

INCREASED IL-8 LEVELS IN THE CEREBROSPINAL FLUID OF PATIENTS WITH AMYOTROPHIC LATERAL SCLEROSIS

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Received October 17, 2008 - Accepted March 9, 2009

Inflammation has been implicated in the pathogenesis of many neurodegenerative diseases. The chemokine IL-8 is thought to have a pathophysiological role in neurodegenerative diseases. IL-8 has recently been shown to induce death of primary cultured motor neurons *in vitro*. We determined IL-8 levels in the cerebrospinal fluid (CSF) from 38 patients with sporadic amyotrophic lateral sclerosis (ALS) compared to patients with other non-inflammatory neurological diseases (cerebrovascular disease, degenerative dementia, Parkinson's disease, compressive radiculo-myelopathy). Multiple sclerosis (MS) patients were used as positive controls. The levels of IL-8 in the CSF of ALS patients were significantly higher than those of patients with other, non-inflammatory neurological conditions and similar to those of MS patients. The only variable influencing IL-8 in ALS patients was sex, with higher levels in men than in women. The presence of the inflammatory cytokine IL-8 in the CSF of patients with ALS at the time of diagnosis strengthens the hypothesis of a role for this chemokine in neurodegenerative disorders.

In the last decade, a role has been proposed for glial-induced neuroinflammation in Amyotrophic Lateral Sclerosis (ALS) (1-5). Although contrasting results on cerebro spinal fluid (CSF) and plasma cytokine levels have been reported in ALS, increased concentrations of interleukin (IL)-6, tumor necrosis factor (TNF) and monocyte chemoattractant protein-1 (MCP-1) do suggest a neuroinflammatory component (6-8). IL-8 is an important chemotactic factor for the recruitment and activation of polymorphonuclear cells at the site of tissue damage during inflammatory reactions (9). IL-8 is synthesized by glial cells in the central

nervous system and is thought to have an important role in neuroinflammatory events and to be involved in rapid signaling in neurons (10). IL-8 has also been proposed to have a pathological role, since it regulates recruitment of PMN and activates PMN functional activities such as cytokine expression (11) and release of tissue damage mediators from cytoplasmic granules (12). Nevertheless, IL-8 has also been detected in the circulation (9) and in CNS tissue (13) from patients with multiple sclerosis (MS), a disease for which PMN infiltration is not considered a hallmark, although both IL-8 levels and infiltrating PMN have been described in

Key words: chemokines, IL-8; CXCR2, amyotrophic lateral sclerosis, cerebro spinal fluid

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1721-727X (2009)

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opticospinal MS (14) and in myelin oligodendrocyte glycoprotein peptide 35-55-induced experimental autoimmune encephalomyelitis in mice (15). Studies in patients with Alzheimer's disease, reporting elevated IL-8 levels in cerebrospinal fluid and microvessels, suggest a role for this chemokine also in neurodegenerative disorders (16-17).

Two receptors with high affinity for IL-8 are known, of these CXCR2, differently from CXCR1, is expressed at high levels in some CNS regions, including motor neurons in the anterior horn of human spinal cord (18). We have recently reported that activation of CXCR2 induces dose-dependent death in cultured motor neurons (19). In this study we tested the hypothesis that CSF levels of IL-8 in ALS patients are higher than those of the general population.

MATERIALS AND METHODS

IL-8 determinations

CSF was obtained in two hospitals (Istituto Nazionale Neurologico Carlo Besta and IRCCS Istituto Auxologico Italiano, Milano) from 38 patients with sporadic ALS, 21 MS patients and 18 patients with non-inflammatory neurological diseases after informed consent. Procedures for sampling and storage (at -80°C) did not differ between the two centres. The protocol of the study was approved by our Ethical Committee.

Table I shows the main demographic and clinical features of the sample. The two control groups were selected to provide the cytokine values expected in the general population (non-inflammatory neurological conditions) and in a chronic CNS immune-mediated disorder (MS). None of the cases and the controls were receiving anti-inflammatory drugs at the time of the spinal tap. IL-8 levels in the CSF were determined using a sandwich enzyme-linked immunosorbent assay (ELISA) for human IL-8 (DuoSet ELISA, R&D Systems, Inc., Minneapolis, MN, USA; sensitivity to 8 pg/ml).

