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Evidence of different mediators of central inflammation in dysfunctional and inflammatory pain — Interleukin-8 in fibromyalgia and interleukin-1 β in rheumatoid arthritis

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Abstract

The purpose of this study was to relate central inflammation to autonomic activity (heart rate variability (HRV)) in patients with rheumatoid arthritis (RA) and fibromyalgia (FM). RA patients had reduced parasympathetic activity and FM patients had increased sympathetic activity compared to healthy controls. Comparisons between RA and FM showed higher cerebrospinal fluid (CSF) interleukin (IL)-1β inversely correlated to parasympathetic activity in RA. The FM patients had higher concentrations of CSF IL-8, IL-1Ra, IL-4 and IL-10, but none of these cytokines correlated with HRV. In conclusion, we found different profiles of central cytokines, i.e., elevated IL-1β in inflammatory pain (RA) and elevated IL-8 in dysfunctional pain (FM).

Abbreviations: HRV, heart rate variability; RA, rheumatoid arthritis; FM, fibromyalgia; CSF, cerebrospinal fluid; IL, interleukin; IL-1Ra, interleukin 1 receptor antagonist; TNF, tumor necrosis factor; CCL-2, chemokine (C–C motif) ligand 2; BDNF, brain-derived neurotrophic factor; NGF, nerve growth factor; SP, substance P; COX-2, cyclooxygenase-2; ACR, American College of Rheumatology; NSAID, non-steroidal anti-inflammatory drug; DAS, disease activity score; ACPA, antibodies to citrullinated peptide antigens; RF, rheumatoid factor; MTX, methotrexate; VAS, visual analogue scale; MFI-20, Multidimensional Fatigue Inventory; PSQI, Pittsburg Sleep Quality Inventory; SF-36, Short Form-36; ELISA, enzyme-linked immunosorbent assay; ECG, electrocardiography; RMSSD, the square root of the mean of the squared differences between adjacent NN intervals; SDNN, the standard deviation of the NN interval; NN interval, the normal-to-normal interval, all intervals between adjacent QRS complexes resulting from sinus node depolarizations; LF, low frequency power; HF, high frequency power; LF/HF, ratio between LF and HF; DMARD, disease-modifying antirheumatic drug

Keywords: Fibromyalgia, Rheumatoid arthritis, Cytokines, Chemokines, Cerebrospinal fluid, Glia cells, Heart rate variability

1. Introduction

Central nervous system (CNS) mechanisms such as central sensitization, facilitation and disinhibition are involved in various forms of chronic pain conditions. The latter was illustrated by findings of widespread allodynia and hyperalgesia in patients with fibromyalgia (FM) (dysfunctional pain) (Kosek et al., 1996), but also other diseases characterized by nociceptive and inflammatory pain, such as osteoarthritis (Kosek and Ordeberg, 2000a,b; Gwilym et al., 2009; Arendt-Nielsen et al., 2010) and rheumatoid arthritis (RA) (Leffler et al., 2002). The mechanisms of sensitization and hyperalgesia involve neuron interaction with activated glia cells (for review see Watkins and Maier, 2005; Milligan and Watkins, 2009). Following activation, glia cells release pro-inflammatory cytokines/chemokines such as tumor necrosis factor (TNF), interleukin-1beta (IL-1β), interleukin-6 (IL-6) and interleukin-8 (IL-8), chemokine (C–C motif) ligand 2 (CCL-2), also known as monocyte chemoattractant protein 1 (MCP-1), as well as brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), glutamate and substance P (SP) (Sofroniew et al., 2001; Watkins and Maier, 2005; Milligan and Watkins, 2009), substances with the potential for pain amplification. Based on data from animal studies, activated glia cells have been proposed to be an important actor also for development and maintenance of chronic pain in humans (Milligan and Watkins, 2009).

