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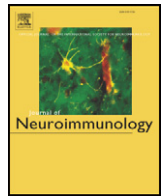


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Evidence of central inflammation in fibromyalgia – Increased cerebrospinal fluid interleukin-8 levels

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ABSTRACT

Activation of glia cells resulting in intrathecal elevation of cytokines and chemokines has been hypothesized in chronic pain syndromes such as fibromyalgia. To our knowledge, this is the first study assessing intrathecal concentrations of pro-inflammatory substances in fibromyalgia. We report elevated cerebrospinal fluid and serum concentrations of interleukin-8, but not interleukin-1beta, in FM patients. This profile is in accordance with FM symptoms being mediated by sympathetic activity rather than dependent on prostaglandin associated mechanisms and supports the hypothesis of glia cell activation in response to pain mechanisms.

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1. Introduction

Recently activation of glia cells has been implicated in the development and maintenance of chronic pain (Watkins and Maier, 2005). Interactions between activated glia and neurons are of importance for the development of central sensitization and hyperalgesia (Ren and Dubner, 2008; Milligan and Watkins, 2009). Therefore, it has been hypothesized that activation of glia could be involved in human chronic pain syndromes characterized by pain amplification, such as fibromyalgia (FM) (Staud, 2004; Gur and Oktayoglu, 2008).

The concept of FM as a pain amplification syndrome relies on the presence of generalized, multimodal allodynia and hyperalgesia (Kosek et al., 1996) and a dysfunction of endogenous pain inhibitory mechanisms (Kosek and Hansson, 1997; Lautenbacher and Rollman, 1997) in FM patients. Recent imaging studies show enhanced transmission and/or processing of nociceptive input and an inability to activate descending pain inhibitory mechanisms in FM patients (Gracely et al., 2002; Jensen et al., 2009). Furthermore, elevated concentrations of substances involved in nociceptive transmission and central sensitization, such as substance P (SP), glutamate, nerve growth factor (NGF) and

brain-derived neurotrophic factor (BDNF), have been reported in the cerebrospinal fluid (CSF) of FM patients (Russell et al., 1998; Giovengo et al., 1999; Sarchielli et al., 2007). Notably, SP, glutamate and BDNF can activate glia cells through receptors localized on microglia and astrocytes (Milligan and Watkins, 2009). Following activation, glia cells release pro-inflammatory cytokines/chemokines such as tumor necrosis factor alpha (TNF-alpha), interleukin-1beta (IL-1b) and interleukin-8 (IL-8) as well as BDNF, NGF, glutamate and SP (Sofroniew et al., 2001; Watkins and Maier, 2005; Milligan and Watkins, 2009). Thereby, the activation of glia cells can further increase pain amplification (Watkins and Maier, 2005) and could thus be implicated in the altered pain modulation in FM patients.

An alternative way to activate glia is through blood-borne, pro-inflammatory cytokines released by peripheral immune cells (Watkins and Maier, 2005) and transported across the blood–brain-barrier by a special transport mechanism (Gutierrez et al., 1993; Banks et al., 1995; Quan and Herkenham, 2002). Increased levels of pro-inflammatory cytokines (Gur et al., 2002; Bazzichi et al., 2007) and reduced concentrations of anti-inflammatory cytokines (Uceyler et al., 2006) have been reported in the blood of FM patients. Furthermore, elevated serum IL-8 concentrations were related to increased pain intensity in FM patients (Gur et al., 2002). However, markers for glial cell activation in the CNS, such as CSF levels of cytokines have, to our knowledge, never been studied in FM patients. The aim of this study was to assess two major pro-inflammatory substances in CSF of FM patients, i.e., IL-1b and IL-8, both recently reported to be elevated in CSF of patients with long-term nociceptive pain (knee osteoarthritis) (Lundborg et al., 2010). Our a priori

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hypothesis was that also patients with a dysfunctional pain syndrome, such as FM, have increased CSF IL-1b and IL-8 concentrations in agreement with the hypothesized glia cell activation in chronic pain.