Statistical analysis

The distribution of the IL-8 CSF values was tested with the Shapiro-Wilk test for normality. The values obtained in patients with ALS and in the two control groups were then compared using the non-parametric Wilcoxon test. A probability value of less than 0.05 was regarded as significant. Multiple comparisons were made among the three groups using the non-parametric Kruskal-Wallis test. To identify the comparisons thought to be significant, the Wilcoxon test was used, considering

a probability value lower than 0.016 as significant. Univariate and multivariate (multiple regression) analyses were performed in patients with ALS to assess the influence of age, sex, disease duration, and functional disability (measured by the ALS Functional Rating Scale, ALSFRS) on the IL-8 values.

RESULTS

The CSF levels (mean \pm SD) of IL-8 are reported in Fig. 1. No statistical differences were found between samples collected in the two participating centres.

ALS patients had IL-8 concentration (23.5 ± 9.2 pg/ml) significantly higher than that found in patients with other, non-inflammatory neurological conditions (15.9 ± 5.5 pg/ml, $p = 0.0026$). The IL-8 levels in MS patients (25.9 ± 11.0 pg/ml) were significantly higher than those of patients with other, non-inflammatory neurological conditions ($p = 0.0009$), and not different to those of ALS patients (Fig. 1). Multivariate analysis showed that in patients with ALS, sex was the only variable found to affect the IL-8 values, while age, disease duration, and functional disability (measured by the ALS Functional Rating Scale, ALSFRS) had no influence on the IL-8 values. The IL-8 values were 25.7 ± 9.5 pg/ml in men and 18.7 ± 6.9 pg/ml in women ($p = 0.0286$) (Fig. 2). In control patients with other, non-inflammatory neurological conditions the mean values were 16.2 ± 6.1 and 15.2 ± 4.8 pg/ml in men and women, respectively ($p = \text{ns}$) (Fig. 2). Stratification by gender showed the difference between ALS cases and controls being significant only in men ($p = 0.0075$).

DISCUSSION

Here we report increased IL-8 levels in the CSF of sporadic ALS patients compared to other non-inflammatory neurological diseases. Of interest, the IL-8 levels in ALS patients were similar to those found in MS patients, already reported to be elevated (14). There was no correlation between IL-8 levels and age, disease severity or duration; the only statistical difference was related to gender, with higher levels found in male patients. This is not surprising since gender is known to significantly influence the serum content and production of chemokines like MCP-1, MIP-1 α , MIP-1 β and IL-8 (20-22).

Table I. General characteristics of patients with ALS (n=38), non-inflammatory neurological conditions (n=18) and MS (n=21).

ALS	
Age (mean \pm SD), years	55 \pm 14.8
Sex (M/F)	26/12
ALS-FRS (mean \pm SD)	33.5 \pm 6.3
Disease duration (mean \pm SD), months	18.3 \pm 14
Controls	
Age (mean \pm SD), years	55 \pm 13.9
Sex (M/F)	11/7
Diagnosis:	
Cerebrovascular disease (n)	10
Degenerative dementia (n)	3
Parkinson's disease (n)	2
Compressive radiculo- myelopathy (n)	3
MS	
Age (mean \pm SD)	35 \pm 7.2
Sex (M/F)	4/17

SD = standard deviation; (n) = number of subjects; M = Male; F = Female

ALS = amyotrophic lateral sclerosis; MS = multiple sclerosis

ALS-FRS = a validated rating instrument for the monitoring of the progression of disability in patients with ALS, ranging from 0 = normal to 40 = maximal deterioration.

Our data are at variance with the report by Tanaka et al. who did not find differences in the CSF levels of IL-8 between ALS patients and controls with other non inflammatory neurological disease, although the positive correlation between MCP-1 and IL-8 led the authors to suggest a "proinflammatory cytokine cascade after microglial activation" (23). One

possibility to explain this discrepancy is that the difference in our study was evident only in men, and the M/F ratio is 2.2 in our cohort and 1.05 in the study by Tanaka et al.

Another point to be considered is that in their study a large number of controls were subjects with neurodegenerative disorders, and one may

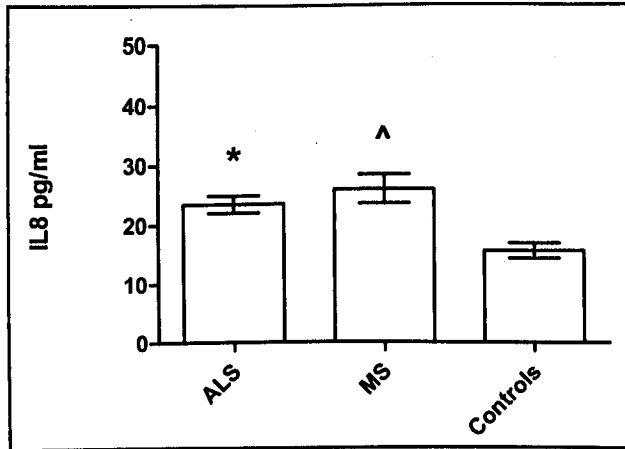


Fig. 1. IL-8 levels in CSF. Data are mean values \pm SD. The number of subjects analyzed is 38, 21 and 18 for ALS, MS and controls, respectively. Controls were subjects with non-inflammatory neurological conditions. Both ALS and MS groups are different from controls (* $p=0.0026$, ^ $p=0.0009$, Wilcoxon test).