Supporting the role of neuroinflammation in human pain patients, elevated cerebrospinal fluid (CSF) concentrations of pro-inflammatory cytokines/chemokines have been reported in patients with chronic nociceptive (Lundborg et al., 2010) as well as neuropathic (Kotani et al., 2004; Backonja et al., 2008) pain. In addition, we have previously documented increased CSF IL-8, but not IL-1β, in FM patients compared to headache controls (Kadetoff et al., 2012) and increased CSF IL-1β levels in patients with RA compared to surgical controls and to patients with multiple sclerosis (MS), respectively (Lampa et al., 2012). These results are in accordance with animal studies showing that the hyperalgesic effects of IL-1β, but not IL-8, were mediated by cyclooxygenase-2 (COX-2) while the hyperalgesic effects of IL-8, but not IL-1β, were mediated by activation of beta-adrenergic receptors (sympathetic activity) (Cunha et al., 2005; Verri et al., 2006).

There are indications that the autonomic nervous system forms an important link for neuro-immune regulation through the cholinergic anti-inflammatory pathway, termed as the inflammatory reflex (Tracey, 2007). Assessment of heart rate variability (HRV) provides a non-invasive

method to assess autonomic function. Previous studies have reported abnormalities in HRV in RA (Janse Van Rensburg et al., 2012) as well as FM (Meeus et al., 2013) patients. However, to our knowledge, no previous study has related autonomic tone to CSF patterns of cytokines in chronic pain patients. In this study, we wanted to profit from our patient cohorts to make a direct comparison between patients with inflammatory, COX-2 driven pain (RA) and patients with dysfunctional pain traditionally regarded as non-inflammatory (FM). Our hypothesis was that RA patients would have reduced parasympathetic activity which would be related to elevated CSF IL-1β and that FM patients would have increased sympathetic activity related to elevated CSF IL-8. Also, we extended our previous CSF assessments with analysis of TNF, IL-4, IL-6, IL-10, CCL-2, BDNF, NGF as well as the IL-1 receptor antagonist (IL-1Ra) in the FM patients and with the analysis of CSF IL-8, CCL-2, BDNF, NGF in the RA patients. In addition to the different concentrations of IL-1β and IL-8, we hypothesized different CSF profiles with higher levels of the pro-inflammatory TNF, IL-6 and CCL-2 and lower concentrations of the anti-inflammatory IL-1Ra, IL-4 and IL-10 in the RA patients compared to the FM patients.

2. Materials & methods

2.1. Subjects

2.1.1. FM patients Fifteen female patients (average age 46.2 years, range 25–60 years, Table 1) participated. They were outpatients at the Department of Rehabilitation Medicine, Danderyds Hospital, Stockholm and fulfilled the classification criteria of the American College of Rheumatology (ACR) 1990 for fibromyalgia (Wolfe et al., 1990). All the patients had normal erythrocyte sedimentation rate, hematology count, liver enzymes, creatinine kinase, thyroid function, rheumatoid factor and anti-nuclear antibodies. No medications were taken on a regular basis and no analgesics or non-steroidal anti-inflammatory drugs (NSAIDs) had been used on the day of assessment. None of the FM patients had other known painful conditions or neurological diseases.

2.1.2. RA patients Fourteen female patients (average age 51.1 years, range 36–59 years, Table 1) participated. They were outpatients at the Unit of Rheumatology, Karolinska University Hospital, Stockholm and fulfilled both the 1987 and 2010 ACR criteria for RA (Arnett et al., 1988; Aletaha et al., 2010) and none fulfilled the ACR criteria for fibromyalgia (Wolfe et al., 1990). The average number (± standard deviation) of swollen and painful joints was 4.9 ± 3.8 and 4.2 ± 3.9 respectively. The average disease activity score for 28 joint count (DAS28) was 3.55 ± 1.3. Twelve patients (86%) had antibodies to citrullinated peptide antigens (ACPA) and ten patients (71%) had positive rheumatoid factor (RF). Seven patients were on methotrexate (MTX) monotherapy, two were on MTX combined with etanercept or infliximab, one was on adalimumab monotherapy, three were on sulfasalazine and one was on hydroxychloroquine. Three patients were also on low-dose prednisone (all below 7.5 mg/d). No NSAIDs were administered within 24 h before CSF sampling and pain and fatigue assessments. No RA patient had any neurological disease.

