Antibodies to Nerve Growth Factor Reverse Established Tactile Allostomy in Rodent Models of Neuropathic Pain without Tolerance

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ABSTRACT

A considerable body of evidence implicates endogenous nerve growth factor (NGF) in conditions in which pain is a prominent feature, including neuropathic pain. However, previous studies of NGF antagonism in animal models of neuropathic pain have examined only the prevention of hyperalgesia and allodynia after injury, whereas the more relevant issue is whether treatment can provide relief of established pain, particularly without tolerance. In the current work, we studied the effects of potent, neutralizing anti-NGF antibodies on the reversal of tactile allo-

proximately day 3 to day 7 after treatment. Effects on thermal hyperalgesia were variable with a significant effect observed only in the spinal nerve ligation model. In the mouse chronic constriction injury (CCI) model, a mouse monoclonal anti-NGF antibody reversed tactile allodynia when administered 2 weeks after surgery. Repeated administration of this antibody to CCI mice for 3 weeks produced a sustained reversal (days 4 to 21) of tactile allodynia that returned 5 days after the end of dosing. In conclusion, NGF seems to play a critical role in models of established neuropathic and inflammatory pain in both rats and mice, with no development of tolerance to antagonism. Antag-
onists of NGF, such as fully human monoclonal anti-NGF anti-

bodies, may have therapeutic utility in analogous human pain conditions.

Nerve growth factor (NGF) was originally discovered as a sarcoma-derived substance that stimulated the outgrowth of dorsal root ganglion (DRG) neurons and is now recognized to be a survival and differentiating factor for neural crest-de-

riv ed sensory neurons and sympathetic and basal forebrain cholinergic neurons (for review, see Levi-Mot alchini and An-
geletti, 1968; Sofroniew et al., 2001). In primary sensory neurons of the DRG, NGF modulates expression of a multi-
tude of pain-related transmitters, receptors, and ion chan-

nels. For example, substance P and calcitonin gene-related

peptide precursors are up-regulated by NGF (Lindsay and Harmar, 1989; Gilchrist et al., 1991), as is the vanilloid receptor TRPV1 (Winston et al., 2001) and sodium channels (Friedel et al., 1997; Fang et al., 2005). NGF can also produce morphological changes, such as the sprouting of sympathetic nervous system neurons and formation of sympathetic “baskets” around the cell bodies of sensory neurons in the DRG (Ramer and Bisby, 1999; Zhou et al., 1999).

A considerable body of evidence implicates endogenous NGF in conditions in which pain is a prominent feature. For example, it is up-regulated in DRG Schwann cells for at least 2 months after peripheral nerve injury (Zhou et al., 1999). NGF levels are elevated in synovial fluid from patients with rheumatoid or other types of inflammatory arthritis (Aloe et al., 1992) [similar to arthritic mice (e.g., Aloe et al., 1993)], and NGF is elevated in inflamed or painful bladders (Lowe et al., 1997; Okragly et al., 1999). In addition, NGF is elevated
in chronic pancreatitis, in pancreatic cancer, and in some melanomas, especially nerve invasive cancers (Zhu et al., 1999). In patients with diabetes, NGF is either elevated (Azar et al., 1999; Diemel et al., 1999) or decreased (Faradje and Sotelo, 1990; Anand et al., 1996) in serum and skin, leaving the role of NGF in this disease less clear, perhaps because these studies did not specifically examine patients with painful diabetic neuropathy.

Not only does local or systemic administration of NGF elicit both hyperalgesia and alldynia in rats (Lewin et al., 1994), but also intravenous infusion of NGF in humans produces a whole body myalgia, whereas local administration additionally evokes injection site hyperalgesia and allodynia (Pety et al., 1994). Concordant with the hypothesis that NGF produces pain in physiological events, it has been demonstrated repeatedly that antagonism of NGF function prevents pain behavior. In models of neuropathic pain, such as nerve trunk or spinal nerve ligation, systemic injection of neutralizing antibodies to NGF prevents both allodynia and hyperalgesia (Ramer and Bisyb, 1999; Ro et al., 1999). Likewise, an antinociceptive effect has been demonstrated to a short-term noxious stimulus (McMahon et al., 1995) in an incision model of inflammatory pain (Banik et al., 2005) and in subacute inflammatory pain (Woolf et al., 1994; McMahon et al., 1995) with no inhibition of the inflammatory process (e.g., edema). Perhaps as predicted by clinical findings (Lowe et al., 1997; Zhu et al., 1999; Okragly et al., 1999), anti-NGF treatment has been shown to prevent pain from developing in response to sarcoma growth in the bone (Halvorson et al., 2005; Sevcik et al., 2005), and Watson et al. (2006) reported an antinociceptive effect of an NGF-neutralizing molecule (trkAd5) in a bladder model of visceral pain.

