Interactive report

Intrathecal anti-IL-6 antibody and IgG attenuates peripheral nerve injury-induced mechanical allodynia in the rat: possible immune modulation in neuropathic pain

Janice L. Arruda a,*, Sarah Sweitzer b, Maria D. Rutkowski a, Joyce A. DeLeo a, b

a Department of Anesthesiology, Dartmouth-Hitchcock Medical Center, HB 7125, 1 Medical Center Drive, Lebanon, NH 03756, USA
b Department of Pharmacology, Dartmouth-Hitchcock Medical Center, HB 7125, 1 Medical Center Drive, Lebanon, NH 03756, USA

Accepted 13 August 2000

Abstract

Interleukin-6 (IL-6) is a pleiotropic cytokine with a diverse range of actions including the modulation of the peripheral and central nervous system. We have previously shown significant IL-6 protein and messenger RNA elevation in rat spinal cord following peripheral nerve injury that results in pain behaviors suggestive of neuropathic pain. These spinal IL-6 levels correlated directly with the mechanical allodynia intensity following nerve injury. In the current study, we sought to determine whether it is possible to attenuate mechanical allodynia and/or alter spinal glial activation resulting from peripheral nerve injury by specific manipulation of IL-6 with neutralizing antibodies or by global immune modulation utilizing immunoglobulin gamma-globulin (IgG). Effects of peripheral administration of normal goat IgG and intrathecal (i.t.) administration of IL-6 neutralizing antibody, normal goat or normal rat IgG on mechanical allodynia associated with L5 spinal nerve transection were compared. Spinal glial activation was assessed at day 10 post surgery by immunohistochemistry. Low dose (0.01–0.001 mg) goat anti-rat IL-6 i.t. administration (P = 0.025) significantly decreased allodynia and trended towards significance at the higher dose (0.08 mg to 0.008 mg, P = 0.062). Low doses (0.01–0.001 mg) i.t. normal goat and rat IgG significantly attenuated mechanical allodynia, but not at higher doses (0.08–0.008 μg; P = 0.001 for both goat and rat IgG). Peripherally administered normal goat IgG (30 or 100 mg/kg) did not attenuate mechanical allodynia. Spinal glial activation was unaltered by any treatment. These data provide further evidence for the role of central IL-6 and neuroimmune modulation in the etiology of mechanical allodynia following peripheral nerve injury.

© 2000 Elsevier Science B.V. All rights reserved.

Theme: Sensory systems

Topic: Pain modulation: pharmacology

Keywords: Astrocytes; Glial fibrillary acidic protein; Interleukin-6; IVIg therapy; Microglia; Neuropathic pain

1. Introduction

Interleukin 6 (IL-6) is a pleiotropic cytokine that is involved in a diverse range of functions within the body including the modulation of proliferation, differentiation and maturation of progenitor cells [28], control of cellular metabolic activities [17], the immune system cascade, and modulation within the nervous system. There is increasing evidence that supports a role of IL-6 in neuronal development, differentiation [31,35], survival [19], regeneration and degeneration in both the peripheral and central nervous systems (CNS).

IL-6 has been implicated as a significant factor in the central nervous system’s response to injury [2,21,23]. This proinflammatory cytokine plays a key role in peripheral nerve injury-induced mechanical allodynia and thermal hyperalgesia in both rodents and humans [13,18,37]. We have previously demonstrated a direct relationship between the increase in both mechanical allodynia (increased sensitivity to a non-noxious stimulus) and IL-6 immunoreactive-like protein in the spinal cord in a sciatic cryoneurolysis model of mononeuropathy [13]. In addition, intrathecally administered human recombinant IL-6 in the

---

*Corresponding author. Tel.: +1-603-650-6205; fax: +1-603-650-4928.

E-mail address: janice.l.pahl@dartmouth.edu (J.L. Arruda).
absence of nerve injury was able to produce touch evoked allodynia in rats and produced thermal hyperalgesia in the sciatic cryoneurolysis model [13]. Furthermore, our laboratory has shown that neurons of the spinal cord produce IL-6 messenger RNA in response to peripheral nerve injury resulting in neuropathic pain behaviors [3]. Recently it has been demonstrated that in the chronic constriction injury model, cutaneous heat and pressure hypersensitivity was not elicited in mice with a null mutation of the IL-6 gene as compared to the wild type controls [33].