2.1.3. 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/RA patients except that no lumbar puncture was performed (for ethical reasons). The subjects were recruited by advertising at public places at Danderyds Hospital.

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

2.2. Procedures

On the first day the RA and FM patients and healthy controls completed all questionnaires and were provided with the device for HRV assessment. The subjects returned the following morning for venous and lumbar (patients only) puncture.

2.2.1. 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”. The RA and FM patients and healthy controls rated fatigue (Multidimensional Fatigue Inventory (MFI-20)) (Lin et al., 2009), sleep disturbance (Pittsburg Sleep Quality Inventory (PSQI)) (Buysse et al., 1989) and health related quality of life (Short Form-36 (SF-36)) (Contopoulos-Ioannidis et al., 2009).

2.2.2. Lumbar puncture and cytokine measurements in CSF and serum Lumbar puncture was performed within 24 h after questionnaires. CSF was sampled in polypropylene tubes. CSF samples were immediately centrifuged and supernatants were frozen and stored in − 80 °C until use. Cytokine levels in CSF and serum were analyzed with enzyme-linked immunosorbent assay (ELISA) (R&D, high sensitivity Quantikine). Sensitivity, expressed as the mean of minimum detectable dose (MDD), for the ELISA kits were as follows: IL-1β 0.14 pg/mL; IL-1ra: 6.26 pg/mL; IL-4: 0.11 pg/mL; IL-6 0.039 pg/mL; IL-8 3.5 pg/mL; IL-10 0.09 pg/mL; and TNF 0.106 pg/mL. Human CSF was tested for CCL-2 (Cat No L451AYA-1), BDNF (Cat No N45ZA-1), and β-NGF (custom made prototype), in a chemiluminescence assay based on the MSD technology (Mesoscale Discovery, Gaithersburg, MA, US). The samples were captured on the pre-coated MSD plates and were detected using a labeled biotinylated antibody directed towards the analyte of interest.

2.2.3. Autonomic activity and heart rate variability Holter electrocardiography (ECG) was applied for 24 h measurements during day and sleep at night. Recordings were manually read, and readings with a high number of ectopic beats were discarded from analysis. The normal-to-normal R–R interval (NN interval) was utilized to perform computation of HRV measures from time and frequency domains. In the time domain analysis heart rate, the square root of the mean of the squares of differences between adjacent NN intervals (RMSSD) and standard deviation of the NN intervals (SDNN) represent the major components of the time domain HRV (Malik et al., 1996) and were selected for the statistical analysis. The power spectrum can be divided into three frequency bands of very low frequency (VLF) 0.003–0.04 Hz, low frequency (LF) 0.04–0.15 Hz and high frequency (HF) 0.15–0.4 Hz. Of these, LF and HF can be related to the controlled and balanced behavior of the two branches of the autonomic nervous system. The efferent vagal activity is a major contributor to the HF component, whereas the major autonomic input on the LF component is not entirely clear. It is considered to be contributed by either sympathetic input only, or in some conditions to reflect a mixture of both sympathetic and parasympathetic inputs (Malik et al., 1996). The LF/HF ratio is related to the sympathetic/parasympathetic balance (Malik et al., 1996).