2. Material and methods

2.1. Subjects

2.1.1. FM patients

Fifteen FM patients (average age 46.2 years, range 25–60 years) participated. They were outpatients at the Department of Rehabilitation Medicine, Danderyds Hospital and fulfilled the classification criteria of the American College of Rheumatology 1990 for fibromyalgia (Wolfe et al., 1990). All patients had normal erythrocyte sedimentation rate, hematology count, liver enzymes, creatinine kinase, thyroid function, rheumatoid factor and antinuclear antibodies. No medications were taken on a regular basis and no analgesics had been used on the day of assessment. None of the FM patients had other known painful conditions or diseases. The patients were recruited consecutively.

2.1.2. Healthy controls

Fifteen healthy sex- and age-matched subjects (average age 44.4 years, range 25–61 years) participated. They were assessed in the same way as the FM patients except that no lumbar puncture was performed (for ethical reasons). The subjects were recruited by advertising at public places at Danderyds Hospital.

2.1.3. CSF controls

Patients with non-inflammatory neurological symptoms (NINS) were used as CSF controls. CSF samples from 11 female patients (average age 41.9 years, range 32–60 years; $p = \text{NS}$ compared to FM patients) that had been investigated for headache at the Department of Neurology at Karolinska University Hospital were analyzed. The routine blood tests, CSF analysis and brain MR showed no signs of inflammatory disease in this cohort. No medications were taken on a regular basis and no analgesics had been used on the day of assessment. None of the headache controls had fibromyalgia symptoms.

The study was approved by the local ethical committee and all subjects gave their informed consent to participate. The study followed the guidelines of the Declaration of Helsinki.

2.2. Pain ratings and questionnaires

Ongoing pain intensity was rated on a 100 mm visual analogue scale (VAS) anchored by the words “no pain” and “worst imaginable pain”. FM patients and healthy controls rated depression and anxiety (Hospital Depression and Anxiety Scale (HADS)) (Bjelland et al., 2002), fatigue (Multidimensional Fatigue Inventory (MFI-20)) (Lin et al., 2009), sleep disturbance (Pittsburgh Sleep Quality Inventory (PSQI)) (Buysse et al., 1989) and health related quality of life (Short Form-36 (SF-36)) (Contopoulos-Ioannidis et al., 2009). In addition, FM patients completed a pain drawing and rated the impact of fibromyalgia (Fibromyalgia Impact Questionnaire (FIQ)) (Bennett, 2005).

2.3. Pressure algometry

Pressure pain thresholds (PPTs) were assessed using a pressure algometer (Somedic Sales AB, Hörby, Sweden) with a probe area of 1 cm² and the chosen rate of pressure increase of approximately 30 kPa/s (Kosek et al., 1993). PPTs were assessed once at 4 different “tender points” according to the ACR-1990 criteria bilaterally (supraspinatus, elbows, gluteus and knees) and the average was calculated as the individual PPT and used for further analysis. Previous studies have shown that assessments of PPTs at a limited number of sites give a good estimate of the overall pain sensitivity (Petzke et al., 2001).

2.4. Lumbar puncture and cytokine measurements in CSF and serum

Lumbar puncture was performed within 24 h after pain and fatigue assessments. CSF was sampled in polypropylene tubes. CSF samples were immediately centrifuged, supernatants frozen and both supernatants and the pellet was stored in -80°C until use. Cytokine levels (IL-1b and IL-8) were analyzed with ELISA (R&D, high sensitivity Quantikine). Serum levels of IL-1b, IL-5, IL-6, IL-8, IL-10 and TNF-alpha were analyzed with ELISA (R&D, high sensitivity Quantikine). Sensitivity, expressed as the minimum detectable dose (MDD), for the ELISA kits were as follows: IL-1beta 0.057 pg/ml; IL-5 0.29 pg/ml; IL-6 0.039 pg/ml; IL-8 3.5 pg/ml; IL-10 0.09 pg/ml; TNF-alpha 0.106 pg/ml. The CSF samples from the NINS patients were taken from a repository. The samples of NINS patients and the samples of control serum were assayed at the same time as the FM patients' samples.

