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Review

Chemokines and peripheral nerve demyelination

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It has been speculated that β-chemokines play a pivotal role in the development of peripheral nervous system (PNS) disorders characterized by mononuclear cell infiltration. In experimental allergic neuritis (EAN), an animal model for human Guillain-Barré syndrome (GBS) with mononuclear cell infiltration, we found by quantitative PCR that β-chemokine messages were upregulated during the active stage. Moreover, an increase in the monocyte chemoattractant protein-1 (MCP-1) message was found in the preclinical stage of EAN, suggesting the critical role of MCP-1 for inducing mononuclear cell infiltrations in this model. Since many cell lineages other than immune cells can produce chemokines, this early upregulation of MCP-1 may be mediated by non-immune cells, probably endothelia or Schwann cells. To date, apart from MCP-1, only RANTES (Regulated on activation, normal T cell expressed and secreted) and macrophage inflammatory protein (MIP)-1α have been examined in EAN and found to have similar kinetics of induction. Therefore, understanding the regulation of production of these chemokines as well as mechanisms of inhibiting chemokine/receptor interactions in the PNS may ultimately lead to disease-specific therapy for GBS and related demyelinating disorders.

Keywords: chemokines; peripheral nervous system; experimental allergic neuritis; Schwann cells

Introduction

The role of chemokines (CK) in peripheral nervous system (PNS) disease is suggested by a number of experimental observations demonstrating leukocyte infiltration around peripheral nerve fibers in inflammatory neuropathies (Prineas, 1981). In many PNS diseases, even in non-inflammatory PNS diseases like hereditary polyneuropathy, ischemic neuropathy or axonal degeneration after sciatic nerve transection, mononuclear cell infiltration is involved during the development of pathological changes (Cornblath *et al*, 1990; Venezie *et al*, 1995). Since CK play a pivotal role in leukocyte trafficking into the CNS (Proost *et al*, 1996; Baggiolini, 1998), it has been hypothesized that they play a similar role in the PNS (Adamus *et al*, 1997; Eng *et al*, 1996; Godiska *et al*, 1995; Karpus *et al*, 1995; Glabinski *et al*, 1997; Ransohoff *et al*, 1996).

The PNS is comprised of relatively fewer cell elements than the CNS: Schwann cells, peripheral nerve axons, fibroblasts, endothelia, pericytes and macrophages (Thomas *et al*, 1993). Among these cell types, Schwann cells (SC) are possibly the only cell lineage that is unique to the PNS. SC have analogous functions to oligodendrocytes and astrocytes in the CNS: myelination of axons and maintenance of the homeostasis of the neuron, respectively. In addition to these roles, SC also have an immunological role, that of antigen presentation (Gold *et al*, 1995; Armati and Pollard, 1996) and cytokine production (Armati and Pollard, 1996; Bolin *et al*, 1995; La Fleur *et al*, 1996). Recently, the ability of SC to produce one of the β-chemokines, monocyte chemoattractant protein (MCP)-1, *in vitro* has been shown (Polydefkis *et al*, 1998). Moreover, cell lineages other than SC found in the PNS such as fibroblasts and endothelia are a well known cell source for CK. However, to date, expression of only MCP-1, MIP-1α and RANTES has been characterized in the PNS using experimental allergic neuritis (EAN), an animal model for Guillain-Barré syndrome. Here we review the present status of CK studies in the PNS, mainly in EAN, and we present our own data concerning β-chemokine expression in EAN.

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Monocyte chemoattractant protein (MCP)-1 production in experimental allergic neuritis

MCP-1, a well characterized β-chemokine, is produced by many cell lineages including monocytes, macrophages, lymphocytes, fibroblasts, endothelia, epithelia (Struyf *et al*, 1998; Douglas *et al*, 1997), and astrocytes (Adamus *et al*, 1997; Glabinski *et al*, 1997; Sun *et al*, 1997). Its target cells are monocytes, T lymphocytes and NK cells, but not neutrophils (Gu *et al*, 1997; Schall, 1994; Vaddi *et al*, 1997) and while a role for MCP-1 in EAE has been proposed (Karpus, 1995) its potential role in experimental allergic neuritis (EAN) has yet to be fully explored. EAN is an animal model for human demyelinating polyneuritis or Guillain-Barré syndrome (GBS). EAN, usually induced in Lewis rats, is mediated by Th1 cells and has a monophasic disease course, with spontaneous recovery (Rostami *et al*, 1985, 1990). Since the major cell populations seen in the PNS of patients with GBS or rats with EAN are macrophages and lymphocytes (Schmidt *et al*, 1996; Asbury *et al*, 1969; Rosen *et al*, 1992; Zettl *et al*, 1996), the involvement of β-chemokines in the development of EAN is strongly suspected.