Taken together, these articles suggest a clear physiological role for NGF in pain, independent of injury or inflammatory processes. Given that NGF is elevated in human and animal painful conditions and that anti-NGF treatment can prevent the development of hyperalgesia in a variety of models, it is reasonable to predict that anti-NGF treatment would successfully reverse hyperalgesia. Indeed, Woolf (1996) demonstrated that anti-NGF could reverse hyperalgesia 5 days after CFA injection to the paw and, more recently, Shelton et al. (2005) demonstrated that anti-NGF treatment can reverse established ankle flexion-induced vocalization in a rat model of CFA-induced arthritis without affecting disease progression. In addition, Delafos et al. (2003) demonstrated reversal of inflammatory colitis with anti-NGF 7 days after TNBS administration.

In contrast to inflammatory pain, previously published studies of NGF antagonism in animal models of neuropathic pain have examined only the prevention of hyperalgesia and allodynia after injury. From a clinical perspective, the more relevant issue is whether treatment can provide relief of established pain. In this study, we showed that acute administration of anti-NGF antibodies reversed established pain behavior in models of neuropathic pain in rats and mice as well as in a rat model of inflammatory pain. Finally, the effects of repeated treatment with an anti-NGF antibody were also examined in a mouse model of neuropathic pain, with the results showing no loss of efficacy after 3 weeks of dosing.

Materials and Methods

**Subjects.** Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing between 200 and 400 g were used. Male C57BL/6 mice (Charles River Laboratories) weighing between 21 and 27 g (approximately 9 weeks of age) were also used. Food and water were available ad libitum, and lights were on for 12 h per day. All testing was performed in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health and the approval of the Institutional Animal Care and Use Committee (IACUC) at Aimgen Inc.

**Test Materials.** A goat polyclonal anti-NGF antibody (AF-556-NA) was purchased from R&D Systems (Minneapolis, MN) and constituted in phosphate-buffered saline (PBS) to a concentration of 2 mg/ml. Intrapitoneal doses were varied by adjusting the injection volume. The control was a nonspecific IgG (Aimgen Inc.), also in PBS.

A commercially available mouse monoclonal Ab (aA-AS-18) was purchased from Exalphia Biologicals, Inc. (Maynard, MA) as a reagent for multiple dose in vivo studies in mice. Because the antibody is a mouse anti-mouse mAb, it was expected that any in vivo effects would not suffer interference from antibodies forming against the mAb. This is a likely problem when administering a goat polyclonal anti-NGF Ab to rats or mice. The Ab was dissolved in PBS for subcutaneous dosing to mice, and PBS also served as the control.

**Determination of in Vitro Potency and Selectivity of Antibodies.** DRG were dissected from embryonic day 19 rats and dissociated into single cells using papain (Worthington Biochemical Corp., Freehold, NJ). The dissociated cells were plated into 96-well plates coated with poly-ornithine (Sigma, St. Louis, MO) and laminin (In-vitrogen, Carlsbad, CA) at a density of 10 x 10³ cells/well in complete medium. Treatments of human NGF (R&D Systems), rat NGF (R&D Systems), mouse NGF (Calbiochem, San Diego, CA), and anti-NGF (R&D or Exalphia Biologicals, Inc., Maynard, MA) were applied 2 h after plating. Based on their concentration-effect curves, the concentration of NGF used for IC₅₀ studies was 0.38 nM for human, rat, and mouse NGF, a submaximal concentration. The treated cells were cultured for 48 h. At the end of the culture, cells were fixed, and neuronal survival was measured by trpV1 expression using trpV1 time-resolved fluorescence. TrpV1 is a specific marker for small diameter sensory neurons in DRG. The fixed cells were incubated with anti-trpV1 antibody (Aimgen Inc.) and followed by a Europium-labeled secondary antibody (PerkinElmer Life Sciences and Analytical Sciences–Wallac Oy, Turku, Finland). The fluorescence signal was measured using a Victor Multilabel Counter (PerkinElmer Life and Analytical Sciences, Boston, MA).