2.3. Statistics

Overall group differences were analyzed by Kruskal–Wallis test and post hoc group differences were assessed by independent samples Mann–Whitney U-test. Correlations were analyzed by Spearmans' 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 characterization

The FM patients rated higher ongoing pain intensity (VAS) ($p < 0.001$) compared to the RA patients. Furthermore, compared to the RA patients and healthy controls, the FM patients reported higher ratings of fatigue (RA; $p < 0.002$, controls; $p < 0.001$), sleep disturbance (RA and controls; $p < 0.001$) and lower ratings of quality of life (mental and physical) (RA and controls; $p < 0.001$). Compared to healthy controls, the RA patients had higher ratings of fatigue ($p < 0.001$), sleep disturbance ($p < 0.001$) and lower quality of life (SF-36: mental $p < 0.014$, physical $p < 0.001$) (Table 1).

3.2. Serum concentrations of cytokines/chemokines

The serum concentrations (mean and standard deviations) are presented in Table 2. There were statistically significant (Kruskal–Wallis test) overall group differences for TNF ($p < 0.001$), IL-1β ($p < 0.001$) and IL-8 ($p < 0.001$), but only a tendency for IL-6 ($p = 0.054$). Post hoc comparisons revealed significantly lower TNF and IL-1β serum concentrations in both patient groups compared to healthy controls ($p < 0.05$). The RA patients had lower serum concentrations of TNF ($p < 0.006$) and IL-1β ($p < 0.001$) compared to the FM patients. In contrast, the FM patients had higher serum IL-8 concentrations compared to the RA patients ($p < 0.001$) and healthy controls ($p < 0.02$), and the RA patients had lower serum IL-8 levels compared to healthy controls ($p < 0.002$). Although the overall group difference was not statistically significant post hoc comparisons, as expected, revealed higher serum IL-6 levels in the RA patients compared to healthy controls ($p < 0.029$).

3.3. Cerebrospinal fluid concentrations of cytokines/chemokines

One FM CSF sample was lost due to technical failure and therefore 14 samples were analyzed. The CSF concentrations (mean and standard deviations) are presented in Table 3. Compared to the FM patients, the RA patients had significantly higher CSF IL-1β, but lower IL-1Ra. The reverse was true for IL-8, with higher levels in the FM patients compared to the RA patients. In addition, the FM patients had higher CSF IL-4 and IL-10 levels compared to the RA patients. Although not statistically significant, the FM patients also tended to have higher CSF TNF concentrations. There were no significant group differences in CSF IL-6 or CCL-2. CSF BDNF and NGF levels were below detection limit in all the FM and RA patients.

3.4. Relationships between serum and CSF levels of cytokines/chemokines

CSF concentrations of IL-1β and IL-8 were higher than corresponding serum concentrations in the FM (IL-1β $p < 0.002$; IL-8 $p < 0.001$) and RA (IL-1β $p < 0.007$; IL-8 $p < 0.004$) patients. TNF was higher in serum compared to CSF in the FM ($p < 0.001$) and RA patients ($p < 0.001$). There were no statistically significant differences between CSF and serum IL-6 concentrations in either group. There were no statistically significant correlations between serum and CSF concentrations for any of the assessed cytokines/chemokines.

3.5. Autonomic activity in patients and controls

Overall group differences concerning time and frequency domain HRV parameters are presented in Table 4. All three time-domain parameters differed significantly between RA and controls, and FM and controls, respectively. There was a decrease in HF in RA compared to controls, in line with decreased parasympathetic activity in RA. In FM there was a significantly increased ratio LF/HF, in line with increased sympathetic activity. There were no statistically significant differences between RA and FM in either time- or frequency domain HRV parameters.

3.6. Relationship between autonomic activity and cytokine levels

Concerning time-domain HRV parameters, there was a strong negative correlation between serum IL-6 levels in RA and SDNN ($r = -0.868$ $p < 0.0001$). Moreover, SDNN in RA correlated negatively with CSF levels of IL-10 ($r = -0.716$, $p < 0.006$), and also serum IL-1beta in FM and SDNN was significantly correlated ($r = 0.646$ $p < 0.01$). There were no other correlations between CSF/serum cytokines and the time-domain HRV parameters.

