SHORT REPORT

Expression of chemokines in cerebrospinal fluid and serum of patients with chronic inflammatory demyelinating polyneuropathy

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Chemokines are likely to contribute to the pathogenesis of chronic inflammatory demyelinating polyneuropathy (CIDP), as evidenced by data from experimental autoimmune neuritis. The α and β chemokines in the cerebrospinal fluid (CSF) and serum from patients with CIDP were analysed using an enzyme linked immunosorbent assay. CXCL9, CXCL10, and CCL3 were raised in the CSF in CIDP compared with controls and non-demyelinating neuropathies ($p < 0.001$). Although the CSF levels of CCL2 were significantly higher than the serum levels for all groups, the difference between groups was not significant. CXCL9, CXCL10, and CCL3 may contribute to the pathogenesis of CIDP by recruiting inflammatory T cells and monocytes to spinal nerve roots, while CCL2 is likely to play a physiological role.

Chronic inflammatory demyelinating polyneuropathy (CIDP) is widely regarded as an autoimmune disease in which one or more components of peripheral myelin are presumed to be the target of an immune attack. Although the aetiology remains unknown, both humoral and cell mediated immune mechanisms contribute to the pathogenesis. Endoneurial inflammatory changes with T cell infiltrates and macrophage associated demyelination are present on nerve biopsies performed during the acute phase of CIDP.[1] These endoneurial lymphocytes and macrophages are associated with an increased expression of mRNA for tumour necrosis factor α (TNFα), γ interferon (IFNγ), and interleukin 2 (IL-2).[2] A subgroup of CIDP patients with an active disease phase have increased levels of serum TNFα.[3] Activated T lymphocytes are increased in the peripheral blood of patients with CIDP,[4] and passive transfer of experimental autoimmune neuritis, using sera and IgG from patients with CIDP, requires activated T lymphocytes.[5] Antibodies and complement also play a role in the pathogenesis of CIDP but only in the presence of blood–nerve barrier breakdown.[6]

Chemokines are involved in the selective recruitment of mononuclear cells to sites of peripheral nerve inflammation and blood–nerve barrier breakdown. The chemokines are chemoattractant cytokines. They are subdivided into four families depending on the amino acids between the terminal cysteine residues,[7] though α and β chemokines form the majority. *CXCL10*, formerly IFNγ inducible protein-10 (IP-10), and *CXCL9*, formerly monokine induced by IFNγ (Mig)—which are both induced by IFNγ—are α chemokines. *CCL2*, formerly macrophage chemoattractant protein 1 (MCP-1), *CCL3*, formerly macrophage inflammatory protein 1α (MIP-1α), *CCL4*, formerly macrophage inflammatory protein 1β (MIP-1β), and *CCL5*, formerly RANTES ("regulated upon activation of normal T cell expressed and secreted") are β chemokines.[7] α Chemokines recruit predominantly T lymphocytes, and β chemokines recruit both T lymphocytes and monocytes. Chemokines also have other functions such as activation of macrophages, induction of nitric oxide synthesis, and differentiation of naive T cells.[7]

In an animal model (experimental autoimmune neuritis), sequential expression of chemokines was found using homogenised sciatic nerve. Increased expression of CCL3 and CCL4 preceded disease, whereas the expression of CXCL10, CCL5, and CCL2 was concomitant with maximum clinical disease.[8] CXCL10 expression was associated with endothelial cells and CCL5 with infiltrating T lymphocytes.[8] Raised cerebrospinal fluid (CSF) concentrations of CXCL10 in CIDP have also been reported.[9]

We designed this study to investigate a possible role for α and β chemokines in patients with CIDP by quantitation of serum and CSF levels.

