Inflammatory cytokines in degeneration, regeneration and maintenance of sciatic nerve

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Chapter 13

INFLAMMATORY CYTOKINES IN DEGENERATION, REGENERATION AND MAINTENANCE OF SCIATIC NERVE

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ABSTRACT

Inflammatory cytokines play important roles in a variety of pathophysiological changes associated with traumatic injury and demyelinating disorders in the peripheral nervous system. After sciatic nerve injury, proinflammatory cytokines such as interleukin 1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNFα) are produced by and act on neurons, Schwann cells, and infiltrating inflammatory cells at the lesion site. Underlying these processes is receptor-mediated activation of intracellular signaling pathways that regulate cell differentiation and proliferation and synthesis of axon and myelin components. This review focuses on roles of inflammatory cytokines in the degeneration and regeneration of the peripheral nerve. In addition, we describe here altered expression of inflammatory cytokines in gradually elongated rat sciatic nerves. This experimental model, which is devoid of Wallerian degeneration, provides insights into the involvement of inflammatory cytokines in the maintenance of myelinated axons. Based on the findings obtained from nerve elongation models and those from conventional nerve injury models, we discuss the molecular mechanism that ensures the integrity of the peripheral nerve.

1. INTRODUCTION

Inflammatory cytokines are small secreted proteins that modulate inflammatory and immune responses (Dinarello, 2000; Borish & Steinke, 2003). A group of inflammatory cytokines is composed of members belonging to distinct cytokine families including TNF-, IL-1-, IL-6-, interferon (IFN)-, and transforming growth factor (TGF)-families (Tracy &

Cerami, 1993; Dinarello, 2009; Hirano et al., 1997; Borden, et al., 2007; Derynck & Miyazono, 2008). Based on their activities, inflammatory cytokines are divided into two types: proinflammatory cytokines, such as TNFα, IL-1, IL-6, IL-12, and IFNγ; and anti-inflammatory ones, such as IL-4, IL-10, IL-13, and TGFβ (Dinarello, 2000; Borish & Steinke, 2003; Shubayev, et al., 2010). Depending on conditions, however, certain proinflammatory cytokines can be anti-inflammatory, and vice versa. Proinflammatory cytokines initiate and promote inflammation by mobilizing and attracting leukocytes, whereas anti-inflammatory cytokines inhibit or terminate inflammation by antagonizing the effects of proinflammatory cytokines.

In the peripheral nervous system (PNS), as well as in the central nervous system (CNS), inflammation is a prerequisite process for degeneration and subsequent regeneration. Although earlier cytokine research was conducted mainly in circulating leukocytes and lymphatic tissues, accumulating evidence has established that they contribute to normal development and pathologic conditions associated with traumatic injury and inflammatory/immune demyelinating neuropathies (Créange et al., 1997; Skundric & Lisak, 2003; Pletz et al., 2003; Leinninger et al., 2004; Martini et al., 2008). Previous studies have shown that inflammatory cytokines are produced not only by infiltrating hematogenous leukocytes but also by neurons, Schwann cells, and resident macrophages. These cells are also known to express various cytokine receptors (Hirota et al., 1996; Grothe et al., 2000; George et al., 2005; Lara-Ramírz t al., 2008; Ozaki t al., 2008) and downstream signaling molecules (Sheu et al., 2000; Zrouri et al., 2004; Girolami et al., 2009; Wang et al., 2009) In concert with neurotrophins, chemokines, and other neurotrophic factors, inflammatory cytokines form an intricate network, thereby participating in the initiation, progression, and termination of many cellular responses including neuronal survival and death, axonal degeneration, loss, regrowth, and remyelination of axons by Schwann cells (Créange et al., 1997; Boyd & Gordon, 2003; Chen et al., 2007; Navaro et al., 2007).

This chapter reviews pathophysiological roles of inflammatory cytokines in the degeneration and regeneration of the peripheral nerve and describes their possible involvement in cellular processes in non-degenerative peripheral nerves.

2. RECEPTORS AND INTRACELLULAR SIGNALING SYSTEMS

TNF-Family

There are two structurally distinct receptors for TNF: TNFR1 (also known as p55) and TNFR2 (also known as p75) (Shuvayev et al., 2010). The cytoplasmic domains of these receptors recruit different proteins that transduce downstream signaling. In one case, the TNFR1 cytoplasmic domain is linked to cell death pathways, but this is not the case with TNFR2. Both receptors, however, lead to the translocation of the nuclear factor-κB (NF-κB), a key transcription factor, into the nucleus. Then NF-κB binds to the promoter regions of diverse genes. TNFR1 has a cell death-related domain and recruits a protein called MORT-1 and other intracellular proteins, referred to as TNFR-associated factors. The TNFR1 cytoplasmic domains also recruit the family of proteins called TNFR-associated death domains (TRADDs). The overexpression of TRADDs evokes cell death and activates NF-κB.

In addition, TRADDs activate the caspase family of intracellular cysteine proteases, some of which are important for processing cytokine precursors, such as proIL-1β and proIL-18.