Along with these direct relationships between IL-6 and the CNS response to injury, recent literature also implicates possible indirect effects of IL-6 through its’ ability to modulate classic pain mediators in the CNS milieu. For example, in a series of related in vivo and in vitro studies, it was shown that the concentration of nitrate increased in rat hippocampus in response to IL-6 treatment, suggesting an overproduction of nitric oxide due to the presence of IL-6 [29,30]. Recent work in our laboratory has also revealed that in the spinal cord of L5 nerve transected animals IL-6 is produced downstream to IL-1 beta and TNF [47]. IL-1 beta is one of the proinflammatory cytokines reported as effecting spinal sensitization possibly through its’ influence on the induction and expression of cyclooxygenase 2, inducible nitric oxide synthase and substance P [22,24,44]. The exact contribution of each cytokine in the response cascade to nerve injury has not yet been determined, but certainly there appears to be an intimate interplay between these cytokines and their modulation of the CNS environment. Pertinent to this study, both the direct and potential indirect effects of IL-6 in nerve injury contribute to the mounting evidence for a seminal role of IL-6 in neuropathic pain and support further investigation into IL-6 targeted interventions to reduce or prevent peripheral nerve injury-induced pain behaviors.

In addition to the interest of elucidating the use of specific interventions like IL-6, in the last few decades there has been an increased focus and use of high dose intravenous immunoglobulin-globulin (IVIg) therapy as global immune modulating therapies. Several immune system related diseases including rheumatoid arthritis [35], thrombocytopenic purpura [25], multiple sclerosis [42], Kawasaki’s disease [34,36] and Guillain–Barre syndrome [49] have been treated with IVIg therapy. It has been found that IVIg therapy is able to relieve symptoms of these diseases, such as pain and morning stiffness in rheumatoid arthritis patients [38]. The mechanism of action for IVIg is currently unknown, but discovery of its beneficial attributes are being expanded continually.

In the present study, the effects of manipulating central neuroinflammation after an L5 spinal nerve transection was investigated by interfering with a specific proinflammatory cytokine, IL-6, and a global immunomodulator, IgG, at the time of peripheral nerve injury and in the initial period following the lesion. In order to elucidate the benefits of these therapies, neutralizing goat anti-IL-6 was administered intrathecally (i.t.) and normal rat or goat IgG antibodies were given either i.t. or via intravenous (i.v.) injection. To assess alterations in this mononeuropathy model due to the various treatments, tactile sensitivity using von Frey filaments and spinal glial activation were assessed. Glial activation, in particular astrocytic, may have a principle role in nociceptive processing and in the thermal and mechanical hyperalgesia and/or allodynia produced by peripheral nerve injury [32]. Efficacy of the pharmacological interventions was determined by their ability to significantly attenuate one nerve-injured induced behavioral modality, mechanical allodynia. Our results reveal a potentially novel mechanism of modulating spinal neuroinflammation that may ultimately translate into new, non-addictive xenobiotics for the treatment of chronic pain syndrome in humans.

2. Materials and methods

2.1. Animal subjects

Male Holtzman rats (Harlan, Indianapolis, IN) weighing at least 200 g at the start of the study were used. Animals were housed individually with a 12/12 h light/dark cycle and free access to food and water. Procedures in this study were approved by the Institutional Animal Care and Use Committee at Dartmouth College. Efforts were made to limit distress and to use the minimum number of animals necessary to achieve statistical significance as set forth by the International Society for the Study of Pain guidelines [8].

2.2. L5 spinal nerve transection

Animal surgery was performed under inhalation anesthesia using halothane in 100% O2, induced at 3% and maintained at 1.5%. Each of the animals sustained an L5 spinal nerve transection as previously described [7]. Briefly, the L4–L5 spinal nerves were isolated following removal of the L6 tranverse processes. The L5 spinal nerve was gently separated from the L4 spinal nerve and then was transected. The wound was closed using 3-0 polyester suture and surgical staples for fascia and skin, respectively.

2.3. Intrathecal injections of anti-rat IL-6, normal goat or rat IgG

Goat anti-rat IL-6 neutralizing antibody, normal goat IgG (R&D Systems, Minneapolis, MN) and normal rat IgG (Sigma, St. Louis, MO) were each diluted in phosphate buffered saline (PBS). Rats were randomly placed into one of seven groups: high dose or low dose of anti-rat IL-6 (n=5 or 8, respectively), normal goat IgG (n=7 or 6, respectively), or normal rat IgG (n=8 or 7, respectively).
and PBS vehicle control (n=8). Each high dose animal was given 0.08 μg of treatment 1 h before surgery and on day 1 post surgery and 0.008 μg on day 3 post surgery. Low dose animals were given 0.01 μg of treatment 1 h before surgery and on day 1 post surgery and 0.001 μg on day 3 post surgery. All intrathecal injections were performed via a direct lumbar puncture under inhalation anesthesia in a total volume of 40 μl.

