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A novel immune-to-CNS communication pathway: Cells of the meninges surrounding the spinal cord CSF space produce proinflammatory cytokines in response to an inflammatory stimulus

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Abstract

Pain is enhanced in response to elevations of proinflammatory cytokines in spinal cerebrospinal fluid (CSF), following either intrathecal injection of these cytokines or intrathecal immune challenge with HIV-1 gp120 that induces cytokine release. Spinal cord glia have been assumed to be the source of endogenous proinflammatory cytokines that enhance pain. However, assuming that spinal cord glia are the sole source of CSF cytokines may be an underestimate, as the cellular composition of the meninges surrounding the spinal cord CSF space includes several cell types known to produce proinflammatory cytokines. The present experiments provide the first investigation of the immunocompetent nature of the spinal cord meninges. Here, we explore whether rat meninges are responsive to intrathecal gp120. These studies demonstrate that: (a) intrathecal gp120 upregulates meningeal gene expression of proinflammatory signals, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin 6 (IL-6), and inducible nitric oxide synthase (iNOS), and (b) intrathecal gp120 induces meningeal release of TNF-α, IL-1β, and IL-6. In addition, stimulation of isolated meninges *in vitro* with gp120 induced the release of TNF-α and IL-1β, indicating that the resident cells of the meninges are able to respond without immune cell recruitment. Taken together, these data document that the meninges are responsive to immunogenic stimuli in the CSF and that the meninges may be a source of immune products detected in CSF. The ability of the meninges to release to proinflammatory signals suggests a potential role in the modulation of pain.
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1. Introduction

The peripheral immune system communicates with the central nervous system (CNS) during systemic inflammation, resulting in CNS-mediated effects collectively referred to as sickness responses (e.g., fever, cognitive impairment, reduced social interaction, and pain enhancement) (Watkins and Maier, 1999, 2000). One of the ways that the peripheral immune system communicates with the CNS is via peripheral nerve afferents, which are activated upon detection of inflammatory mediators. This pathway leads to CNS glial activation and proinflammatory cytokine release in discrete CNS regions, thereby inducing sickness responses (Cunningham et al., 2005; Godbout et al., 2005; Nadeau and Rivest, 2000; Semmler et al., 2005; Soulet and Rivest, 2003).

With regards to sickness-induced pain enhancement, proinflammatory cytokines rise in the spinal cord in response to peripheral infection, inflammation, and trauma, and, in so doing, mediate enhanced pain

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(Meller et al., 1994; Sweitzer et al., 2001; Watkins et al., 1997). In such studies, proinflammatory cytokine elevations have been assumed to reflect release by glia within the spinal cord. Here, we explore a different concept; that is, whether there may be another potential source for spinal proinflammatory cytokines, namely the spinal cord meninges.

The meninges are most often thought of as simply a protective barrier between the CNS and the periphery, composed of fibroblasts and connective tissue. However, what is often overlooked about connective tissue in general is that the cellular composition includes immune cells such as mast cells and macrophages. Consistent with this description, the meninges surrounding the brain and spinal cord are also composed of fibroblasts, macrophages, mast cells and dendritic cells, but not glial cells (Artico and Cavallotti, 2001; Braun et al., 1993; Fischer and Reichmann, 2001; McMenamin, 1999; Mercier and Hatton, 2004; Rozniecki et al., 1999; Zenker et al., 1994). This cellular composition suggests that the meninges may be immunocompetent, and thus would be predicted to respond to immune challenges by producing and releasing proinflammatory mediators. The close proximity of the spinal cord meninges to the spinal cord parenchyma suggests that, were the meninges able to produce and release proinflammatory products, they would be well-positioned to modulate spinal cord neuronal functions including pain. Given the meninges are considered independent of the CNS parenchyma and thus part of the periphery, such a mechanism would provide a novel pathway for immune-to-CNS communication.