IL-1-Family

Two distinct binding proteins (IL-1 receptors: IL-1Rs) and one IL-1R accessory protein (IL-1R-AcP) have been identified. The extracellular domains of the two IL-1Rs and the IL-1R-AcP share a common structural feature. Binding of IL-1 to its receptor induces several transcription factors. In most instances, induction of IL-1-related genes is dependent on nuclear translocation of NF-κB and another nuclear factor AP-1. TNF- and IL-1-related cytokines activate NF-κB and RAS/mitogen-activated kinase (RAS/MAPK) pathways (Ishihara & Hirano, 2002).

IL-6-Family

The IL-6-family of cytokines share a common receptor subunit designated gp130 (Ishihara & Hirano, 2002; Ernst & Jenkins, 2004). Through binding to their specific receptors in many tissues, these cytokines activate intracellular signaling cascades and alter gene expression levels in target cells. Many ILs and IFNs activate Janus kinase-signal transducers and activators of transcription (JAK/STAT) pathway (Heinrich et al., 2003; Dziennis & Alkayed, 2008).

One important aspect is that some receptors are secreted as a soluble form (Levine, 2008). In general, soluble cytokine receptors are generated by proteolytic cleavage, by alternative splicing, or by expression of distinct genes that encode secreted cytokine-binding proteins (Levine, 2008). Importantly, these soluble receptors bind their target ligands and modulate their activity in either an antagonistic or agonistic fashion (Levine, 2008).

3. Effects on Degeneratiion and Regeneration of Peripheral Nerve

Traumatic injury, toxicity, autoimmune, and metabolic damages elicit a sequence of cellular responses including neuronal cell death or survival, axonal degeneration, axonal regrowth, dedifferentiation and proliferation of Schwann cells, and remyelination (Waller, 1850; Fawcett & Keynes, 1990; Ide, 1996; Fu & Gordon, 1997; Hall, 2005; Makwana & Raivich, 2005; Raivich & Makwana, 2007). During these responses, various inflammatory cytokines exhibit upregulated expression in many types of cells, such as motor and sensory neurons, Schwann cells, resident and infiltrating macrophages, lymphocytes, mast cells, and fibroblasts (Okamoto et al., 2001; Stoll, et al., 2002). Proinflammatory cytokines, such as IL-1β, IL-6, and TNFα, are produced by and act on neurons, Schwann cells, and infiltrating inflammatory cells in autocrine and paracrine manners, forming an intricate network with other factors.

Responses in the Nerve Cell Body

After nerve injury, a proportion of motor neurons in the spinal cord and primary sensory neurons in dorsal root ganglia (DRG) undergo cell death, and surviving cells exhibit changes to promote regeneration at both the cellular and molecular levels (Lieberman, 1974; Richardson et al., 2009). Several distinct pathways convey signals from injured sites to cell bodies (Snider et al., 2002; Abe & Cavalli, 2008). At the lesion site, Schwann cells produce LIF and CNTF, both of which belong to the IL-6 family and activate the gp130/JAK/STAT signaling. The resulting phosphorylated form of STAT3 (pSTAT3) is then transported retrogradely into the nucleus and upregulates the transcription of axonal regeneraton-related genes (Lee et al., 2004). This process is facilitated by a broad spectrum of trophic factors secreted by many types of cells. Such factors include neurotrophins, neuropoietic cytokines, insulin-like growth factors (IGFs), and glial cell line-derived neurotrophic factors (GDNFs).

Upon injury, IL-6 is induced in neurona cell bodies (Kiefer et al., 1993; Murphy et al., 1995; Hirota et al., 1996; Osamura et al., 2005) as well as in Schwann cells (Bolin et al., 1995). IL-6 is a well-known pleiotrophic cytokine that is involved in neuronal survival (Gadient and Otten, 1997; Hirano et al., 1997; Heinrich et al., 1998). IL-6 further induces the expression of various genes associated with degeneration and regeneration (Lee et al., 2009a & b). Transgenic mice expressing both IL-6 and IL-6 receptor (IL-6R) showed accelerated regeneration of axotomized hypoglossal nerve (Hirota et al., 1996). In IL-6 knockout mice, its functional recovery was delayed after the crush lesion of the sciatic nerve (Zhong et al., 1999). When the sciatic nerve was transected, retrograde death of L5 DRG neurons was 45% greater in IL-6 knockout mice than in wild-type ones (Murphy et al., 1999). In addition, exogenously applied human IL-6 and soluble human IL-6R promoted the survival of DRG neurons cultured from newborn rats (Thier et al., 1999). IL-6 is thus thought to moderate the death of neurons that undergo Wallerian degeneration. Other factors including IL-1β also have protective effects on neurons (Ikeda et al., 1996; Carlson et al., 1999; Diem et al., 2003)

Axonal Degeneration and Phagocytosis

In degenerated peripheral nerve, both resident endoneural and infiltrating blood-derived macrophages serve as scavengers of myelin debris. Upon peripheral nerve injury, Schwann cells rapidly and transiently synthesize TNFα, which initiates the cytokine network and recruits macrophages from the blood circulation (Wagner & Myers, 1996; Oka et al., 1998; Liefner et al., 2000; Kiefer et al., 2001; Leonhard et al., 2002; Mueller et al., 2003; Mäurer et al., 2003; Siebert & Brück, 2003; George et al., 2004; Cheepudomwit et al., 2008). The activated macrophages at injured and demyelinated sites further produce TNFα and phagocytose myelin, resulting in Wallerian degeneration (Stoll, et al., 1993). TNFα has been shown to induce inflammatory demyelination and Wallerian degeneration by activating the transcription of genes in Schwann cells (Bonetti et al., 2000). In TNFα-deficient mice, macrophage recruitment into transected, degenerating sciatic nerve, and clearance of myelin debris was delayed, without significant loss of phagocytotic activity (Liefer et al., 2000). This observation indicates that the major role of TNFα is recruitment of macrophage from

peripheral blood into the injury site. The production of TNFα is suppressed by IL-10 (Wagner et al., 1998) and erythropoietin (Li et al., 2005; Campana et al., 2006).