To assess potential inhibition of BDNF, mesencephalic dopaminergic neurons from ventral midbrain (Worthington Biochemical Corp.). The dissociated cells were plated into 96-well plates coated with poly-ornithine and laminin at a density of 100,000/cm² as described previously (Louis et al., 1992). BDNF (Aimgen Inc.) at a submaximal concentration of 1 nM was added to the cells 2 h after plating, followed by serial concentrations of anti-NGF samples (R&D or Exalphia Biologicals). Anti-BDNF antibody (Aimgen Inc.) was used as a positive control. After 6 days, high-affinity dopamine uptake capacity of the mesencephalic neurons was assessed as described previously (Friedman and Mytilineou, 1987).

**Rat CFA Model of Inflammatory Pain.** Rats (200 g) were lightly anesthetized with isofo lurean inhalant anesthetic, and the left hind paw was injected with complete Freund’s adjuvant (CFA) emulsion (Sigma), 0.15 ml. This procedure results in tactile allodynia and spreading pain, including injection site pain. All testing was performed in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health and the approval of the Institutional Animal Care and Use Committee (IACUC) at Aimgen Inc.
injection, and PWT and thermal paw withdrawal latency (PWL) were determined each day thereafter without purposeful blinding.

Rat Chung Model of Spinal Nerve Ligation. Rats (200 g) were anesthetized with isoflurane inhalant anesthesia, and the left lumbar spinal nerves at the level of L5 and L6 were tightly ligated (4-0 silk suture) distal to the DRG and before entrance into the sciatic nerve, as first described by Kim and Chung (1992). The incisions were closed, and the rats were allowed to recover. This procedure results in tactile allodynia in the left hind paw. Rats were included in the study only if they did not exhibit motor dysfunction (e.g., paw dragging or dropping) and their PWT was below 39.2 mN (equivalent to 4.0 g). Spinal nerve ligation also leads to the development of thermal hyperalgesia in the injected hind paw. At least 7 days after surgery, rats were treated with polyclonal anti-NGF antibody (3 or 10 mg/kg) or control IgG (3 mg/kg) once by i.p. injection, and PWT and PWL were determined each day thereafter without purposeful blinding.

Rat STZ-Induced Neuropathic Pain Model. Streptozotocin (STZ) is a pancreatic β cell toxin, often used to produce a diabetic state by depleting the supply of insulin, and also produces tactile allodynia. The dose of STZ used here to produce tactile allodynia in male Sprague-Dawley rats of approximately 350 g b.wt. was 50 mg/kg i.p. Body weight was monitored after injection as an indication of diabetes production (failure to gain at control rate) and to ensure that no rat lost excessive body weight during the course of the study. Other signs of diabetes were noted, particularly increased urine output; however, blood glucose levels were not assessed. In general, tactile allodynia developed and reached a stable level 4 weeks after STZ administration and then lasted for another 4 weeks. The success rate for producing rats with neuropathic pain symptoms was 80 to 90%. Rats were included in the study only if their PWT was below 90%. Rats were closed, and the mice were allowed to recover for a minimum of 2 weeks before dosing. This model produces symptoms of tactile allodynia similar to the rat Chung model of neuropathic pain although presurgery PWT is much lower than that in rat. Mice were treated with anti-NGF antibody (100 mg/kg) or vehicle by i.p. injection by an investigator blinded to the treatment, and PWT was determined each day thereafter by an observer also blinded to the treatment.

Mouse Chronic Constriction Injury Model. To test the in vivo activity of the mouse monoclonal anti-NGF antibody (Exalpha Biologicals, Inc.), the mouse chronic constriction injury (CCI) (aka Bennett model) model of neuropathic pain was used. In brief, the animals was anesthetized using isoflurane inhalant anesthetic, and the sciatic nerve was loosely ligated (6-0 silk suture) in the mid-thigh as described for rats by Bennett and Xie (1988). The incisions were closed, and the mice were allowed to recover for a minimum of 2 weeks before dosing. This model produces symptoms of tactile allodynia similar to the rat Chung model of neuropathic pain although presurgery PWT is much lower than that in rat. Mice were treated with anti-NGF antibody (100 mg/kg) or vehicle by i.p. injection by an investigator blinded to the treatment, and PWT was determined each day thereafter by an observer also blinded to the treatment.

Anesthesia and surgical technique. After anesthesia, rats were treated with polyclonal anti-NGF antibody (3 or 10 mg/kg) or control IgG (3 mg/kg) once by i.p. injection, and PWT and PWL were determined each day thereafter without purposeful blinding.