The frequency domain of HRV is known to reflect sympathetic/parasympathetic balance. In RA, CSF IL-1β correlated positively with LF/HF ($r = 0.64$; $p < 0.05$). Moreover, and in line with these data, RA CSF IL-10 correlated negatively with LF ($r = -0.58$; $p < 0.05$). On the contrary, in the FM patients no significant correlations between CSF cytokines and HRV were found. In serum, IL-1β and IL-10 in the RA patients was not correlated to HRV, but serum IL-6 was correlated inversely to LF both in RA ($r = -0.55$ $p < 0.05$) and controls ($r = -0.46$; $p < 0.05$). There were no correlations between CSF IL-6 or other CSF or serum cytokine levels and frequency domains of HRV in the RA and FM patients.

4. Discussion

In accordance with our a priori hypothesis, the RA patients had a reduced parasympathetic tone compared to controls and an inverse correlation between CSF IL-1β levels and parasympathetic activity. Compared to controls, the FM patients had increased sympathetic activity, but contrary to our hypothesis, we did not find a positive correlation between CSF IL-8 and HRV measures of sympathetic tone. The RA patients had higher CSF concentrations of the pro-inflammatory IL-1β and lower anti-inflammatory IL-1Ra, IL-4 and IL-10 compared to the FM patients, whereas the FM patients had higher CSF levels of the pro-inflammatory chemokine IL-8 compared to the RA patients. Thus we found evidence of different CSF cytokine profiles in the patients with inflammatory, COX-2 dependent pain (RA) and dysfunctional, possibly sympathetically mediated pain (FM). The CSF concentrations of IL-1β and IL-8 were significantly higher than serum concentrations in both groups indicating a central inflammatory response involving these cytokines.

An important link for neuro-immune regulation is the cholinergic anti-inflammatory pathway, termed as the inflammatory reflex, which has been shown to impact immune regulation in experimental inflammatory conditions and arthritis (Tracey, 2007). Moreover, these vagus-mediated neuro-immune mechanisms have also been shown to be dysfunctional in several inflammatory diseases including RA (Tracey, 2007). In the present study, we could confirm a decreased vagus activity in RA, with a marked decrease in HF. It has been discussed how much peripheral inflammation may influence the parasympathetic dysfunction in RA and a relation to inflammatory state was indicated by the coupling between parasympathetic dysfunction and clinical response to anti-TNF therapy (Holman and Ng, 2008). Whereas the peripheral inflammatory reflex is dependent upon activation of the alpha7 nicotinic receptor (Tracey, 2007), CNS activation of the efferent, cholinergic vagus nerve is mediated through muscarinic receptors (Pavlov et al., 2006). Interestingly, earlier investigations have shown that interleukin IL-1β may cause dysfunction in cholinergic neurotransmission (Schliebs et al., 2006). Thus, our findings of an inverse correlation between elevated IL-1β levels in RA CSF and parasympathetic activity may indicate that autonomic activity in RA is centrally regulated through action of inflammatory cytokines, such as IL-1β.

Interestingly, and in the RA patients only, we found an inverse correlation between intrathecal levels of the anti-inflammatory cytokine IL-10 and LF. Whereas the major autonomic component of LF is not exactly clear, LF has in some studies been related directly to sympathetic activity. Thus, in a post-infarction study beta-blockers were shown to reduce LF, stressing the importance of adrenergic stimulation for this component of HRV (Sandrone et al., 1994). In addition, central nervous sympathetic outflow and intracerebral cathecolamine release has been shown related to action of inflammatory cytokines (Szelenyi and Vizi, 2007), and it is possible that intrathecal IL-10 may thus have a regulating function in this context. When correlating the serum levels of cytokines to HRV, we found an inverse correlation between serum IL-6 and LF in both RA and controls. This pattern has earlier been described in healthy controls (von Känel et al., 2008), and confirms the association of systemic inflammation in relation to autonomic activity also under physiological conditions.