IL-1α, IL-1β, ICE, and type 1 IL-1 receptor (IL-1RI) are constitutively expressed in a normal adult sciatic nerve. Upon injury, IL-1 is locally produced by Schwann cells, endoneural and infiltrating macrophages and fibroblasts in the peripheral nerve, and dorsal root ganglion (DRG) neurons (Lisak et al., 1997). IL-1 promotes phagocytosis by macrophages and the synthesis of other factors, such as nerve growth factor (NGF), leukemia inhibitory factor (LIF), and macrophage chemoattractant protein-1 (MCP-1), by Schwann cells and sensory neurons (Lindholm et al., 1987; Rotshenker et al., 1992; Shadiac et al., 1993; Carlson et al., 1996; Basu et al., 2004; Perrin et al., 2005). Schwann cells of denervated nerves attract macrophages by secretion of MCP-1 in a process regulated by IL-6 and LIF (Tofaris et al., 2002).

Regrowth of Axons

In injured nerve, neuropoietic cytokines, including IL-1β, IL-6, LIF, and TGF-β1, are upregulatedly expressed and promote axonal growth. Sprouted axons then establish the contact with Schwann cells. During this period, axotomized neurons transit from a transmitting mode to a growth mode, and produce growth-associated proteins, such as GAP-43, tubulin, and actin, as well as diverse neuropeptides and cytokines that promote axonal regeneration. Macrophages also can produce neurite-promotoing factors (Luk et al., 2003). IL-1β promotes axonal growth of sensory neurons (Horie et al., 1997; Temporin et al., 2008a), by deactivating RhoA through the p38 MAP kianse pathway (Horie et al., 1997; Temporin et al., 2008b). Inhibition of TNFα-activeted p38 MAP kinase enhances the axonal regeneration (Myers et al., 2003). In organotyoic mouse DRG explants, IL-4 increased outgrowth induced by neurotrophin-4 (NT-4); IL-6 stimulated outgrowth by neurotrophin-3 (NT-3) and NT-4; and IFNγ stimulated neurite extension in the presence of NT-3 plus NT-4 and NT-3 plus NGF (Gölz et al., 2006). In contrast, TNFα inhibited outgrowth induced by NT-3, NT-3 and/or plus NGF (Gölz et al., 2006). TNFα has been also reported to inhibit the neurite outgrowth of DRG explants through down-regulation of myelin associated glycoprotein (Schneider-Schaulies et al., 1991). However, TNFα supposedly induces sensory nerve growth, possibly leading to pain (Hayashi et al., 2008), although part of this action might be mediated by the stimulatory effect of TNFα on the production of NGF (Hattori et al., 1993).

Dedifferentiation and Proliferation of Schwann Cells

In both normal development and pathologic states, Schwann cells provide trophic support for peripheral neurons by producing inflammatory cytokines, as well as neurotrophins and other cytokines (Bhatheja & Field, 2006; Lisak & Benjamins, 2007; Mirsky et al., 2008; Madduri & Gander, 2010). After axonal degeneration, Schwann cells dedifferentiate and proliferate (Livesey et al., 1997). Conti et al. (2002) have shown that IL-1 promotes proliferation and apoptosis in cultured Schwann cells. Subsequently, as degenerated axons start regrowing, Schwan cells quit dividing and start myelination. Schwann cells in the distal

stump undergo proliferation and phenotypical changes to prepare the local environment to be favorable for axonal regeneration. In addition to producing many neurotrophic factors and their receptors, Schwann cells produce cell-surface adhesion molecules, which serve as components of the basement membrane, along with many extracellular matrix proteins, such as laminin, fibronectin, and tenascin.

Remyelination

Schwann cells are responsible for myelination and ensheathment of myelinated axons and synthesis of myelin components (Garbay et al., 2000; Jessen & Mirsky, 2005; Chen et al., 2007; Svaren & Meijer, 2008). It is well known that myelination is regulated by various endogenous modulators including extracellular matrix proteins, neurotrophins, and cytokines (Boyd & Gordon, 2003; Notterpek, 2003; Rosenberg, et al., 2006; Chen, et al., 2007). Of these, the IL-6 family of cytokines, such as ciliary neurotrophic factor (CNTF), LIF, and IL-11, are thought to have substantial importance, because mice genetically depleted of the gp130, a common receptor subunit for these cytokines, undergo degeneration of peripheral myelin sheath (Betz et al., 1998).