The aim of the present studies was to explore whether the spinal cord meninges are immunocompetent, by exploring its response to HIV-1 gp120. gp120 was chosen for test as it is known to produce profound pain facilitation upon injection into spinal CSF (intrathecal; i.t.), an effect mediated by the release of proinflammatory cytokines (Milligan et al., 2001). While prior studies have assumed that i.t. gp120-induced elevations of proinflammatory cytokines in CSF were due solely to spinal cord glia, the potential contribution of the meninges are investigated here. That the meninges are a potential source of proinflammatory mediators able to influence pain states has not previously been considered. The goal of the first experiment was to evaluate if i.t. gp120 upregulates gene expression for proinflammatory mediators in the meninges. The second experiment assesses if, in addition to upregulating gene expression, there is a concurrent increase in the release of proinflammatory cytokine proteins in response to this immune challenge. The third experiment evaluates the response of isolated naïve meninges in response to *in vitro* gp120. Finally, the fourth experiment uses immunohistochemistry to identify IL-1β in meningeal cells. The results demonstrate the immunocompetent nature of the meninges, suggesting that these tissues that may exert immunoregulatory effects on spinal cord functions, such as pain.

2. Materials and methods

2.1. Subjects

Pathogen-free adult male Sprague–Dawley rats (300–450 g; Harlan Laboratories, Madison, WI) were used in all experiments. Rats were housed in temperature (23+/− 3C) and light (12:12 light:dark; lights on at 0700 h) controlled rooms with standard rodent chow and water available *ad libitum*. The Institutional Animal Care and Use Committee of the University of Colorado at Boulder approved all procedures.

2.2. gp120

Frozen solutions of recombinant gp120 (product 1021, lot number 2S3/1.5; from ImmunoDiagnostics, Inc.) were thawed, aliquoted at 1 μg/μl, and stored at −75 °C. Vehicle, 0.2% rat serum albumin (Accurate Chemical & Scientific Corp, Westbury, NY) in PBS, was aliquoted and stored at −75 °C. The gp120 was endotoxin free (documented by manufacturer) and handled using sterile technique. Frozen aliquots of gp120 and vehicle were thawed immediately before administration, and gp120 was diluted to concentrations specified in each experiment, in a final solution containing 0.1% rat serum albumin to facilitate gp120 administration. We have previously shown that the rat serum albumin does not interfere with the actions of gp120 (Milligan et al., 2000). Aliquots were kept on ice during use and discarded within 30-min.

2.3. Acute intrathecal lumbar puncture

Rats were briefly anesthetized with isoflurane (Phoenix Pharmaceuticals, St. Joseph, MO) for insertion of a sterile PE-10 catheter as previously described (Ledeboer et al., 2005). This placed the open end of the catheter such that it rested approximately at spinal level L5, the injection site previously employed in studies of i.t. gp120-induced pain facilitation (Milligan et al., 2000). The sterile PE-10 catheter, pre-loaded with 3.5-μg gp120+ 0.1% RSA or equivolume physiological saline was briefly inserted into the open end of the 18-gauge cannula, up to the mark at 7.75 cm and removed upon completion of the microinjection so to avoid the potential of a chronic indwelling foreign body altering the results. Total time for microinjections averaged 3-min and rats showed full recovery within 10 min of being removed from isoflurane. Rats did not display any impaired motor behavior following injection and were returned to their home cages upon full recovery.

2.4. Meninges isolation

Rats were deeply anesthetized with sodium pentobarbital (Abbott Laboratories, North Chicago, IL) prior to whole-body transcardial perfusion with ice cold heparinized (1 U/ml) 0.9% saline for 3 min. The meninges-enwrapped spinal cord was exposed intact by laminectomy. All meningeal samples collected in these experiments consisted of the dura mater and the arachnoid layers of the meninges. As the pia is securely attached to the underlying spinal cord, to collect this layer by dissection would present too high a risk of contamination of the results by spinal cord microglia and astrocytes. Hence, pia was intentionally excluded from the analyses. Meningeal samples were dissected away from the spinal cord by first making a superficial incision along the ventral midline deep enough to penetrate the meninges, but not disrupt the pia or underlying spinal cord tissue. In Experiments 1 and 2, meninges were collected starting from the juncture of vertebrae L3/L4 and extending 7.5 mm caudally. This was done so to collect meninges predicted to be exposed to i.t. gp120, as has previously been shown in studies using gp120 induced pain states (Milligan et al., 2000, 2001). These samples were collected 4 h after i.t. gp120 or i.t. vehicle injection and frozen in liquid nitrogen, or collected at 2 h after i.t. gp120 or i.t. vehicle and then incubated in culture media. These time points were chosen based on prior time course studies of i.t. gp120-induced elevations in proinflammatory cytokine proteins and mRNAs (Holguin et al., 2004; Ledeboer et al., 2005; Milligan et al.,

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