Results

In Vitro Activity of Antibodies. The IC_{50} of the goat polyclonal antibody from R&D Systems was determined to be 129 nM against human NGF (0.38 nM) and 0.5 nM against rat NGF (0.38 nM). The IC_{50} of the mouse monoclonal antibody from Exalpha Biologicals was determined to be 0.48 nM against mouse NGF (0.38 nM). Neither antibody was capable of inhibiting the activity of BDNF (1 nM) when tested at concentrations up to 10 μM.

Rat CFA Model of Inflammatory Pain. Behavior indicative of inflammatory pain was induced by the injection of CFA into the hind paw of rats. One week after CFA injection, a single i.p. injection of anti-NGF antibody (R&D Systems) or control IgG was administered, and tactile allodynia and thermal hyperalgesia were assessed daily. For tactile allodynia, ANOVA revealed a significant main effect of treatment (F = 11.4, p < 0.0001) and of time (F = 37.6, p < 0.0001) with a significant interaction (F = 17.4, p < 0.0001). Subsequent Bonferroni corrected t tests determined that allodynia was significantly reversed by 1 and 3 mg/kg beginning on day 3 after treatment and that this effect persisted through day 7 after treatment. At a dose of 0.3 mg/kg, allodynia was significantly reversed on days 4 and 5 (Fig. 1A). For thermal hyperalgesia, ANOVA revealed a significant main effect of time (F = 8.4, p < 0.016) but not of treatment (F = 2.5, p = 0.114).

Thermal Hyperalgesia was induced by the injection of CFA into the hind paw of rats. One week after CFA injection, a single i.p. injection of anti-NGF antibody (R&D Systems) or control IgG was administered, and tactile allodynia and thermal hyperalgesia were assessed daily. For tactile allodynia, ANOVA revealed a significant main effect of treatment (F = 11.4, p < 0.0001) and of time (F = 37.6, p < 0.0001) with a significant interaction (F = 17.4, p < 0.0001). Subsequent Bonferroni corrected t tests determined that allodynia was significantly reversed by 1 and 3 mg/kg beginning on day 3 after treatment and that this effect persisted through day 7 after treatment. At a dose of 0.3 mg/kg, allodynia was significantly reversed on days 4 and 5 (Fig. 1A). For thermal hyperalgesia, ANOVA revealed a significant main effect of time (F = 8.4, p < 0.016) but not of treatment (F = 2.5, p = 0.114).

Statistical Analysis. Data were analyzed for significant differences by ANOVA and, if warranted, followed by t tests with Bonferroni’s correction of p values for multiple comparisons using the computer program Prism (GraphPad, San Diego, CA). The p value for significance was set at 0.05. Graphs were drawn with the same program.

Fig. 1. Time course for reversal of tactile allodynia (A) and thermal hyperalgesia (B) in the CFA model of inflammatory pain. Rats were administered CFA (0.15 mg) into the hind paw 5 days before administration of a single i.p. injection of either anti-NGF antibody or a control IgG (arrows indicate injection). Tactile allodynia was assessed with von Frey filaments, and thermal hyperalgesia was assessed with a Hargreaves style hot-box. Points and bars represent the mean and S.E.M., respectively. Asterisk indicates Bonferroni-corrected p < 0.05. n = 6/group.
injected with a single dose of the pancreatic cell toxin STZ, developed in the hind paws within 2 to 3 weeks. After 4 weeks, a single i.p. injection of anti-NGF antibody (10 mg/kg) or control IgG was administered and tactile allodynia was assessed daily. ANOVA revealed a significant main effect of treatment \( (F = 61.46, p < 0.0001) \) and of time \( (F = 4.7, P = 0.0001) \), with a significant interaction \( (F = 2.65, p = 0.014) \). Subsequent Bonferroni-corrected \( t \) tests determined that a statistically significant reversal of allodynia was observed beginning on day 2 and that this effect persisted through day 6 after treatment (Fig. 3). Thermal hyperalgesia was not assessed.

**Mouse Chronic Constriction Injury Model.** The mouse mAb from Exalpha Biologicals produces single-dose, repeated-dose, and sustained reversal of allodynia in the CCI model as shown in Figs. 4 and 5. Approximately 2 weeks after CCI surgery, an i.p. dose of anti-NGF Ab (100 mg/kg i.p.) or vehicle was administered twice, with each administration separated by 2 weeks. Tactile allodynia data were analyzed by ANOVA, and significant main effects of treatment \( (F = 8.01, p = 0.02) \) and time \( (F = 4.18, p < 0.0001) \) were observed, as well as a significant interaction \( (F = 3.95, p < 0.0001) \) (Fig. 4). In additional single-dose studies (data not shown), a dose of 50 mg/kg also was effective, but 10 mg/kg was not.