IL-6 is involved in various pathophysiological processes in the PNS. Several studies have demonstrated that the expression of IL-6 and IL-6 receptor α subunit (IL-6Rα) is upregulated in dorsal root ganglion (DRG) neurons after axonal injury and elongation (Lara-Ramiréz et al., 2008). IL-6 is also known to promote myelination of Schwann cells. In cultured mouse and rat embryonic DRG, IL-6 fused to soluble IL-6 IL-6R stimulated the expression of many genes for myelin-associated proteins including P0 and myelin basic protein (MBP) (Haggiag et al., 1999; Zhang et al., 2007). Intraperitoneal injection of IL-6 along with IL-6R increased 4-fold the number of myelinated axons and thickness of myelin sheaths following suture of transected rat sciatic nerves (Haggiag et al., 2001). Subcutaneous injection of IL-6 prevented cisplatin-induced degeneration of rat sciatic nerve (Callizot et al., 2008) and improved reduced nerve conduction velocity of the sciatic nerve of streptozotocin-induced diabetic rats (Cotter et al., 2010).

As already established in non-neuronal tissues, these cytokines exert their effects through the JAK/STAT and RAS/MAPK pathways (Ishihara & Hirano, 2002; Ernst & Jenkins, 2004). The balance or interplay of these pathways is thought to determine the cell type-specific responses. Although receptor subunits specific for these cytokines and gp130 are expressed in Schwann cells, very little is known about their downstream effector molecules.

In a study using cultured rat Schwann cells, we demonstrated that IL-6 elevated the mRNA level of PMP22 but not those of MPZ and MBP, and that the increase in PMP22 mRNA is and accompanied by phosphorylation of JAK2 and STAT3 (Ito et al., 2010). The increase in PMP22 mRNA was JAK2 inhibitor-sensitive, but MAPK kinase-insensitive (Ito et al., 2010). PMP22 is a tetraspan glycoprotein vigorously produced by myelinating Schwann cells. Despite its abundant presence in the compact myelin, the physiological role of PMP22 has not been fully clarified. In good correlation with myelin formation, PMP22 expression in the sciatic nerve is strongly upregulated during the postnatal period and during nerve regeneration after injury. Duplication of the PMP22 genes is associated with hereditary demyelinating neuropathies, such as Charcot-Marie-Tooth disease type 1A, and deletion of a single gene copy leads to hereditary neuropathy with liability to pressure palsies. Amici et al.

(2006) showed that PMP22 forms a complex with α6β4 integrin and laminin, suggesting its involvement in the interaction between Schwann cells and the surrounding basal lamina. Therefore, together with the observation that conditional knockout of the mouse gp130 gene caused degeneration of myelin sheath, we surmise that IL-6-signaling through the gp130/JAK/STAT pathway may regulate PMP22 expression and thereby contribute to the formation or stabilization of the compact myelin.

Earlier studies have revealed that PMP22 expression is regulated transcriptionally by cyclic AMP and post-transcriptionally by microRNA-29a (Verrier et al., 2009). The relationship between these regulatory processes and the present results is currently unclear. In DRG neurons, levels of IL-6 mRNA and IL-6 increase rapidly within a day and gradually decrease over a period of 1–2 weeks after transection and elongation of the sciatic nerve (Gadient et al., 1997; Osamura et al., 2005). Also, Sheu et al. (2000) have shown that phosphorylation of STAT3, in both proximal and distal ends, peaks within a day but remains for more than 2 weeks (See also Fig.1). In contrast, the PMP22 mRNA level declines immediately after nerve injury and then is upregulated after 1 week (Snipes et al., 1992). Conceivably, the IL-6-signaling through the gp130/JAK/STAT pathway may participate in the delayed upregulation of PMP22; however, the initial reduction could not be explained by the same mechanism. More detailed analysis is required to determine the contribution of the IL-6 and gp130/JAK/STAT signaling to the peripheral nerve regeneration. If this pathway is critical for in vivo myelin synthesis, the recent challenges for pharmacological interventions targeting the gp130-mediated signaling (Fischer et al., 2008) might benefit the treatment of neuropathies associated with abnormal myelin formation. Also, upon Schwann cell transplantation into injured neural tissues (Kocsis & Waxman, 2007; Woodhoo et al., 2007), it would be important to control the gp130/JAK/STAT pathway to ensure proper myelination.

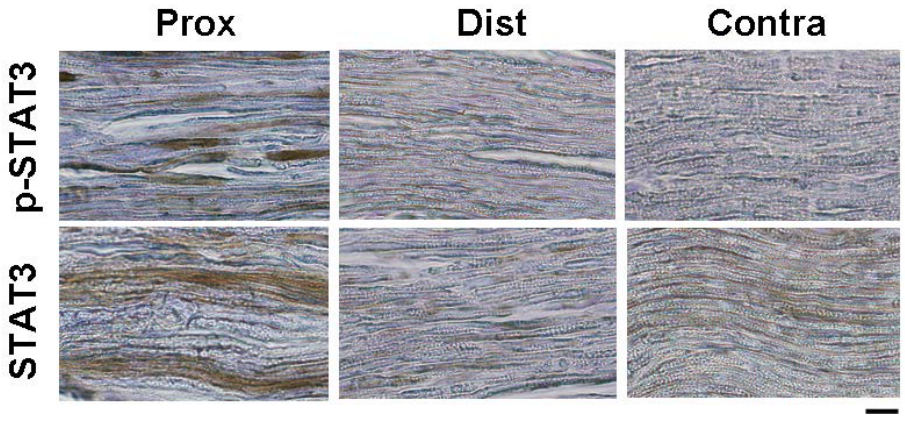

Figure 1. Immunoperoxidase staining for phosphrylated STAT3 (pSTAT3) and STAT3. At 1 day after transection of the left sciatic nerve, proximal (*Prox*) and distal (*Dist*) nerve stumps, and contralateral (*Contra*) were subjected to staining with anti- pSTAT3 and anti-STAT3 antidodies. In the proximal stump, intense immunoreactivity (IR) for pSTAT3 is detectable in cells located along axons. Scale bar, 20 μm.

