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T lymphocytes containing β-endorphin ameliorate mechanical hypersensitivity following nerve injury ☆

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ABSTRACT

Neuropathic pain is a debilitating consequence of nerve injuries and is frequently resistant to classical therapies. T lymphocytes mediate adaptive immune responses and have been suggested to generate neuropathic pain. In contrast, in this study we investigated T cells as a source of opioidergic analgesic β-endorphin for the control of augmented tactile sensitivity following neuropathy. We employed in vivo nociceptive (von Frey) testing, flow cytometry and immunofluorescence in wild-type and mice with severe combined immunodeficiency (SCID) subjected to a chronic constriction injury of the sciatic nerve. In wild-type mice, T lymphocytes constituted approximately 11% of all immune cells infiltrating the injury site, and they expressed β-endorphin and receptors for corticotropin-releasing factor (CRF), an agent releasing opioids from leukocytes. CRF applied at the nerve injury site fully reversed neuropathy-induced mechanical hypersensitivity in wild-type animals. In SCID mice, T cells expressing β-endorphin and CRF receptors were absent at the damaged nerve. Consequently, these animals had substantially reduced CRF-mediated antinociception. Importantly, the decreased antinociception was fully restored by transfer of wild-type mice-derived T lymphocytes in SCID mice. The re-established CRF antinociception could be reversed by co-injection of an antibody against β-endorphin or an opioid receptor antagonist with limited access to the central nervous system. We propose that, in response to CRF stimulation, T lymphocytes accumulating at the injured nerves utilize β-endorphin for activation of local neuronal opioid receptors to reduce neuropathy-induced mechanical hypersensitivity. Our findings reveal β-endorphin-containing T cells as a crucial component of beneficial adaptive immune responses associated with painful peripheral nerve injuries.

1. Introduction

Chronic pain is frequently associated with inflammation, and it is increasingly evident that there is a close interplay between the nervous and immune systems. Immune cells are not only the source of mediators promoting pain but they also produce analgesic molecules (Machelska, 2007; Stein et al., 2003). In somatic inflammatory pain the best characterized and clinically relevant are leukocyte-derived opioid peptides such as β-endorphin and enkephalins. Circulating opioid-producing immune cells accumulate in peripheral inflamed tissues where they secrete the opioids (Brack et al., 2004; Cabot et al., 1997; Labuz et al., 2006; Machelska et al., 1998, 2002, 2004; Mousa et al., 2004; Rittner et al., 2001, 2006; Schäfer et al., 1994; Sitte et al., 2007; Stein et al., 1990, 1993). The released opioid peptides activate opioid receptors on peripheral sensory neurons resulting in inhibition of inflammatory pain in animals and in patients with arthritis (Likar et al., 2007; Machelska et al., 2003; Mousa et al., 2007; Rittner et al., 2006; Stein et al., 1990, 1993).

Neuropathic pain is a common consequence of peripheral nerve injuries following a body part amputation, nerve entrapment or compression, and represents one of the most devastating forms of chronic pain. It is characterized by spontaneous burning or shooting sensations, heightened responses to normally noxious (hyperalgesia) and innocuous stimuli (allodynia). Mechanical hypersensitivity can be especially distressing to patients, as tactile stimulation (for example skin to clothes contact), and therefore pain, is inevitable (Scholz and Woolf, 2002). Such pain can persist long after the cessation of its initial cause and it is often unresponsive to conventional treatments (Dworkin et al., 2007; Stein and Kopf, 2009). Activation of the immune system gains increasing attention as a component of nerve injury. Interestingly, however, the currently prevalent opinion considers leukocytes as cells enhancing neuropathic pain (Marchand et al., 2005; Moalem and Tracey, 2006; Scholz and Woolf, 2007; Sommer and Kress, 2004; Watkins and Maier, 2002). The blockade of interleukin-1 receptors or of tumor necrosis factor-α production, deletion of interleukin-6 or CCR2 chemokine receptor genes (Abbadie et al., 2003; Murphy et al., 1999; Sommer and Kress,

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2004), or depletion of leukocytes (Liu et al., 2000; Perkins and Tracey, 2000; Zuo et al., 2003) have been reported to decrease experimental neuropathic pain. In contrast, we have recently shown that upon stimulation with corticotropin-releasing factor (CRF), opioid peptide-expressing CD45$^+$ hematopoetic cells reduce such pain (Labuz et al., 2009).

T lymphocytes mediate adaptive immune responses and have been suggested to be involved in neuropathic pain. Thus, animals with genetic absence of T lymphocytes such as athymic nude rodents, CD4-knock-out (KO) as well as the recombination-activating gene-1 (RAG-1)-KO mice were reported to develop less hyperalgesia, defining T cells as enhancers of pain following nerve damage (Cao and DeLeo, 2008; Costigan et al., 2009; Kleinschnitz et al., 2006; Moalem et al., 2004).

In the current study, using a combination of in vivo pain testing, biochemical and histochemical methods in wild-type (WT) and mice with severe combined immunodeficiency (SCID), we demonstrate substantial evidence that T cells accumulating at the damaged nerve are the source of β-endorphin, an endogenous opioidergic pain killer. Stimulation of T cells with CRF led to β-endorphin-mediated activation of local opioid receptors and ultimately hindered the exaggerated tactile sensitivity following nerve injury.