2.4. Peripheral injections of IgG

Animals were divided into 3 groups: PBS control (n=8), low dose (30 mg/kg, n=8) and high dose (100 mg/kg, n=7) normal goat IgG in PBS. Treatments were administered via tail vein injection on day 1 before surgery (to allow ample time to penetrate the central nervous system) and on days 1 and 3 post L5 transection surgery. These doses were based on previously reported human doses for IV Ig therapy [15,38,41,43] with modifications made for the bolus injections utilized in our model.

2.5. Mechanical allodynia

Tactile sensitivity was measured as the frequency of foot-withdrawals elicited by a defined normally non-noxious mechanical stimulus. Each rat, under non-restrained conditions, was placed singly beneath an inverted ventilated Plexiglas cage upon an elevated aluminum screen surface with 1 cm mesh openings. Animals were previously acclimated to this environment and to the experimenter. In each blinded testing session, rats were subjected to 10 tactile stimulations on the ipsilateral hind paw using 2 g and 12 g von Frey filaments (Stoelting Co., Wood Dale, IL). Baseline (pre-lesion) responsiveness was minimal (0 to 1 responses out of 30 stimulations) as confirmed from testing sessions prior to nerve lesion. Mechanical allodynia was assessed by recording the total number of responses elicited during three successive trials (10 stimulations/each filament) separated by at least 10 min for a total possible maximum response of 30. Mechanical allodynia was measured on days 1, 3, 5, 7, and 10 post surgery.

2.6. Spinal cord tissue preparation

All rats were perfusion-fixed for immunohistochemistry (IHC). Under deep anesthesia (sodium pentobarbital, 150 mg/kg, intraperitoneal injection) rats were euthanized by transcardiac perfusion with 250 ml PBS followed by 250 ml of 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Following perfusion and laminectomy, the lesioned root or nerve was verified and traced to its site of entry into the spinal cord. Appropriate (L5) spinal cord segments were retrieved and post-fixed for 4 h in the above fixative and then cryoprotected for at least 72 h in 30% sucrose/PBS at 4°C. Segments were freeze-mounted in OCT embedding medium on cork blocks for cryostat sectioning

2.7. Immunohistochemical staining

IHC was performed by the avidin–biotin technique (ABC Elite Kits, Vector Labs, Burlingame, CA) on free floating 20 μm sections. Elimination of the primary antibody was performed in each run as a negative control. Monoclonal OX-42 antibody to CR3/CD11b was used as a microglial marker at a dilution of 1:1 (Dr. W.F. Hickey, Dartmouth) and rabbit anti-glial fibrillary acidic protein (GFAP) was used as an astrocytic marker at a dilution of 1:20,000 (DAKO, Carpinteria, CA).

2.8. Scoring of IHC slides

Assessment of IHC slides was performed by an experimenter blinded to the treatment groups. Following staining of lumbar spinal cord sections with the specific marker under consideration, three or more sections from each animal were surveyed under low and medium magnification to arrive at a score. Microglia and astrocytes were scored based on the following scale as previously described [6]: baseline staining (−), mild response (+), moderate response (++), intense response (+++). The activation response is based on the observed cell morphology, local cell density, and intensity of GFAP and CR3/CD11b immunoreactivity.

2.9. Statistical methods

STATA™ 5.0 (Stata Corp., College Station, TX) was used to perform all statistical tests. To determine the existence of a difference in overall behavioral outcomes (mechanical sensitivities) due to surgical or pharmacologic treatment, a one-way ANOVA was performed with subsequent specific comparisons of behaviors between treatment groups accomplished with the Bonferroni multiple-comparisons test. A P value of <0.05 was considered significant.

3. Results

3.1. Effects of i.t. antibody administration on mechanical allodynia

3.1.1. IL-6 neutralizing antibody

Low dose anti-IL-6 intrathecal injections significantly attenuated 2 g von Frey measurement of mechanical allodynia as compared to the PBS control group (P = 0.025) while the high dose anti-IL-6 injections trended towards significance (P = 0.062). With the 12 g von Frey filaments, low dose anti-IL-6 trended toward significance when compared to the PBS control animals (P = 0.085)
while the high dose did not attenuate allodynia ($P=0.318$) (see Fig. 1).