Recently the kinetics of β-chemokines (MIP-1α, MCP-1 and RANTES) in EAN peripheral nerves has been clarified (Spies *et al*, 1997). In this study, the messages of β-chemokines were found to increase in concert with the clinical signs of EAN. In our studies, we have assessed the kinetics of β-chemokine mRNA expression in rat cauda equina using quantitative competitive RT–PCR. To do this, we immunized Lewis rats with synthetic peptide SP26 corresponding to the 53–78 amino-acid sequence of the bovine myelin P2 protein to obtain severe clinical disease (Rostami *et al*, 1990) (Figure 1). We found that MCP-1 message increases significantly at day 7 post immunization (p.i.), and that the message remained at a high level during the active disease stage (from days 10–17 p.i.), then returned to a normal level at day 20 p.i. (Figure 2). Since the influx of mononuclear infiltrating cells such as macrophages and lymphocytes begins at the clinical onset of EAN (Rosen *et al*, 1992; Zettl *et al*, 1996), our data suggest that the upregulation of MCP-1 preceding the clinical onset of EAN may attract these mononuclear cells and cause cell infiltration.

MCP-1 can be produced by SC *in vitro* upon stimulation by TNF-α or IFN-γ, or it may appear within 1 day after nerve transection *in vivo* (Polydefkis *et al*, 1998). In addition, MCP-1 production by other cell lineages is stimulated by IL-1 (Schwarz *et al*, 1997) and IL-6 (Biswas *et al*, 1998). Interestingly, MCP-1 expression is suppressed by Th2 cytokines IL-4, IL-10 and IL-13 (Kucharzik *et al*, 1998).

These studies raise the question of the relationship between other pro-inflammatory cytokines and MCP-1 in the induction of EAN. Most pro-inflammatory cytokines are upregulated during the acute stage while anti-inflammatory cytokines like TGF-β and IL-10 are upregulated just before clinical recovery starts (Fujioka *et al*, 1998; Zhu *et al*, 1998). Therefore it is possible that MCP-1 is upregulated by pro-inflammatory cytokines during the disease-accelerating stage (from preclinical to peak stages), while it is downregulated by anti-inflammatory cytokines during disease recovery stage. Our data suggest that the production of MCP-1 in the PNS during EAN is strongly regulated by cytokines: specifically, upregulation by pro-inflammatory cytokines and downregulation by anti-inflammatory cytokines. This hypothesis can explain how MCP-1 is regulated after clinical onset; however, it remains unclear what first triggers the upregulation of MCP-1 before overt mononuclear cell infiltration. Production of IL-1β from recruiting neuritogenic T cells is a possible explanation since IL-1β is upregulated in the early stage of EAN (Zhu *et al*, 1998) and can upregulate MCP-1 production (Schwarz *et al*, 1997). In EAN, neuritogenic T cells must invade the PNS to initiate tissue-specific immune response and demyelination before clinical onset. Once these neuritogenic T cells meet specific antigens in the PNS they may produce pro-inflammatory cytokines as well as chemokines. The pro-inflammatory cytokines upregulate other pro-inflammatory cytokines and chemokines, increase the permeability of the blood-nerve barrier, activate invading cells, and sometimes cause tissue damage

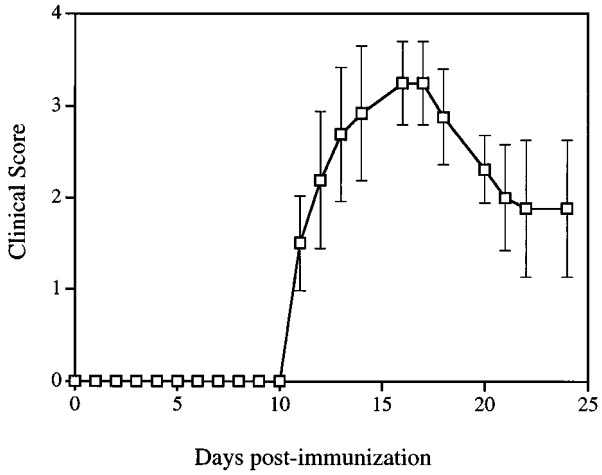

Figure 1 Clinical course of EAN. Average score is shown by solid line. Bars indicate s.d. n=24 (days 0–7 post immunization, p.i.), 20 (days 8–10 p.i.), 16 (days 11–13 p.i.), 12 (days 14–17 p.i.), 8 (days 18–20 p.i.) and 4 (days 21–24 p.i.), respectively. Female Lewis rats were immunized with 150 mg of HPLC-purified SP26. Clinical onset of EAN was day 11 p.i. Peak of disease was observed at days 16 and 17 p.i.; thereafter clinical recovery took place. Clinical scores were given as follows: 0 = normal; 1 = limp tail; 2 = paraparesis; 3 = paraplegia; 4 = quadriplegia (Rostami *et al*, 1990).