In addition to its degenerative action, TNFα has also been reported to aid regeneration by protecting injured neurons (Cheng et al., 1994) or by up-regulating synthesis of myelin-specific proteins, such as myelin basic protein (Huang et al., 2002). For all these findings, elucidation of regulatory mechanisms for peripheral myelination is still in its infancy; there still remain unidentified regulatory factor(s) involved in Schwann cell myelination (Monk et al., 2009).

4. POSSIBLE ROLES IN PRE- OR NON-DEGENERATIVE PERIPHERAL NERVE

Most experiments using peripheral nerves, especially the sciatic nerve, have been conducted toward the elucidation of degeneration, regeneration, and pain. Besides, inflammatory cytokines play roles in pre- or non-degenerative myelinated fibers, although the number of reports is limited. This section reviews the alterations observed in gradually elongated sciatic nerves and those in contralateral sciatic nerves after unilateral sciatic nerve elongation and injury.

Gradual Sciatic Nerve Elongation

Gradual nerve elongation was originally developed for the reconstruction of segmental nerve defects in peripheral nerve surgery. Although transplantation of autologous nerve grafts has been a standard operation, the outcome is not always satisfactory due to sensory deficit in the donor site, the limited length of donor nerves, and the requirement for axonal regeneration across two anastomotic sites (Doi et al., 1993, Orbay et al., 1993; Taylor, 1976). These problems are inevitable in most instances, and thus, alternative procedures are awaited. Recently, several studies have demonstrated that gradual nerve elongation can achieve results comparable to nerve grafting not only in experimental animals (De Bastiani et al., 1987; Orbay et al., 1993; Ohkaya et al., 2000; Kroeber et al., 2001; Jiang et al., 2004; Matsuzaki et al., 2004), but also in clinical applications (Manders et al., 1987). This procedure has advantages over nerve grafting, as a defect can be bridged by an elongated nerve without donor-site problems and anastomosed at one site. Besides these studies in vivo, nerve elongation can be applied to producing transplant materials in vitro (Pfister et al., 2004).

More importantly, in contrast with conventional sciatic nerve injury models, the gradual elongation model is devoid of Wallerian degeneration. During successful elongation, the peripheral nerve exhibits an adaptative response, in which stretched axons escape degeneration and retain electrical impulse conduction. Previous studies have shown that axonal degeneration occurs in acutely elongated nerves, but not in gradually elongated nerves (Galardi et al., 1990; Polo et al., 1997; Ikeda et al., 2000; Abe et al., 2004). Gradual elongation at a rate within 1 mm/day does not interrupt nerve conduction (Skoulis et al., 1998; Ikeda et al., 2000; Yokota et al., 2003). Gradual elongation at the rate of 2 mm/day causes neither axonal transport insufficiency nor destruction of the blood–nerve barrier function (Ikeda et al., 2001). This is also the case with the current rat model (Osamura et al., 2007). Upon elongation, individual Schwann cells cover a lengthened internode by cell body

extension, rather than by cell proliferation (Abe et al., 2002; Yokota et al., 2003). Providing that myelin components are supplemented to elongated myelin sheaths, cytokines and neurotrophins that are known to regulate myelination would also play roles during the adaptation.

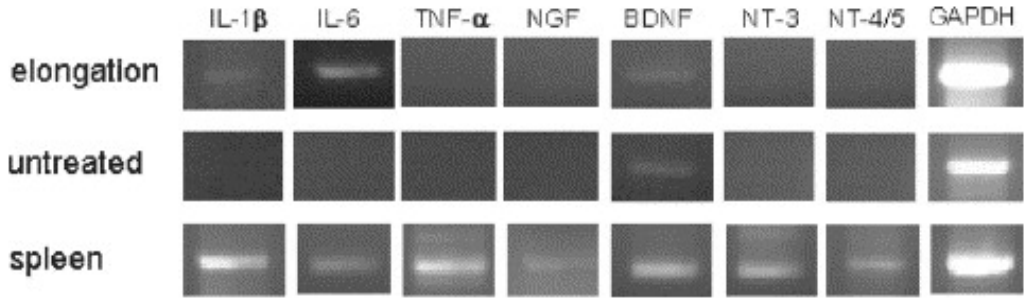

Figure 2. RT-PCR analysis for various cytokines and neurotrophins. Total RNA was obtained from L4–6 DRG of the 2-mm/day group 1 day after sciatic nerve elongation (*top*), untreated control (*middle*), and positive control (*bottom*). After reverse transcription, cDNA was subjected to amplification with specific primers for the indicated factors. Note that IL-1β and IL-6 mRNA are detected only in the nerve elongation model. (Reproduced with permission from Osamura et al., 2005. Copyright 2005 Elsevier Ltd.)