Table 1 Details of patients with chronic inflammatory demyelinating polyneuropathy and control groups

	CIDP	Headache	NDN
Median age (years)	58	46	58
Age range (years)	22 to 84	20 to 59	34 to 76
No of patients	9	10	10
Sex (F/M) (n)	3/6	7/3	3/7
Mean CSF protein (mg/l)	586	104	219
Median CSF WCC/mm^3 (range)	0 (0 to 10)	0 (0 to 2)	0 (0 to 2)
Disease duration (months) (range)	14 (4 to 96)	0.06 (0 to 0.13)	NA
Median Hughes score (range)	2 (1 to 4)	–	–
Clinical status: previous attacks/no previous attacks	3/6	–	–
Clinical signs: symmetrical/asymmetrical	2/7	–	–
Median diffusion ratio (range)	0.6 (0.4 to 1.2)	NA	0.5 (0.4 to 0.7)

CIDP, chronic inflammatory demyelinating polyneuropathy; headache, non-specific headache controls; NA, not assayed, not available; NDN, non-demyelinating neuropathy controls; WCC, white cell count.

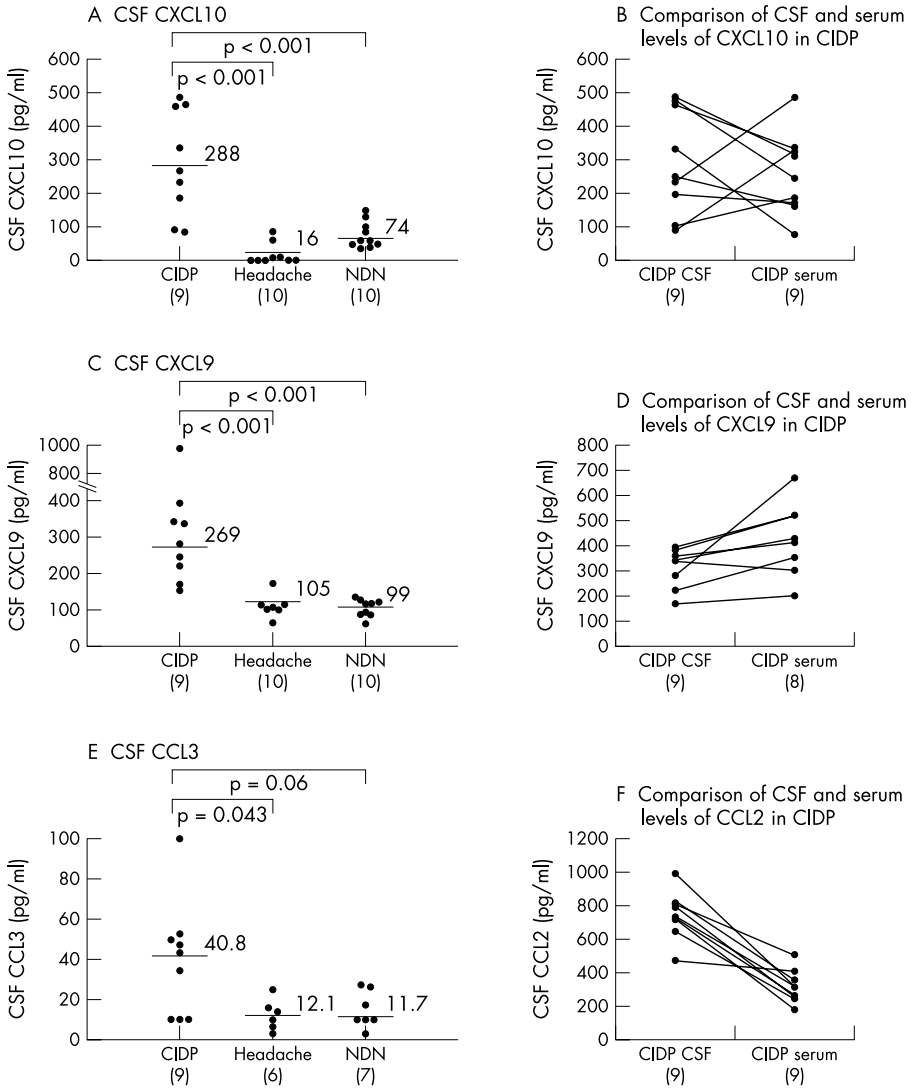

Figure 1 Chemokine levels in cerebrospinal fluid (CSF) and serum. CIDP, chronic inflammatory demyelinating polyneuropathy; Headache, patients with non-specific headache; NDN, non-demyelinating neuropathy. Horizontal bars on panels A, C, and E represent mean concentrations in pg/ml; n values are indicated on the x axis.