2.5. Procedures

On the first day FM patients and healthy controls completed all questionnaires and pressure algometry was performed. The subjects returned the following morning for venous and lumbar (FM patients only) puncture. The CSF samples from NINS patients were taken for diagnostic purposes. However these patients had given their permission for these samples to also be used for research. No corresponding serum samples were available for analysis in this group.

2.6. Statistics

Group differences were analyzed by Mann–Whitney *U*-test. Differences between IL-1b and IL-8 concentrations between serum and CSF in FM patients were analyzed by Wilcoxon Signed Ranks Test. Correlations were analyzed by Spearman's correlation coefficient. $p < 0.05$ was considered as a statistically significant difference. Means and standard deviations are presented in the text.

3. Results

3.1. Subject characteristics

FM patients had an average duration of FM corresponding to 2.9 years (range 1–10 years) and an average duration of pain corresponding to 10.3 years (range 2–30 years). They rated ongoing pain intensity as 65.8 mm (range 42–87 mm), while all healthy controls were pain free ($p < 0.001$). PPTs were lower in FM patients compared to healthy controls ($p < 0.001$). Compared to healthy controls, FM patients had higher ratings of fatigue ($p < 0.001$), sleep disturbance ($p < 0.001$), depression ($p < 0.001$) and anxiety ($p < 0.001$) and lower ratings of quality of life ($p < 0.001$) (Table 1).

3.2. Serum concentrations of cytokines/chemokines

The serum concentrations (mean and standard deviations) are presented in Table 2. FM patients had higher average serum concentrations of IL-8 compared to healthy controls ($p < 0.02$), but lower serum IL-1b ($p < 0.007$), IL-5 ($p < 0.001$) and TNF-alpha ($p < 0.04$) concentrations. No statistically significant group differences were found regarding serum concentrations of IL-6 and IL-10. Since previous publications have found increased TH1 to TH2 interleukin ratios in depressed patients (Kim et al., 2007; Li et al., 2010), we wanted to investigate whether the TH2 interleukin IL-5 was influenced by depression in FM patients. We found a statistically significant negative correlation between serum IL-5 and ratings of depression (HAD-D) ($r = -0.518$; $p < 0.05$) in our FM patients. IL-6 levels, also previously shown to be elevated in depressed patients, did not correlate with ratings of depression in our FM patients ($r = 0.243$; $p = 0.4$).

Table 1

Descriptive data for FM patients (n = 15) and healthy controls (n = 15). SD = standard deviation, NA = not applicable, NS = non significant, VAS = visual analogue scale, PPTs = pressure pain thresholds, MFI-20 = Multidimensional Fatigue Inventory 20 item, PSQI = Pittsburgh Sleep Quality Inventory, HADS = Hospital Depression and Anxiety Scale, FIQ = Fibromyalgia Impact Questionnaire, SF-36 = Short Form-36; phys = physical components; ment = mental components (original 0–100 scoring algorithms based on the summated ratings method).

Average and SD	FM patients	Healthy controls	Group differences
Age	46.2 ± 11.1	44.4 ± 10.7	NS
Pain dur. (years)	10.3 ± 8.2	NA	–
FM dur. (years)	2.9 ± 2.7	NA	–
Pain (mm VAS)	65.8 ± 13.2	0 ± 0	p < 0.001
PPTs (kPa)	99.5 ± 36.7	471.6 ± 104.5	p < 0.001
Fatigue (MFI-20)	18.1 ± 1.4	5.1 ± 1.0	p < 0.001
Sleep (PSQI)	13.2 ± 3.7	1.8 ± 1.7	p < 0.001
Depression (HADS)	6.9 ± 3.4	0.5 ± 1.1	p < 0.001
Anxiety (HADS)	9.8 ± 4.0	1.9 ± 1.8	p < 0.001
FIQ (%)	72.5 ± 12.5	NA	–
SF-36phys	26.4 ± 7.6	97.5 ± 2.7	P < 0.001
SF-36ment	40.3 ± 21.2	90.4 ± 6.3	P < 0.001