In our study, the expression of IL-6 was prominently induced in DRG neurons after gradual elongation of rat sciatic nerves (Fig. 2). The induction was detected not only in the acutely elongated 20-mm/day group, in which nuclear eccentricity in the cell body and degenerated axons were observed, but also in the gradually elongated 1- and 2-mm/day groups, in which no degenerative change was detectable. These data indicate that peripheral sensory neurons perceptively react to axonal elongation by producing IL-6. Although NGF, BDNF, and NT-3 are known to be involved in the process of myelination (Mattson, 2003; Notterpek, 2003), mRNAs for these factors did not show any change in the DRG of the gradually elongated models (Fig. 2). The selective induction of IL-6 may provide a molecular explanation for the adaptation of gradually elongated 1- and 2-mm/day groups. In the 20-mm/day group, in which a fraction of fibers are degenerated, the neuroprotective action of IL-6, as described in the transected sciatic nerve, may be effective.

In addition, we have also demonstrated in this study that IL-6 can stimulate the expression of mRNA for PMP22, a major myelin component that exhibits expression changes similar to myelin protein zero (MPZ) during normal development and after peripheral nerve injury (Welcher et al., 1991; Kuhn et al., 1993; Notterpek et al., 1999; Suter, 2004). Together with the observation that the expression of P0 gene is up-regulated by gradual elongation of rat sciatic nerves (Hara et al., 2003), it is conceivable that IL-6, at least in part, mediates the expression of genes for myelin-specific proteins in gradually elongated nerves. The resulting proteins may be supplied to the Schwann cell body lengthened by the nerve elongation procedure.

Occasionally, the adaptation is preceded by minimal damage around the node of Ranvier. When a rabbit sciatic nerve was elongated at the rate of 2 mm/day, the myelin sheath in the paranodal region was straightened and nodes of Ranvier became wider, resulting in reversible partial conduction block (Ikeda et al., 2000). Yokota et al. (2003) also reported similar

changes in teased myelinated fibers. We assume that IL-6 is also involved in repairing extended paranodal myelin sheath. Murphy et al. (1999) suggested that, in axotomized DRG neurons, the induction of IL-6 mRNA is initiated by a factor released from mast cells at the injury site. In contrast with the strong and relatively brief induction in the axotomized group, the 1- and 2-mm/day groups exhibited a more sustained induction, keeping much lower mRNA levels. The difference might reside in whether or not Wallerian degeneration occurred. IL-6 induction in the acutely elongated 20-mm/day group, which was accompanied by Wallerian regeneration, appears to have both characteristics. We consider that DRG neurons employ two distinct mechanisms for IL-6 induction: an axonal-injury-dependent one as described by Murphy et al. (1999) and an axonal-elongation-dependent one that is free from degeneration.

In another study, we demonstrated that the expression of TNFα mRNA is induced in rat sciatic nerves after gradual elongation, whereas mRNAs for IL-1β, IL-6, NGF, BDNF, NT-3 and NT-4/5 remain undetectable (Fig. 4). Our data indicate that TNFα is produced by non-proliferating Schwann cells. In contrast with transaction and acute elongation of the sciatic nerve, gradually elongated nerves lacked in a prominent rise in TNFα mRNA immediately after 20-mm lengthening. Moreover, as shown in the histochemical staining, most TNFα-producing cells in gradually elongated nerves are not macrophages, but Schwann cells in the non-proliferative state. These histological findings sharply contrast with those in Wallerian degeneration after usual injury. Hence, it is unlikely that TNFα produced in gradually elongated nerves mediates degenerative responses as in injured nerves. It is rather conceivable that TNFα acts as a mediator of regenerative responses. As already mentioned above, IL-1β, IL-6, TNFα, NGF, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5), are involved not only in neuronal survival and neurite outgrowth (Hattori et al., 1993; Boyd & Gordon, 2003), but also in myelin formation by Schwann cells (Chan et al., 2001; Cosgaya et al., 2002). We surmise that TNFα acts on Schwann cells in a paracrine or autocrine manner, stimulates myelin synthesis necessary for insulation of lengthened internodes, and thereby facilitates the adaptation of peripheral nerve to gradual elongation. In the acute elongation model, the expression of TNFα mRNA is probably influenced by both injury and elongation. Provided that expression glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA was unchanged by elongation, the level of TNFα mRNA in the 20-mm/d group was significantly higher than those in the 1- and 2-mm/d groups. One possibility is that damages in surrounding tissues contribute to the marked increase in TNFα mRNA at day 1, as described in transected sciatic nerves (Wagner et al., 1996; Oka et al., 1998; Liefner et al., 2000; Taskinen et al., 2000; Shamash et al., 2002; Siebert et al., 2003; George et al., 2004). At 1 day after acute 20-mm elongation, TNFα might play a role in inflammatory degenerative processes. In summary, peripheral sensory neurons sense gradual nerve elongation and produce IL-6, which may serve as an active signal in the process of the adaptation of its length. Also, gradual nerve elongation by limb lengthening elicits production of TNFα by Schwann cells. In addition to the well-studied degenerative actions in Wallerian degeneration, TNFα could play a critical role in the adaptative response in elongated peripheral nerves. These results provide a molecular basis for peripheral nerve elongation as a treatment for a segmental peripheral nerve defect. Further studies would clarify whether controlling TNFα action could reduce nerve damage after elongation and ameliorate the complications of limb lengthening.