question that increased cytokine levels could also be found in the controls, as part of the degenerative process. Indeed in our study we have only 6 subjects with neurodegenerative disease. In spite of this low number, if we stratified our controls into two groups, one including non-degenerative conditions (cerebrovascular disorders, compressive radiculomyelopathy) and the other including dementia and Parkinson's disease, only in the first case the IL-8 levels are still significantly higher in patients with ALS compared to controls ($p=0.0009$).

The role of increased IL-8 levels in the CSF of ALS patients is not clear, since samples from affected patients can be obtained only at the time of diagnosis, when symptoms are already fully developed. For this reason, it is unclear whether IL-8 is involved in the pathogenesis of the disease or it better reflects an inflammatory reaction to motor neuron damage. The elective IL-8 increased levels in men seem to favour the former hypothesis.

Interestingly, studies from our group documented that activation of CXCR-2 induces a dose-dependent death on cultured motor neurons (19), thus suggesting that increased IL-8 production by activated glial cells could contribute to motor neuron degeneration

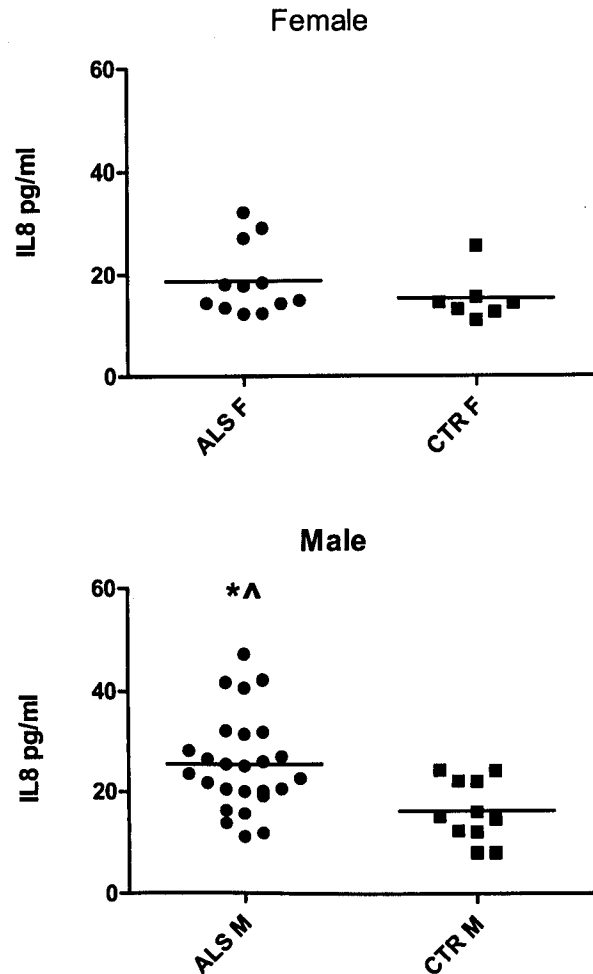


Fig. 2. IL-8 levels in CSF in male and female ALS patients or and controls.

Data are expressed as pg/ml. Each point is the mean value of duplicate samples from each subject. Male ALS patients have significantly higher values than females (^ $p=0.0286$, Wilcoxon test) and than control patients with other non-inflammatory neurological diseases (* $p=0.0075$, Wilcoxon test).

in ALS patients. The efficacy of reparixin, an orally active CXCR1/2 inhibitor, to prevent CXCL2-induced death of motor neurons (25), warrants future investigations about the possible neuroprotective role of CXCR2 inhibitors.

ACKNOWLEDGEMENTS

This work was supported by the Italian Ministry of Instruction, University and Scientific Research

(grants RBNE01B5WW003 and RBIP06LSS2-006), the Istituto Superiore di Sanità (contract n° 526/A31), and the Italian Ministry of Health (Malattie Neurodegenerative, Ex art 56, 2004, 533F/N/1).

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