3.3. Cerebrospinal fluid concentrations of IL-1b and IL-8

One CSF FM sample was lost due to technical failure and therefore 14 samples were analyzed. FM patients had significantly higher CSF IL-8 levels (62.3 ± 26.3 pg/ml) compared to NINS patients (16.0 ± 8.6 pg/ml) ($p < 0.001$) (Fig. 1). There was no statistically significant difference in CSF IL-1b levels between FM patients (2.6 ± 2.0 pg/ml) and NINS patients (6.4 ± 6.1 pg/ml) (Fig. 2).

3.4. Relationships between serum and CSF levels of IL-1b and IL-8 in FM patients

CSF concentrations of IL-1b and IL-8 were higher than corresponding serum concentrations (IL-1b; CSF 2.6 ± 2.0 pg/ml, serum 0.6 ± 0.1 pg/ml, $p < 0.005$ and IL-8; CSF 62.3 ± 26.3 pg/ml, serum 21.4 ± 5.5 pg/ml, $p < 0.001$). CSF concentrations of IL-1b or IL-8 did not correlate with serum concentrations.

4. Discussion

FM patients had elevated CSF and serum IL-8 and the average CSF concentration of IL-8 was three times higher than the average serum IL-8 concentration. To our knowledge, this is the first report of intrathecal inflammatory mediators in FM patients. Our results indicate a central inflammatory response involving the pro-inflammatory chemokine IL-8. The findings tally previous reports of increased CSF IL-8 in patients with postherpetic neuralgia (Kikuchi et al., 1999; Kotani et al., 2004), osteoarthritis (Lundborg et al., 2010) and post-operative pain (Buvanendran et al., 2006). However, while the CSF IL-8 concentrations in our CSF controls were comparable to previously reported control values (Backonja et al., 2008; Lundborg et al., 2010), the average concentration of CSF IL-8 in our FM patients was at least 40% higher than any of the previously reported patient concentrations, indicating the relevance of CSF IL-8 for the pathophysiology of FM.

Table 2

Serum cytokine and chemokine concentrations in FM patients (n = 15) and healthy controls (n = 15). SD = standard deviation, NS = non significant.

Mean and SD	FM patients	Healthy controls	Group diff.
Serum IL-1b	0.59 ± 0.08	0.83 ± 0.24	p < 0.007
Serum IL-5	9.36 ± 3.37	28.89 ± 15.59	p < 0.001
Serum IL-6	1.44 ± 0.76	1.09 ± 0.55	NS
Serum IL-8	21.36 ± 5.54	15.53 ± 4.83	p < 0.02
Serum IL-10	33.44 ± 61.18	16.33 ± 11.76	NS
Serum TNF-alpha	2.77 ± 1.62	4.41 ± 2.29	p < 0.04

