-cell isolation, 1×10^8 splenocytes/mL were prepared in PBS + 2% FBS. EasySep negative selection mouse $CD4^+$ T-cell enrichment cocktail with magnetic nanoparticles (Stem Cell Technologies, Vancouver, B.C., Canada) was used to isolate $CD4^+$ T cells. The supernatant containing $CD4^+$ T cells was collected, cells were resuspended in PBS, and each mouse received 5×10^6 cells/200 μL IP. Cell purity as determined by flow cytometry using anti-mouse CD4 (LT34) monoclonal antibody (BD Pharmingen, San Jose, CA) was approximately 90%.

Colorectal Distention

Electromyographic (EMG) electrodes were surgically implanted under sterile conditions in the anterior abdominal muscle wall of mice anesthetized with ketamine (Ketalean; Bimeda-MTC, Cambridge, ON, Canada; 90 mg/kg) and xylazine (Rompun; Bayer, Toronto, ON, Canada; 20 mg/kg) IP, and a chronic fistula was exteriorized. Mice were then allowed to recover for a period of at least 7 days.

Mice were briefly anesthetized with enflurane (Enflurane USP, Abbott Laboratories, Saint-Laurent, QU, Canada) and a custom-made balloon catheter (20 × 10 mm) was gently inserted into the distal colon. A recording cable was connected to the chronic fistula, and mice were placed into Bollman restrainers. After connecting the catheters and cables to the barostat and EMG acquisition system, respectively, the mice were allowed a 5-minute rest. Colorectal distention (CRD) was then performed in a stepwise fashion. Each 10-second distention was followed by a 5-minute resting period. Each level of distention (30 and 60 mmHg) was repeated 3 times. EMG activity of the abdominal muscle was continuously recorded using customized software (Acquire 5.0; A. Bayatti). The area under the curve was calculated for 10 seconds before and after the beginning of each distention period using customized software (GrafView 4.1; A. Bayatti). The median value for each distention level per mouse was then calculated; this value is described as the visceromotor response (VMR) and is plotted as area under the curve. Naloxone methiodide (Sigma-Aldrich, Oakville, ON, Canada) was administered (30 mg/kg IP) 30 minutes prior to CRD testing. This experiment was carried out 2 days after week 12 CRD testing.

Flow Cytometry

To confirm reconstitution, 1×10^6 isolated splenocytes were incubated with phycoerythrin-conjugated antibody to CD3 and fluorescein isothiocyanate-conjugated antibody to B220 (BD Pharmingen) was then analyzed by flow cytometry (FACScan, BD Pharmingen) using CellQuest software (BD Pharmingen).

Myeloperoxidase Assay

Acute inflammation was assessed by myeloperoxidase (MPO) activity in distal colonic tissue. The assay was performed on frozen samples as previously described.[7]