An extended repeated dose study was conducted to assess possible tolerance to the long-term depletion of NGF. Anti-NGF Ab (100 mg/kg i.p.) or vehicle (i.p.) was administered thrice weekly for 3 weeks, and tactile allodynia was assessed at regular intervals for 28 days. ANOVA revealed a significant main effect of treatment \( (F = 84.34, p < 0.0001) \) and time \( (F = 4.27, p < 0.0001) \), as well as a significant interaction \( (F = 4.06, p < 0.0001) \). Subsequent Bonferroni corrected \( t \) tests determined that a statistically significant reversal of allodynia was observed from days 4 to 21 (Fig. 5). No behavioral consequences were observed other than this continued reversal of tactile allodynia throughout the dosing period.

**Discussion**

Although inflammatory pain can be both prevented (Woolf et al., 1994) and reversed (Woolf, 1996; Shelton et al., 2005) with anti-NGF treatment, previous studies of neuropathic pain. Rats had the left L5 and L6 spinal nerves ligated at least 7 days before spinal nerve ligation surgery, rats were administered a single i.p. injection of anti-NGF antibody (R&D Systems) or control IgG and monitored for tactile allodynia and thermal hyperalgesia. For tactile allodynia, ANOVA revealed a significant main effect of treatment \( (F = 18.6, p < 0.0001) \) and of time \( (F = 17.7, P < 0.0001) \) with a significant interaction \( (F = 15.6, p < 0.0001) \). Subsequent Bonferroni-corrected \( t \) tests determined that allodynia was significantly reversed by 10 mg/kg beginning on day 2 after treatment and that this effect persisted through day 7 after treatment. At a dose of 3 mg/kg beginning on day 2 after treatment and that this trend toward reversal for the dose of 3 mg/kg but the variance was too large to discern this effect statistically (Fig. 1B).

**Rat Chung Model of Spinal Nerve Ligation.** One week after spinal nerve ligation surgery, rats were administered a single i.p. injection of anti-NGF antibody (R&D Systems) or control IgG and monitored for tactile allodynia and thermal hyperalgesia. For thermal hyperalgesia, ANOVA revealed a significant main effect of treatment \( (F = 15.64, p < 0.0001) \) and of time \( (F = 7.2, P < 0.0001) \). Therefore, further post hoc analysis was not performed, although a significant interaction \( (F = 7.2, p = 0.107) \). Therefore, further post hoc analysis was not performed, although a significant interaction \( (F = 7.2, p = 0.107) \). Therefore, further post hoc analysis was not performed, although a significant interaction \( (F = 7.2, p = 0.107) \). Therefore, further post hoc analysis was not performed, although a significant interaction \( (F = 7.2, p = 0.107) \). Therefore, further post hoc analysis was not performed, although a significant interaction \( (F = 7.2, p = 0.107) \). Therefore, further post hoc analysis was not performed, although a significant interaction \( (F = 7.2, p = 0.107) \). Therefore, further post hoc analysis was not performed, although a significant interaction \( (F = 7.2, p = 0.107) \). Therefore, further post hoc analysis was not performed, although a significant interaction \( (F = 7.2, p = 0.107) \). Therefore, further post hoc analysis was not performed, although a significant interaction \( (F = 7.2, p = 0.107) \). Therefore, further post hoc analysis was not performed, although a significant interaction \( (F = 7.2, p = 0.107) \).
pain have demonstrated the prevention of pain behavior only when the anti-NGF treatment was applied before, or concur-
rent with, the pain-inducing stimulus (Ramer and Bisby, 1999; Ro et al., 1999). Here, we demonstrate for the first time
that anti-NGF treatment reversed established pain behaviors in rat and mouse models of neuropathic pain when the
treatment was applied well after the pain-inducing stimulus. Furthermore, there was no development of tolerance to the
antiallodynic effects of anti-NGF treatment over 3 weeks in the mouse CCI model of neuropathic pain.

Although the closely related growth factors BDNF and
NT-3 have a possible role in pain, the effects observed here are likely to be due to the selective sequestration of NGF. The
two antibodies used here were tested in vitro against NGF
and BDNF and were found to be potent (subnanomolar)
inhibitors of NGF but not of BDNF at concentrations up to 10
μM. Actual plasma levels were not determined here, but a 10
mg/kg i.v. dose of another antibody to rat resulted in a maxi-
mum plasma concentration of only 1.6 μM (L. Nguyen, un-
published observations). The activity against NT-3 was not
tested here, but given that the antibodies can discriminate
NGF from BDNF, the epitopes are likely to be on regions of
NGF not well conserved in this family of growth factors (see
Wiesmann et al., 1999).