METHODS

Patients

The local research ethics committee approved the study and 29 patients were included, following their informed consent (table 1).

Nine patients fulfilled the research criteria of the American Academy of Neurology for probable CIDP.[10] The clinical, electrophysiological, and CSF findings were typical of CIPD in all patients. However, the absence of biopsy evidence prevented a definite diagnosis of CIPD.

Of the nine patients, three had had disease of 6, 84, and 96 months' duration, and had been treated with intravenous immunoglobulin and steroids. In these three patients, the treatment was completed at least three months before their second lumbar puncture, which was carried out at the time of presentation with active disease because the protein levels on the initial CSF samples were within the normal range. Lumbar punctures were done during investigation at the time of presentation with active disease.

The remaining patients had received no specific treatment and had a gradual onset of progressive symptoms before nerve conduction studies and diagnostic lumbar punctures were done. The median Hughes score for patients with CIDP was 2, with a range of 1 to 4. Two patients had scores of 1 and another two had a score of 4. The remaining patients had a score of 2.

Ten patients with non-demyelinating neuropathies and 10 patients with benign headache were included as controls. Patients with benign headaches had sudden onset of headache without evidence of subarachnoid haemorrhage, and following investigations had no diagnosis other than non-specific headache. None had received immune modulatory treatment in the three months preceding the lumbar puncture.

CSF was centrifuged within 20 minutes of the lumbar puncture and CSF supernatant and serum were stored at –70°C.

Serum and CSF chemokine assays

The α chemokines CXCL9 and CXCL10 and the β chemokines CCL2, CCL3, and CCL5 were analysed using an enzyme linked immunosorbent assay (ELISA). For α chemokines, capture and detection antibody concentrations were optimised using recombinant chemokines from R&D Systems, Minneapolis, USA. For β chemokines, ELISA kits were used (R&D Systems, UK), following the manufacturer's guidelines. The assay detection limits were 7.6 pg/ml for α chemokines, 5 pg/ml for CCL2, and 10 pg/ml for CCL3 and CCL5. Interassay variability was 10% and intra-assay variability, 7%.

Statistics
Statistical significance between the mean values was tested using Kruskal-Wallis and Mann-Whitney tests (SPSS version 10).

RESULTS
α Chemokine levels in CSF
The mean (SEM) CSF concentration of CXCL9 was 269 (79.8) pg/ml and of CXCL10, 288 (41.9) pg/ml in patients with CIDP (fig 1, panels A and C). CSF CXCL9 and CXCL10 concentrations were significantly raised in patients with CIDP in comparison with the controls with non-specific headache and non-demyelinating neuropathy ($p < 0.001$). Six of nine patients with CIDP had higher concentrations of CXCL10 in CSF than in the paired serum samples (fig 1B), while seven of eight CXCL9 concentrations were lower in CSF than in the paired serum samples (fig 1D).

β chemokine levels
The mean (SEM) CCL3 concentrations were 40.8 (9.5) pg/ml for CIDP, 12.1 (2.7) pg/ml for headache controls, and 11.7 (3.1) pg/ml for non-demyelinating neuropathy (fig 1E). CSF CCL3 levels in CIDP patients were greater than in the headache group ($p = 0.043$). CCL2 concentrations were not significantly different between groups for either serum or CSF (data not shown). However, the CSF concentrations were significantly higher than the serum concentrations for all patient groups ($p < 0.001$, fig 1F). The mean level of CCL5 in CSF was 12.8 (2.1) pg/ml for CIDP, 10.2 (0.6) pg/ml for headache controls, and 15.7 (2.0) pg/ml for controls with non-demyelinating neuropathy. CCL5 levels were not significantly different between the groups (data not shown, $p = 0.46$).