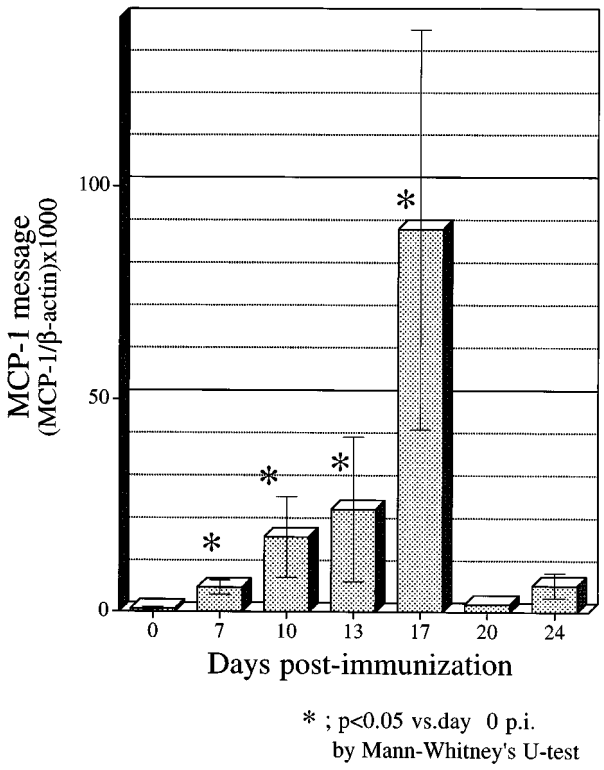

Figure 2 The expression of MCP-1 message in the cauda equina of Lewis rats with experimental allergic neuritis. Total RNA was isolated from caudae equinae removed from rats at different time points, reverse transcribed by Molony Murine Leukemia Virus reverse transcriptase with Oligo (dT) primer. Replicated cDNA was amplified using competitive quantitative PCR (Fujioka *et al*, 1998). Message of MCP-1 is expressed as a ratio of β-actin message in the same rats. In this graph, MCP-1 message is shown as an average of four rats in each time point. Bars indicate s.e. Message of MCP-1 increased from days 7–17 p.i. significantly. There was no statistically significant difference within these days (days 7–17 p.i.). MCP-1 message at days 20 and 24 p.i. declined to normal level.

(Lisak *et al*, 1997). Upregulated CK attract monocytes/macrophages from the blood stream to the PNS, together with cytokines, resulting in intense inflammation in the PNS. This process can be initiated by IL-1β or MCP-1, although the initial signal for this process remains unclear.

As mentioned above, MCP-1 can be produced not only by immune cells but by many other cell lineages found in both the CNS and PNS. While it is assumed that these cells (SC, fibroblasts, endothelia, epithelia, smooth muscle, monocytes, macrophages, lymphocytes and fibroblasts) are the source of β-chemokines in the PNS, this hypothesis has yet to be confirmed. Thus, important questions concerning the origin and regulation of chemokine expression in the PNS remain, and are the focus of current investigations in our laboratory.

Macrophage inflammatory protein (MIP)-1α and Schwann cells

MIP-1α acts as prostaglandin pathway-independent pyrogen in addition to its role in inducing chemotaxis (Minano *et al*, 1996; Armengol *et al*, 1997), suggesting pleiotropic effects. Notably, a suppressor role for MIP-1α against astrocyte growth as well as stimulation of SC growth has been demonstrated (Khan and Wigley, 1994). These effects on glial cell growth by MIP-1α may be important for the remyelination of peripheral nerve during the recovery stage of EAN. Thus, upregulation of MIP-1α may, as with other β-CKs, cause an influx of mononuclear cells but, unlike other β-CKs, MIP-1α may also help remyelination.

Finally, the β-CK RANTES (Regulated on activation, normal T cell expressed and secreted) may also have a role in EAN. Although its cell source is still unclear, the stimuli for RANTES upregulation and inhibition, and its target cells are similar to those of MCP-1 (Schall, 1994). In fact, in EAE, the animal model for human multiple sclerosis and the CNS counterpart of EAN, RANTES production is similar to MCP-1 (Glabinski *et al*, 1997; Godiska *et al*, 1995; Ransohoff *et al*, 1996). In EAN, RANTES shows a similar pattern of secretion as MCP-1 and MIP-1α (Spies *et al*, 1997). Thus, evidence suggests a role for multiple β-chemokines in the pathogenesis of EAN and, similar to recent studies in EAE, further analysis of the role of β-chemokines in peripheral demyelination may lead to the rational design of targeted pharmacological therapeutics for demyelinating peripheral nerve disorders (Ransohoff, 1997).

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