The release of pro-inflammatory cytokines/chemokines (including IL-8), by glia cells can be triggered by stress, immune activation and afferent nociceptive input (Milligan and Watkins, 2009). Glia cells, as well as neurons, express IL-8 receptors (Yamamoto et al., 1998) and CNS injections of CINC-1 (the rat equivalent to human IL-8) (Sachs et al., 2002) induced hyperalgesia and pain behavior (Yamamoto et al., 1998; Ahn et al., 2005). Furthermore, IL-8 has been shown to stimulate astrocyte production of NGF (Kotani et al., 2004). Elevated concentrations of NGF have been reported in CSF of FM patients (Giovengo et al., 1999; Sarchielli et al., 2007) and would support an involvement of activated glia in FM (Sarchielli et al., 2007). Blood-borne, pro-inflammatory cytokines released by peripheral immune cells (Watkins and Maier, 2005) can be transported across the blood–brain–barrier (BBB) by special transport mechanisms (Gutierrez et al., 1993; Banks et al., 1995; Quan and Herkenham, 2002). However, we are not aware of a specific transport mechanism for IL-8 over BBB and our FM patients had three times higher IL-8 concentrations in CSF compared to serum. Therefore, it is likely that the elevated CSF IL-8 concentrations in our FM patients depend on IL-8 production within the CNS most likely by activated glia. Contrary to IL-8, we found no statistically significant group difference in CSF IL-1b concentrations.

The CSF profile of increased IL-8 but not IL-1b in FM patients was reproduced in serum. Compared to healthy controls, FM patients had elevated serum IL-8 concentrations, but decreased concentrations of the pro-inflammatory cytokines IL-1b and TNF-alpha, while IL-6 and the anti-inflammatory cytokine IL-10 was normal. Previous studies have reported elevated (Wang et al., 2009; Gur et al., 2002; Bazzichi et al., 2007; Wallace et al., 2001; Ortega et al., 2009) or normal (Uceyler et al., 2006; Togo et al., 2009) IL-8 serum concentrations in FM patients as well as normal concentrations of IL-1b (Gur et al., 2002; Bazzichi et al., 2007; Wallace et al., 2001; Togo et al., 2009), IL-6 (Gur et al., 2002; Bazzichi et al., 2007) and elevated (Bazzichi et al., 2007) or normal TNF-alpha (Uceyler et al., 2006; Wallace et al., 2001; Togo et al., 2009). We have no reliable explanations for the discrepancies regarding IL-1b and TNF-alpha between these studies and our own. Conflicting results have been reported regarding serum IL-10 concentrations in FM, with both elevated (Bazzichi et al., 2007; Togo et al., 2009) and reduced (Uceyler et al., 2006) levels. In our study, the levels of the anti-inflammatory cytokine IL-10 were normal, but FM patients had reduced levels of the anti-inflammatory Th2 cytokine IL-5. To our knowledge, IL-5 has not been assessed previously in FM, however reduced Th2 concentrations have been reported in depressed patients (Kim et al., 2007; Li et al., 2010). In accordance with this, we found a negative correlation between IL-5 and depression in our FM patients. Therefore, further studies comparing depressed and non-depressed FM patients are needed to establish if IL-5 is reduced only in depressed FM patients or, if as is the case with increased serum IL-8, the finding applies also to FM patients free from depression and anxiety disorders (Bazzichi et al., 2007; Gur et al., 2002).

There is evidence that the two pro-inflammatory cytokines IL-1b and IL-8 contribute to pain and hyperalgesia by different mechanisms (Cunha et al., 1991; Sachs et al., 2002).

Peripheral as well as central (intrathecal) IL-1b stimulates COX-2 activity (Bartfai, 2001; Samad et al., 2001) and the IL-1b mediated increase in pain sensitivity can be prevented by administration of COX-2 inhibitors (Cunha et al., 1991; Sachs et al., 2002; Samad et al., 2001), but not by beta-adrenergic receptor antagonists (Cunha et al., 1991; Sachs et al., 2002) or by the adrenergic neurone-blocking agent guanethidine (Cunha et al., 1991). In contrast, peripherally administered IL-8 induced local hyperalgesia (Bartfai, 2001; Oh et al., 2001) that could be blocked by beta-adrenergic receptor antagonists (Cunha et al., 1991; Sachs et al., 2002) as well as by guanethidine (Cunha et al., 1991), but was not influenced by local administration of COX-2 inhibitors (Cunha et al., 1991; Sachs et al., 2002). In analogy, centrally administered CINC-1 (the rat analogue of human IL-8), reduced