### 3.1.2. Normal goat IgG

Normal goat i.t. IgG administration significantly attenuated mechanical allodynia at the low dose when compared to the PBS control (2 and 12 g, $P=0.001$) while the high dose did not decrease nerve injury-induced allodynia (2 and 12 g, $P=1.0$) (see Fig. 2).

### 3.1.3. Normal rat IgG

Normal rat i.t. IgG administration significantly attenuated mechanical allodynia at the low dose when compared to the PBS control (2 g, $P=0.001$; 12 g, $P=0.003$) while the high dose did not decrease nerve injury-induced allodynia (2 and 12 g, $P=1.0$) (see Fig. 3). There was no significant difference between the goat and rat IgG treatments with either the 2 or 12 g von Frey filaments. This result implies that the species of IgG used is not critical for modulating the immune response to peripheral nerve-injury induced allodynia.

---

**Fig. 1.** Time course for mechanical allodynia in the ipsilateral hind paw following L5 spinal nerve transaction with PBS, high dose normal goat-IgG, or low dose normal goat-IgG intrathecal treatment 1 h before and on day 1 (high dose=$0.08$ µg; low dose=$0.01$ µg) and day 3 (high dose=$0.008$ µg; low dose=$0.001$ µg) post surgery. All animals were exposed to 30 stimulations with 12 g (A) or 2 g (B) von Frey filaments at each time point. Results are reported as the average response out of 30 stimulations per group±S.E.M. *Represents statistically significant difference between treatment and PBS control at indicated time points ($P<0.05$).

**Fig. 2.** Time course for mechanical allodynia in the ipsilateral hind paw following L5 spinal nerve transaction with PBS, high dose normal goat-IgG, or low dose normal goat-IgG intrathecal treatment 1 h before and on day 1 (high dose=$0.08$ µg; low dose=$0.01$ µg) and day 3 (high dose=$0.008$ µg; low dose=$0.001$ µg) post surgery. All animals were exposed to 30 stimulations with 12 g (A) or 2 g (B) von Frey filaments at each time point. Results are reported as the average response out of 30 stimulations per group±S.E.M. *Represents statistically significant difference between treatment and PBS control at indicated time points ($P<0.05$).

### 3.2. Effects of peripheral IgG administration on mechanical allodynia

Intravenous goat IgG administration at 30 and 100 mg/kg doses did not significantly attenuate nerve injury-induced allodynia (see Fig. 4).

### 3.3. Immunohistochemical staining

There were no observable differences in GFAP or OX-42 immunoreactivity between any of the treatment groups (see Fig. 5 and Tables 1 and 2). Astrocytic and microglial activation mirrored previously reported glial changes in both the treated and untreated groups [3]. Microglial cells were no longer highly ramified (Fig. 5A). The processes were concentrated to the cell body but did not appear phagocytic (Fig. 5B and C). Astrocytes demonstrated hypertrophy in both the dorsal and ventral horn of the treated groups (Fig. 5E and F). This was an increase from that seen in normal animals (Fig. 5D).
Fig. 3. Time course for mechanical allodynia in the ipsilateral hind paw following L5 spinal nerve transection with PBS, high dose normal rat IgG, or low dose normal rat IgG intrathecal treatment 1 h before and on day 1 post surgery (high dose=0.08 µg; low dose=0.01 µg) and on day 3 post surgery (high dose=0.008 µg; low dose=0.001 µg). All animals were exposed to 30 stimulations with 12 g (A) or 2 g (B) von Frey filaments at each time point. Results are reported as the average response out of 30 stimulations per group±S.E.M. *Represents statistically significant difference between treatment and PBS control at indicated time points (P<0.05).

4. Discussion

In the present study, we demonstrated significant attenuation of mechanical allodynia following L5 spinal nerve transection by intrathecal injection of neutralizing anti-IL-6. We also found in the same model that low doses of intrathecally administered normal goat and rat IgG were able to significantly attenuate enhanced tactile sensitivity. These data suggest that both specific and global central immune manipulation alters nociceptive sensory processing after a peripheral nerve injury.