Contralateral Effects — Transmedian Signaling

Following unilateral sciatic nerve injury, the expression of inflammatory cytokines is often increased in the contralateral non-lesioned side (Koltzenburg er al., 1999). The magnitude of this transmedian effect is smaller, and duration is shorter.

We observed that, after injury to the left sciatic nerve, the expression of IL-6 mRNA was upregulated in the right DRG, although the extent was less than that in the ipsilateral side (Figs. 3 & 5; Osamura et al., 2005). Transection of unilateral sciatic nerve accelerated the neurite outgrowth of neurons cultured from the contralateral DRG (Yamaguchi et al., 1999). Ryoke et al. (2000) demonstrated that sciatic nerve transection enhanced the expression of IL-1β and TGF-β in the contralateral DRG neurons, and promoted regeneration of the contralateral nerve. Also, Ruohonen et al. (2002) reported that sciatic axotomy significantly raised the expression levels of IL-1β, TGF-β1, TNFα, and IL-10 in uninjured contralateral sciatic nerves. These phenomena appear to share some common features with the conditioning lesion that enhances ipsilateral nerve regeneration capacity (McQuarrie & Grafstein, 1973; Smith & Skene, 1997).

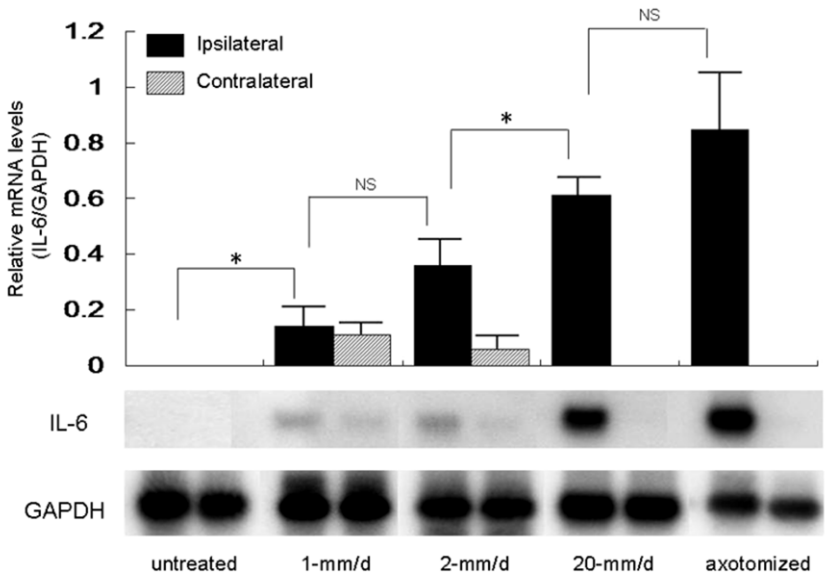

Figure 3. Expression levels of IL-6 mRNA in DRG 1 day after sciatic nerve elongation and transection. Total RNA was extracted from ipsilateral and contralateral L4-6 DRG, and subjected to RT-PCR followed by Southern blot hybridization analysis. A bar graph represents densitmetrically analyzed band intensities for IL-6. Representative autoradiograms for IL-6 and GAPDH are given. Intensities for IL-6 were normalized to those for GAPDH. Note that IL-6 mRNA is also induced in the contralateral side of the 1- and 2-mm/d groups. In the 20-mm/d and axotomized groups, levels of IL-6 mRNA are significantly high compared with the 1- and 2-mm/d groups. Data show mean ± SEM obtained from 5 independent experiments. Fisher's *post-hoc t*-test was used to compare IL-6 mRNA level of each group 1 day after lengthening or transection (* $P < 0.05$; ** $P < 0.01$). (Reproduced with permission from Osamura et al., 2005. Copyright 2005 Elsevier Ltd.)

More recently, IL-6 has been shown to be necessary or sufficient for conditioning-injury-induced regeneration of dorsal column axons (Cafferty et al., 2004; Cao et al., 2006). Given the regenerating activity of IL-6, it is tempting to postulate that gradual nerve elongation, like conditioning lesion, may increase the nerve regenerating capacity or stimulate the myelination of Schwann cells on the opposite side. Also, this phenomenon might have clinical importance. For example, in a case that requires nerve elongation in bilateral extremities, IL-6 could be a useful indicator in deciding the appropriate timing for surgery at the contralateral side following the initial elongation.

We also detected the increasing of TNFα mRNA in contralateral sciatic nerves that had not been elongated (Figs. 4 & 5; Hagiwara et al., 2005). The induction of TNFα mRNA was not detected in ipsilateral L4-6 dorsal root ganglia of the same gradual elongation model (Osamura et al., 2005). Although the precise role of TNFα in this phenomenon is currently unclear, our data raise the possibility that gradual elongation, like axotomy, might enhance the regenerating capacity of peripheral nerves. In the chronic constriction injury model, mRNA levels for IL-1β, IL-10 and MCP-1, but not for TNF-α, were elevated in the contralateral side (Kleinschnitz et al. 2005). This upregulation was inhibited by MK-801, an *N*-methyl-D-aspartate (NMDA) receptor antagonist, indicating the involvement of glutaminergic transmission (Kleinschnitz et al., 2005).