Correlation of chemokine levels with disease indices
There were no differential patterns of chemokine expression in the serum or CSF in patients with previous relapses compared with patients without previous relapse or treatment. Neither CSF CXCL9 nor CXCL10 levels correlated significantly with CSF protein level, diffusion ratio, disease duration, or Hughes score (data not shown). However, there was a non-significant trend towards a lower CSF CXCL10 concentration with longer disease duration. In addition, patients with a higher Hughes score tended to have a higher CXCL10 level in the CSF, though again this was non-significant. The lack of correlation of serum or CSF chemokine levels with the Hughes score and the electrophysiological findings may be a reflection of the relatively small number of patients studied ($n = 9$).

DISCUSSION
CSF concentrations of CCL3, CXCL9, and CXCL10 were significantly greater in CIDP patients than in the control groups. The higher levels of CCL2 and CXCL10 in the CSF than in paired serum samples in patients with CIDP provides evidence for intrathecal synthesis of these chemokines, as passive diffusion the blood–nerve barrier is unlikely to lead to higher levels in the CSF than in the blood. The higher serum concentrations of CXCL9 in CIDP in comparison with the paired CSF levels suggests a possible pathogenic role for CXCL9 in the peripheral nerves.

A recent study by Kieseier *et al* identified perineurial endothelial cells in peripheral nerves as the source of CXCL10 in patients with CIDP.[9] Raised levels of CXCL10 in the CSF have also been reported in patients with relapsing remitting multiple sclerosis.[11–13] CXCL10 has been identified as a key chemokine in the recruitment of lymphocytes into the CSF across the blood–brain barrier in multiple sclerosis.[11 13] The absence of CSF lymphocytosis in CIDP, despite a similar increase in CSF CXCL10, contrasts with the proposed chemoattractive role of CXCL10 in multiple sclerosis. Both the expression of CXCR3, the receptor for CXCL10, on peripheral lymphocytes from patients with CIDP (personal observations) and the percentage of peripheral $CD4^+$ $CXCR3^+$ T cells are similar in CIDP and multiple sclerosis.[11 13] Hence the lack of CSF lymphocytosis in CIDP despite raised CSF CXCL10 levels does not result from the absence of CXCR3 on peripheral blood T cells.

The chemoattractive role of CXCL10 in multiple sclerosis may be dependent on breakdown of the blood–brain barrier, which does not occur in CIDP. Alternatively, the CXCL10 levels determined in CSF obtained at lumbar puncture may not reflect the local concentration in the peripheral nerve. The concentration of CXCL10 at the blood–brain barrier may be much greater in multiple sclerosis than in CIDP, as CXCL10 is produced mainly by the astrocyte foot processes at the blood–brain barrier.[11]

Other possible reasons for the dissimilarity in CSF pleocytosis between multiple sclerosis and CIDP may be differences in permeability at the blood–brain and blood–nerve barriers,[14] a greater degree of apoptosis of CSF lymphocytes in CIDP, differing functional activity of CXCL10 and CXCR3, and disparities in the serum to CSF CXCL10 gradient.

There is little evidence for intrathecal synthesis of CXCL9 as all CSF levels were lower than serum levels. None of the chemokines in the CSF correlated significantly with CSF protein concentrations or the diffusion ratio, which provides evidence against passive diffusion of chemokines from the serum to CSF. As the Hughes score is dependent on both spinal root and peripheral nerve lesions in CIDP, the lack of correlation of CSF chemokine levels with Hughes score is not unexpected.

The finding of a raised CSF concentration of CCL3 in our study adds to the existing evidence from experimental autoimmune neuritis that CCL3 is linked to the spinal root pathology in CIDP because of its recruitment of T cells and monocytes.[8] Intrathecal CCL2 is likely to play a physiological role as the CSF levels were raised compared with serum in all patient groups and as CSF CCL2 levels in CIPD were not significantly different from control groups. CCL2 and CCL5 may still be involved in the pathogenesis despite the lack of evidence from CSF analysis, as this does not exclude significant expression of chemokines at local sites of inflammation in spinal roots.