It is noteworthy that our studies seem to show that lower
doses of the same anti-NGF antibody were effective in the
CFA model of inflammatory pain (Fig. 1) compared with the
Chung or STZ models of neuropathic pain (Figs. 2 and 3) in
rats. Although this could be a result of the different stimulus
intensity of these models, it could also be due to a higher level
of NGF produced in nerve injury compared with inflammatory
conditions or a larger role of NGF in inflammatory pain.
Unfortunately, the literature does not resolve this issue; no
other studies have compared the same antibody in these
different models. The nearest comparison is a study of anti-
NGF in both the CCI and Chung models of neuropathic pain,
reported by Ramer and Bisby (1999), in which tactile allo-
dynia was prevented more effectively in the CCI model than
in the Chung model. Further studies will be required to
determine differential sensitivity of different types of pain.

It is also noteworthy that thermal hyperalgesia was not
robustly reversed in the CFA and Chung models where it was assessed. However, the observed variability in the thermal
hyperalgesia data were higher than in the tactile allodynia
data, limiting the statistical power. A main effect of treatment
was observed for thermal hyperalgesia in the Chung model as well as a trend in the CFA model. Furthermore,
reversal of thermal hyperalgesia has been reported in the
literature (Woolf, 1996). Viewed from a mechanistic perspec-
tive, it may be more surprising that tactile allodynia was
significantly reversed by anti-NGF treatment. Allodynia is
believed to be mediated by large Aβ fibers, which are not
normally regarded as NGF-sensitive. Perhaps reversal of
tactile allodynia is mediated through interruption of a C-
fiber-mediated central sensitization process or of an interme-
siately signaling process from trkA-positive C fibers to Aβ
fibers, such as BDNF (Woolf, 1996). However, it should also
be noted that some Aβ fibers do express the high-affinity
NGF receptor trkA, resulting in apparent control of Nav 1.8
expression and function (Fang et al., 2005), a sodium channel
believed to be important in neuropathic pain.

The time course and duration of anti-NGF efficacy is of
interest with regard to possible mechanisms of action. The
biological effect of anti-NGF Ab in these rat studies is con-
sistent with the 11 day half-life reported in the literature for
an IgG1 Ab (Davis et al., 1995). In our mouse studies using a
mouse monoclonal anti-NGF Ab, the duration of action was
shorter than observed in rat (Fig. 4), but a study of the
normal duration of IgG in mouse plasma found the half-life to be
4 days (Paherty and Robinson, 1963), which can be shortened
to 2 days by increasing the plasma concentration of IgG
beyond normal, such as with large exogenous doses. Because
mice would not be expected to generate a neutralizing im-
mune response to a mouse monoclonal Ab, the observed time
courses are probably due to normal pharmacokinetic behav-
ior. Still, sustained sequestration of NGF could lead to up-
regulation of NGF, overcoming and terminating the antibody
effect. This possibility was addressed in the mouse CCI
model, where a second administration of anti-NGF was ef-
sective shortly after the effect of the first administration had
ended (Fig. 4). Furthermore, repeated dosing of the antibody
in mice led to a sustained reversal of pain behavior for more
than 3 weeks (Fig. 5). Thus, it seems unlikely that increased
production of NGF in response to the Ab explains the termi-
nation of effect in either rat or mouse; i.e., there is no apparent tolerance to sustained depletion of NGF. It is noteworthy that tactile allodynia returned after 3 weeks of suppression with anti-NGF treatment, indicating that anti-NGF treatment does not lead to a permanent reversal of pain in this period, perhaps because the underlying nerve injury is still present.

In conclusion, NGF seems to be a critical mediator of all types of pain, including short-term pain (McMahon et al., 1995), surgical pain (Banik et al., 2005), inflammatory pain (Woolf et al., 1994), visceral pain (Watson et al., 2006), and, as we report here, neuropathic pain. It is noteworthy that a lack of tolerance to NGF sequestration was demonstrated over a 3-week period. Thus, NGF may be an excellent therapeutic target for these short- and long-term indications. Either a polyclonal or a monoclonal Ab was shown here to reverse pain behavior, improving the likelihood of producing and developing an effective therapeutic agent, such as a fully human monoclonal anti-NGF antibody.

References