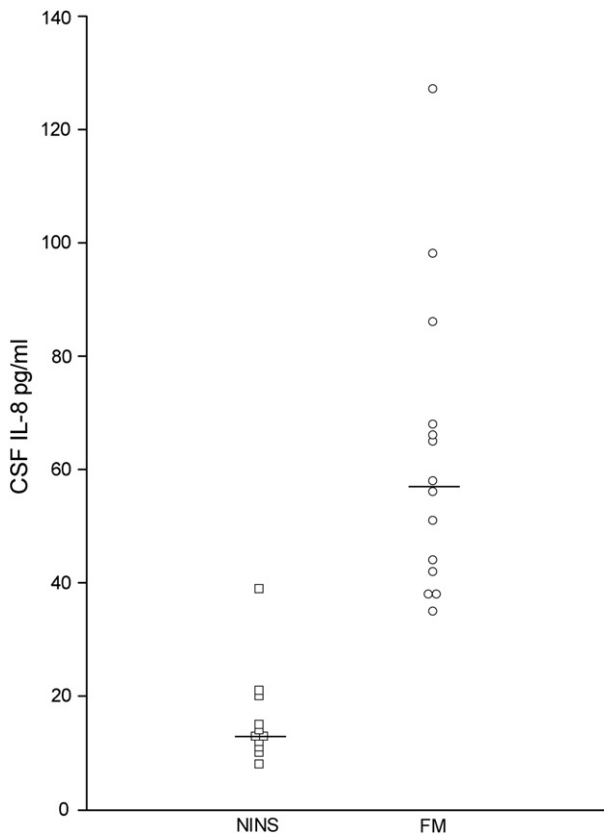


Fig. 1. Cerebrospinal fluid (CSF) concentrations of interleukin-8 (IL-8) in 14 female fibromyalgia (FM) patients and 11 age-matched patients with non-inflammatory neurological symptoms (NINS). The medians are shown. FM patients had significantly higher CSF concentrations of IL-8 compared to NINS patients ($p < 0.001$).

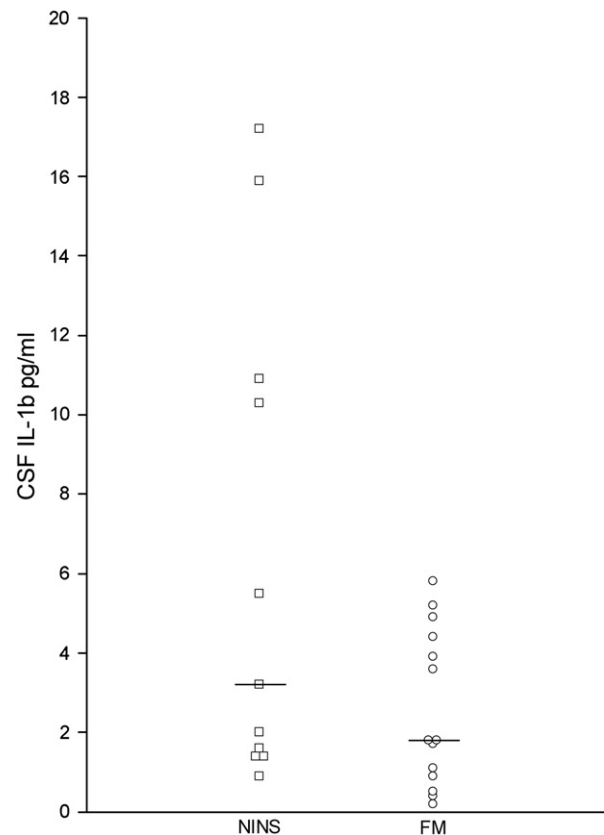


Fig. 2. Cerebrospinal fluid (CSF) concentrations of interleukin-1 beta (IL-1b) in 14 female fibromyalgia (FM) patients and 11 age-matched patients with non-inflammatory neurological symptoms (NINS). The medians are shown. No statistically significant group difference was found (NS).