Conclusions
Our study provides evidence for the involvement of CXCL9, CXCL10, and CCL3 in the pathogenesis of CIDP, and these chemokines and their receptors—CXCR3 and CCR5—may be targets for therapeutic agents in the treatment of CIDP. Indeed, neutralisation of chemokines with anti-chemokine antibodies in the animal model (experimental autoimmune neuritis) has been effective in reducing the level of inflammation [15].

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REFERENCES

1 **Schimdt B**, Toyka KV, Kiefer R, *et al*. Inflammatory infiltrates in sural nerve biopsies in Guillain-Barré syndrome and chronic inflammatory demyelinating polyneuropathy. *Muscle Nerve* 1996;**19**:474–87.
2 **Mathy KM**, Pollard JD, Armati PJ. TNFα, IFNγ and IL-2 mRNA expression in CIDP sural nerve biopsies. *J Neuro Sci* 1999;**163**:47–52.
3 **Misawa S**, Kuwabara S, Mori M, *et al*. Serum levels of tumour necrosis factor-α in chronic inflammatory demyelinating polyneuropathy. *Neurology* 2001;**56**:666–9.
4 **Van den Berg LH**, Mollee I, Wokke JHJ, *et al*. Increased frequency of HPRT mutant T lymphocytes in patients with Guillain-Barré syndrome and chronic inflammatory demyelinating polyneuropathy: further evidence for a role of T cells in the etiopathogenesis of peripheral demyelinating diseases. *J Neuroimmunol* 1995;**58**:37–42.
5 **Yan WX**, Taylor J, Andrias-Kauba S, *et al*. Passive transfer of demyelination by serum or IgG from chronic inflammatory demyelinating polyneuropathy patients. *Ann Neurol* 2000;**47**:765–75.
6 **Hughes RAC**. Chronic inflammatory demyelinating polyradiculoneuropathy. *Ann Neurol* 2001;**50**:281–2.
7 **Zlotnik A**, Yoshie O. Chemokines: a new classification system and their role in immunity. *Immunity* 2000;**12**:121–7.
8 **Kieseier BC**, Krivacic K, Jung S, *et al*. Sequencial expression of chemokines in experimental autoimmune neuritis. *J Neuroimmunol* 2000;**110**:121–9.
9 **Kieseier BC**, Tani M, Mahad D, *et al*. Chemokines and chemokine receptors in inflammatory demyelinating neuropathies – a central role for IP-10. *Brain* 2002;**125**:823–34.
10 **Ad Hoc Subcommittee**. American Academy of Neurology AIDS Task Force. Research criteria for diagnosis of chronic inflammatory demyelinating polyneuropathy. *Neurology* 1991;**41**:617–18.
11 **Sorensen TL**, Tani M, Jensen J, *et al*. Expression of specific chemokines and chemokine receptors in the central nervous system of multiple sclerosis patients. *J Clin Invest* 1999;**103**:807–15.
12 **Franciotta D**, Martino G, Zardini E, *et al*. Serum and CSF levels of MCP-1 and IP-10 in multiple sclerosis patients with acute and stable disease and undergoing immunomodulatory therapies. *J Neuroimmunol* 2001;**115**:192–8.
13 **Mahad DJ**, Howell SJL, Woodroofe MN. Expression of chemokines in the CSF and correlation with clinical disease activity in patients with multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2002;**72**:498–502.
14 **Allt G**, Lawrenson JG. The blood–nerve barrier: enzymes, transporters and receptors. A comparison with blood–brain barrier. *Brain Res Bull* 2000;**52**:1–12.
15 **Zou LP**, Pelidou SH, Abbas N, *et al*. Dynamics of production of MIP-1 alpha, MCP-1 and MIP-2 and potential role of neutralization of these chemokines in the regulation of the immune responses during experimental autoimmune neuritis in Lewis rats. *J Neuroimmunol* 1999;**98**:168–75.