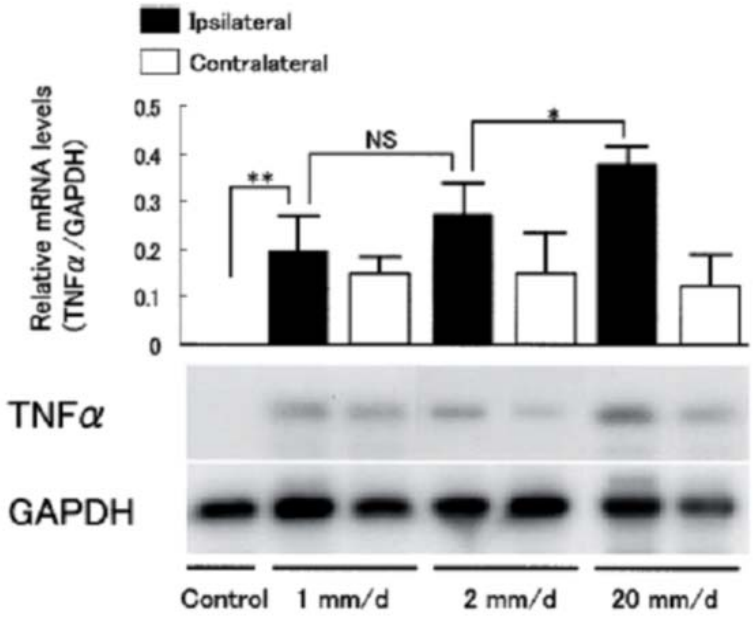

Figure 4. Expression levels of TNFα mRNA in sciatic nerves 1 day after 20-mm lengthening. Total RNA extracted from ipsilateral and contralateral sciatic nerves was processed for RT-PCR followed by Southern blot hybridization analysis. A bar graph represents densitometrically analyzed band intensities for TNFα. The intensities were normalized to those for GAPDH. Given are representative autoradiograms for TNFα and GAPDH. Data show mean ± SEM obtained from 4 independent experiments. Fisher's *post-hoc t*-test was used to compare TNFα mRNA level of each group 1 day after lengthening (* $P < 0.05$; ** $P < 0.01$; *NS*, not significant). (Reproduced with permission from Hagiwara et al., 2005. Copyright 2005 Japanese Orthopaedic Association)

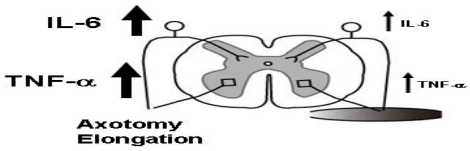

Figure 5. Schematic representation of contralateral effects. Expression of mRNA for IL-6 and TNF-α are depicted based on our previous studies (Osamura et al., 2005; Hagiwara et al., 2005). IL-6 mRNA in DRG and TNF-α mRNA in sciatic trunk are increased in both ipsilateral and contralateral side, although the contralateral elevation is less in magnitude.

At present, little is known about the underlying signaling mechanism that connects the left and right sides of the PNS. There are two putative peripheral mechanisms by which contralateral change might occur: (i) Contralateral effects mediated by circulating factors and (ii) those by transmedian sprouting (Koltzenburg et al., 1999). In the former hypothesis, breakdown products from the damaged nerve or denervated tissue are thought to circulate via the bloodstream and induce changes in contralateral neuronal populations. In the latter hypothesis, unilateral nerve lesions promote collateral, transmedian sprouting of neurons in peripheral tissues that receive bilateral inputs. Such collateral sprouting across the midline might cause other changes in these contralateral neurons. Koltzenburg et al. (1999) have also proposed central mechanisms whereby the contralateral effects are mediated by dendrites of motor neurons, central termini of primary sensory neurons, or spinal interneurons.

The pathophysiological significance of the contra-lateral effect is also largely unknown. This transmedian signaling could account for bilateral loss of distal skin innervation after unilateral injury (Oaklander & Brown, 2004) and mirror-image pain that arises in a proportion of patient with neuropathic pain (Mogli, 2009; Huang & Yu, 2010).

5. CONCLUSION

Extensive studies over decades have revealed that inflammatory cytokines exert multiple effects on many types of cells in degenerating and regenerating peripheral nerves. Some effects are beneficial and others detrimental, for precise renervation of target organs and restoration of nerve conductivity. With the recent advances in the development of pharmacological interventions, such as neutralizing monoclonal antibodies and receptor antagonist (Kato et al., 2009; Kato et al., 2010), it will be easier to optimize cytokine effects toward accelerated and controlled functional recovery from traumatic injury and demyelinating neuropathies.

In addition to the well-known pathologic states, recent studies have suggested that inflammatory cytokines may play roles in pre- or non-degenerative nerves. In the future, more effort should be directed toward clarifying the molecular mechanisms and pathophysiological significance of conditioning effects and transmedian signaling.

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