Neuroimmune interactions are highly complex and multi-faceted. Recently reported findings indicate that a variety of cytokines are involved in neuropathic pain including IL-6, Interleukin 1-β (IL-1β), and Tumor Necrosis Factor (TNF). In addition to the previously mentioned studies indicating a role for IL-6, Ramer et al. [39] have shown that IL-6 knockout mice have delayed mechanical allodynia in response to L5 spinal nerve tight ligation and transection when compared to the parent strain. In addition to IL-6, we have also reported spinal increases in immunoreactive-like TNF and IL-1β following nerve injury [14]. Sommer et al. [45] demonstrated dose-dependent attenuation of both hyperalgesia and mechanical allodynia in mice with experimental neuropathy by i.t. administration of neutralizing antibodies to the IL-1β receptor (10–80 µg/animal). Based on these results, it is evident that there are several neuroimmune factors influencing pain-related behavioral responses following peripheral nerve injuries.

Therefore, it is of particular interest that intrathecally administered normal goat and rat IgG were able to significantly attenuate the mechanical allodynia following an L5 spinal nerve transection. Over the past twenty years normal human IVIg therapy has been used with success in humans for a variety of autoimmune disorders, primary immunodeficiencies, and Kawasaki disease [5,16,25] to both ameliorate disease and alleviate symptoms. For example, IVIg treatment in rheumatoid arthritis (RA) patients has been shown to decrease plasma IL-6 levels, morning stiffness, and pain levels [38] associated with RA. Given the nature of the illnesses treated with IVIg, it is
Fig. 5. (A–C) Representative microglial responses as visualized by anti-OX42 immunohistochemistry. (A) normal, untreated animal, (B) intrathecal saline treatment with L5 spinal transection (SPTS), and (C) intrathecal high dose normal goat IgG treatment with SPTS. (D–F) Representative astrocytic responses as visualized by anti-GFAP immunohistochemistry. (D) normal, untreated animal, (E) intrathecal saline treatment with SPTS, and (F) intrathecal high dose anti-IL-6 treatment with SPTS.
Table 1
Astrocytic responses (illuminated by GFAP ir) of individual animals at the L5 spinal cord level at day 10 post L5 spinal nerve transection with intrathecal treatment on days 0, 1, and 3

<table>
<thead>
<tr>
<th>Intrathecal treatment</th>
<th>Ipsilateral (injured) side</th>
<th>Contralateral (uninjured) side</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DH</td>
<td>VH</td>
</tr>
<tr>
<td>Normal, untreated</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Surgery alone</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td>Saline</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>LD anti-IL6</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>LD goat IgG</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>LD rat IgG</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>HD anti-IL6</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td>HD goat IgG</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>HD rat IgG</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

*Staining/morphology key: baseline (−), mild (+), moderate (++), or intense (+++ +) responses. VH = ventral horn, DH = dorsal horn, LD = low dose, HD = high dose.

conceivable that the effective attenuation seen with normal IgG treatment in our rodent model is related to the therapy’s immunomodulatory actions.

A thorough understanding of the mechanisms of action of IVIg treatment is currently unknown, but there are several proposed mechanisms of action, some of which may apply to the reduction in allodynia we observed in the present study. Of interest in our model is the ability of IVIg therapy to block cytokines and growth factors. IVIg has been shown in human immune disorders to decrease the levels and/or production of interferon [40], TNF, IL-1β, transforming growth factor-β [1,46] and IL-6 [38]. The blockade could act directly from antibodies in the IVIg preparation against the cytokines or indirectly by disruption of precursor components of the immune cascade. The presence of low levels of anti-cytokine antibodies has been shown in normal, healthy humans [20]. Based on this knowledge, it is conceivable that in our study, the normal IgG donor animals also had antibodies that had direct and/or indirect effects on the cytokine levels in the spinal cord milieu following nerve injury. Other proposed mechanisms for IVIg therapy include the neutralization of disease related autoantibodies [12,16], inhibition of complement binding and the resultant mem-branolytic attack complex [4,10,11], modulation and blocking of the antibody’s Fc (constant fragment) receptor [26,27], and neutralizing effects of superantigen epitopes [48].