2. Materials and methods

2.1. Animals

Experiments were performed in male mice (25–30 g) bred at the Charité-Universitätsmedizin Berlin, and they were C57Bl/6J WT and SCID (C57BL/6J-Prkdc^{scid}; Jackson Laboratory, Bar Harbor, ME). Animals were housed in groups of 8–10 per cage lined with ground corncob bedding, under specific pathogen-free conditions. They were kept on a 12 h light/dark schedule with food pellets and water *ad libitum*. Room temperature was maintained at 22 ± 0.5 °C and a relative humidity between 60–65%. The experiments were approved by the local animal care committee (Landesamt für Gesundheit und Soziales Berlin, Germany) and were carried out in accordance with the guidelines of the International Association for the Study of Pain (Zimmermann, 1983). The experimenters were blinded to the genotypes and treatments.

2.2. Induction of neuropathy and nociceptive testing

Chronic constriction injury (CCI) was induced in deeply anesthetized mice with isoflurane (Abbott, Wiesbaden, Germany). The sciatic nerve was exposed at the level of the right mid-thigh and three loose silk ligatures (4/0) were placed around the nerve with about 1 mm spacing. The ligatures were tightened until they elicited a brief twitch in the respective hind limb, and the wound was closed using silk sutures. Mechanical allodynia was evaluated with calibrated von Frey filaments (Stoelting, Wood Dale, IL) using an up–down method, as in our previous study (Labuz et al., 2009). Briefly, testing began using a 3.9 mN hair (0.4 g) and if the animal withdrew the paw the weaker hair was applied. In the absence of paw withdrawal the next stronger hair was used. The maximal number of applications was 6–9 and the cut-off was 39.2 mN (4 g), as previously (Labuz et al., 2009).

2.3. Flow cytometry

Mice (n = 6 per group) were killed at 3 or 15 days following CCI and the ligated parts of the sciatic nerves (approximately 1 cm-long including the ligation site and sites both proximal and distal to it) were collected (Labuz et al., 2009). Single cell suspensions were prepared (Labuz et al., 2009; Machelska et al., 2002; Rittner et al., 2001) and samples were stained with rat anti-mouse CD45 phytoerythrin-cyanine dye 5-conjugated monoclonal Ab (mAb; 4 μg/ml; BD Biosciences, Heidelberg, Germany) to label all hematopoetic (CD45$^+$) cells. To differentiate between leukocyte subpopulations, cell suspensions were stained with phytoerythrin-conjugated rat anti-mouse mAbs recognizing either granulocytes (Ly6; 2 μg/ml) or T cells (CD3; 4 μg/ml) (BD Biosciences), or with fluorescein isocyanate (FITC)-conjugated mAbs recognizing monocytes/macrophages (F4/80; 6 μg/ml; Serotec, Oxford, UK) or B cells (CD19; 10 μg/ml; BD Biosciences). For intracellular stains cells were prepared and incubated with 3E7 phytoerythrin-conjugated mAb recognizing the pan-opioid sequence Tyr-Gly-Gly-Phe at the N-terminus of opioid peptides (10 μg/ml; Gramsch Laboratories, Schwabhausen, Germany). The specificity of the staining was verified by incubation of cell suspensions with appropriate isotype-matched control Abs. Absolute numbers of cells were calculated using Tru-COUNT tubes with known numbers of fluorescent beads. Data were acquired using a FACSCalibur and analyzed using the CellQuest software (all from BD Biosciences), as described earlier (Labuz et al., 2009; Machelska et al., 2002; Rittner et al., 2001).

2.4. T cell isolation

At 7 days following CCI of the sciatic nerve, WT mice were killed and the inguinal lymph nodes from the site of nerve injury were collected. Single cell suspensions were prepared and samples were incubated with F4/80 and CD19 Abs, as described for flow cytometry (Section 2.3). The cell suspensions were labeled with anti-FITC microbeads, loaded onto MACS separation columns 25MS (Miltenyi Biotec, Bergisch Gladbach, Germany), and unlabeled T cells were separated for immediate injections (see Section 2.6). Some aliquots of separated cells were analyzed by flow cytometry (see Section 2.3) and showed a T cell purity of 85–90%.

2.5. Immunostaining

At 2 or 14 days following CCI mice (n = 3 at each time point) were deeply anesthetized and perfused. Ligated parts of the sciatic nerves were dissected (as described in Section 2.3) and 10 μm-thick longitudinal sections were mounted on gelatin-coated slides, as described previously (Labuz et al., 2009). To assess the expression of β-endorphin and CRF receptors in T cells the sections were incubated with hamster CD3ε Ab (staining T cells) and rabbit CRF receptor Ab (both at 1:400; Santa Cruz Biotechnology, Santa Cruz, CA) or with rabbit β-endorphin Ab (1:500; Bachem, Weil am Rhein, Germany). The sections were then incubated with the respective secondary Abs: goat anti-hamster conjugated with FITC and goat anti-rabbit conjugated with Texas red (both at 1:200; Vector Laboratories, Burlingame, CA). Thereafter, the sections were washed, mounted in Mowiol 4-88 (Carl Roth, Karlsruhe, Germany) and viewed under a fluorescence microscope (Zeiss) with appropriate filters. Specificity control staining included omission of the primary Abs or preabsorption (for 4 h) of the primary Abs with the respective antigenic peptides (fivefold excess), and showed no staining (data not shown).

2.6. Drug treatments and behavioral experimental protocols

Mice received CRF at the site of nerve injury at 2 days (20 ng) and 14 days (100 ng) after CCI. CRF was injected alone or together with Ab against β-endorphin (0.25 μg) or with naloxone methiodide (NLXM; 5 μg). In T cell reconstitution experiments, the T cells were isolated from WT mice (see Section 2.4) and injected (100×10^3 cells in RPMI buffer) at the CCI site followed 10 min later by CRF (100 ng) applied alone or together with anti-β-endorphin (0.25 μg) or NLXM (5 μg) at 14 days following CCI. The doses