mechanical pain thresholds (Yamamoto et al., 1998; Ahn et al., 2005) and increased pain related behavior in rats (Ahn et al., 2005). The latter could be prevented by central pre-treatment with beta adrenergic receptor antagonists, but was not influenced by COX-2 inhibitors (Ahn et al., 2005). Furthermore, activation of the sympathetic nervous system has been shown to increase the production of IL-8 in peripheral tissues through a beta adrenergic receptor dependent mechanism (Elenkov et al., 2000; Black, 2002). Finally, stress related release of the IL-8 analogue CINC from the hypothalamic-pituitary region has been shown in animal experiments (Sakamoto et al., 1996; Matsumoto et al., 1997). Taken together, these studies suggest the involvement of sympathetic nervous system in the regulation of IL-8 release as well as in IL-8 induced hyperalgesia.

There are several lines of evidence that FM patients have an increased baseline sympathetic activation (Petzke and Clauw, 2000; Cohen et al., 2001). In fact, some researchers regard FM as a sympathetically maintained pain syndrome (Martinez-Lavin, 2007) and increased catecholamine availability has been proposed as an animal model for human pain syndromes such as FM (Nackley et al., 2007). Stress induced hyperalgesia is mediated by release of epinephrine, an endogenous beta-adrenergic receptor ligand (Khasar et al., 2009). Activation of beta-adrenergic receptors on primary nociceptive afferents can produce hyperalgesia in animals, which is reversed by treatment with beta-adrenergic receptor antagonists (Nackley et al., 2007). The latter is in accordance with two studies showing analgesic effects of treatment with beta-adrenergic receptor antagonists in FM patients (Wood et al., 2005; Light et al., 2009). In addition, FM patients show evidence of desensitization or down-regulation of beta-adrenergic receptors, which would be in accordance with chronic stimulation (Maekawa et al., 2003). Finally, a specific adrenergic receptor gene polymorphism has been associated with an increased frequency of FM (Vargas-Alarcon et al., 2009), also supporting involvement of beta-adrenergic receptors in

pathogenesis of FM. Therefore, FM could be regarded as a sympathetically maintained pain syndrome related to increased IL-8 levels, rather than dependent on prostaglandin associated mechanisms. The lack of efficacy of NSAIDs and corticosteroids in FM treatment would support the latter (Carville et al., 2008), although other factors explaining the lack of IL-1b increase in our FM patients cannot be excluded.

The present study suffers from several limitations. The small number of subjects makes this a pilot study and future studies using larger patient cohorts are needed. The FM patients were recruited from a tertiary care clinic and might not be representative for the FM population as such. For ethical reasons we were not able to take CSF samples from healthy controls, however, we used a patient group where no organic abnormalities were found in spite of extensive medical exams.

In conclusion, we found elevated CSF concentrations of IL-8 in FM patients in agreement with the hypothesis of glia cell activation in chronic pain syndromes such as FM. The profile of elevated pro-inflammatory cytokine IL-8, but not IL-1b, in CSF and serum, is in accordance with FM symptoms being mediated by sympathetic activity rather than dependent on prostaglandin associated mechanisms. If this is the case, then patients with inflammatory, prostaglandin associated, pain would be expected to have the reverse pattern of cytokine expression, i.e., increased IL-1, but not IL-8 CSF concentrations. Comparisons of FM patients and patients with inflammatory pain, i.e., rheumatoid arthritis, are currently underway in our laboratory. The long-term goal is to identify differential patterns of pro-inflammatory substances in the periphery and the CNS, related to specific pain mechanisms. These substances could be valuable for diagnostic purposes and facilitate development of mechanism based pain treatment. Furthermore, increased understanding of interactions between pain related signalling in the periphery and the CNS would potentially open up for development of truly new treatment strategies.

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