Based on the many potentially diverse effects of IVIg therapy and our previous work showing that nerve injury appears to induce a robust spinal neuroimmune response, it is not surprising that intrathecal administration of IgG would be able to significantly attenuate mechanical al-lodynia in our current study. Interestingly, the higher doses of either rat or goat IgG were unable to elicit the same effect, demonstrating a reverse U-shaped dose–response...
Table 2
Microglial responses (as illuminated by OX42 ir) of individual animals at the L5 spinal cord level at day 10 post L5 spinal nerve transection with intrathecal treatment on days 0, 1, and 3

<table>
<thead>
<tr>
<th>Intrathecal treatment</th>
<th>Ipsilateral (injured) side</th>
<th>Contralateral (uninjured) side</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DH*</td>
<td>VH</td>
</tr>
<tr>
<td>Normal, untreated</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Surgery alone</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Saline</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>LD anti-IL6</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>LD goat IgG</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>LD rat IgG</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>HD anti-IL6</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>HD goat IgG</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>HD rat IgG</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

a Staining/morphology key: baseline (–), mild (+), moderate (++), or intense (+++) responses. VH = ventral horn, DH = dorsal horn, LD = low dose, HD = high dose.

curve. The reasons for this have not yet been elucidated, but it is possible that when the system is overwhelmed with IgG it is no longer able to exert its beneficial actions on the resultant immune cascade, i.e. may induce a compensatory proinflammatory cascade. Clearly, a more complete understanding of the mechanism(s) by which the immune system is being effected to reduce mechanical allodynia by i.t. IgG therapy is needed if this potential method of therapy is to be utilized and/or optimized for clinical treatment.

Due to the difficulties of intrathecal administration, peripheral therapies are preferable to intrathecal ones. For this reason, we chose to explore the option of peripheral IL-6 and IgG therapy to alter mechanical allodynia in our L5 transection model. However, unlike i.t. IgG administration, peripheral IgG therapy was unable to attenuate mechanical allodynia. There are several plausible reasons for this result. First, we employed lower doses of systemic IgG in our model than those currently used in human disease [9]. In many of the human dosing schedules, IgG was gradually administered over an extended period of time through a continuous intravenous drip and, thereby, attained much higher doses than those employed in the present study. High doses of IgG given in a short time period are associated with adverse effects such as headache, chills, myalgia, and chest discomfort [9]. In view of the difficulty to accurately monitor side effects, administration limitations, and the animals' overall well being, we chose to utilize conservative, instead of high doses of IgG via intravenous injections. Our inability to attenuate mechanical allodynia, therefore, could be due to the concentration administered. Secondly, the lack of attenuation could be the result of the time course we chose to use.

Third, and perhaps of most importance, the concentration that was able to penetrate the CNS from the periphery may not have been adequate for spinal modula-
tion of the cascade resulting from L5 spinal nerve injury. Clearly, further research on the use of IgG therapy needs to be explored to elucidate the optimal dosing concentration/schedule, its mechanism of central action, and its potential role in the alleviation of neuropathic pain.

The pharmacological interventions used in this study were unable to alter robust spinal astrocytic or microglial activation that occurs following a peripheral nerve injury suggesting distinct mechanisms of IL-6 and IgG modulation and spinal glial changes. In addition, the sole use of GFAP and the CR3/CD11b antigen may be inadequate markers to fully assess the contribution of this large glial sub-population of the CNS. At the present time, the complexities of the spinal neuroimmune response to a nerve injury are incompletely elucidated. It appears, however, that the major events in a peripheral inflammatory response following tissue damage also occurs in the CNS after a distant nerve lesion. This innate immunologic reaction in the CNS is comprised of anatomic, physiologic and inflammatory processes that, if left unabated, may facilitate spinal hypersensitization, the pathophysiologic correlate of persistent neuropathic pain. Future plans include the investigation of centrally administered spinal anti-IL-6 and IgG antibodies on cellular adhesion molecule and Major Histocompatibility Complex expression after a peripheral nerve injury.

In conclusion, this study showed that i.t. administration of neutralizing IL-6 antibody and normal rat and goat IgG were effective in attenuating mechanical allodynia in a rat mononeuropathy model. These data are supportive of our hypothesis that a complex central neuroimmune cascade is involved in the development of nerve injury-induced mechanical allodynia. Further research needs to be done to determine specific mechanisms of action, optimally effective doses, dosing schedules, and administration routes of anti-IL6 or IgG therapy while minimizing side effects if this approach is to be considered in the treatment of neuropathic pain in humans.

Acknowledgements

The authors would like to graciously acknowledge Dr. Nancy Birkmeyer for statistical analyses and consultation and Tracy Wynkoop for editorial assistance. The research was supported by: National Institute of Drug Abuse grant DA11276 (JAD).

References