In FM, we detected an increased sympathetic-to-parasympathetic balance, which is well in line with previous data (Meeus et al., 2013), however, no correlation was found between cerebrospinal cytokines/chemokines and HRV. The fact that we failed to confirm a statistically significant correlation between CSF IL-8 and HRV measures of sympathetic activity in our FM cohort should be interpreted with caution due to the small sample size. Previously, pain and stress have been directly associated with increased central nervous sympathetic outflow (Li et al., 1996). Furthermore, in animal studies stress and activation of sympathetic NS have been reported to increase the release of the IL-8 analogue CINC-1 from the hypothalamic–pituitary region (Sakamoto et al., 1996; Matsumoto et al., 1997). The combination of elevated stress levels (Schmidt-Wilcke and Clauw, 2011) and autonomic dysfunction (Meeus et al., 2013) is in line with the proposal to regard FM as a sympathetically mediated pain syndrome (Martinez-Lavin, 2007). The latter is in agreement with our findings of higher CSF and serum IL-8 in the FM patients compared to the RA patients and also the increased sympathetic activity in the FM patients, but not the RA patients, compared to controls.

The different CSF cytokine patterns, i.e., higher CSF IL-1β and lower anti-inflammatory IL-1Ra, IL-4 and IL-10 in an inflammatory painful condition (RA) and higher CSF and serum IL-8 in a dysfunctional pain syndrome (FM) tally reports from animal studies showing that the two pro-inflammatory cytokines IL-1β and IL-8 contribute to pain and hyperalgesia by different mechanisms (Cunha et al., 1991; Sachs et al., 2002). Administration of IL-1β in peripheral tissues or intrathecally stimulates COX-2 activity (Bartfai, 2001; Samad et al., 2001) and IL-1β mediated increase in pain sensitivity can be prevented by COX-2 inhibitors (Cunha et al., 1991; Sachs et al., 2002; Samad et al., 2001). Pharmacological agents counteracting the biological effects of IL-1β (in the form, of anakinra, IL-1Ra) have been established in the treatment of RA (Nam et al., 2010; Neurath and Finotto, 2011) and COX-2 inhibitors have shown good pain relieving effects (Shi and Klotz, 2008). In contrast, administration of IL-8 (or the rat analogue CINC-1) in peripheral tissues or intrathecally causes an increase in pain sensitivity (Yamamoto et al., 1998; Bartfai, 2001; Oh et al., 2001; Ahn et al., 2005) that can be reversed by beta-adrenergic receptor antagonists (Ahn et al., 2005) or guanethidine (Cunha et al., 1991) but not COX-2 inhibitors (Ahn et al., 2005), which fits well with the inefficacy of COX-2 inhibitors in FM (Carville et al., 2008). Contrary to our hypothesis, we found no group difference regarding CSF CCL-2. Despite the fact that CCL-2 has been implicated in the activation of astrocytes, promoting their release of IL-1β (Gao and Ji, 2010) we found no evidence that this would be the mechanism responsible for the elevated CSF IL-1β in our RA patients.

The RA patients had higher serum IL-6 compared to healthy controls (but not the FM patients) and there was a positive correlation between serum IL-6 levels and disease activity assessed by DAS28 ($r = 0.585$, $p = 0.028$), as reported previously (Madhok et al., 1993; Nishimoto et al., 2004, 2007). However, all the RA patients in the present study were on DMARD medications, which might have influenced the serum levels of pro-inflammatory cytokines (Eklund et al., 2007; Chen et al., 2011) and could explain the lower TNF, IL-1β and IL-8 serum concentrations compared to the healthy controls and the FM patients. Only serum IL-8 levels were higher in our FM patients compared to healthy controls, which is in accordance with other studies (Wallace et al., 2001; Gur et al., 2002; Bazzichi et al., 2007; Wang et al., 2009; Ortega et al., 2009; Kadetoff et al., 2012).

Despite the fact that cytokine/chemokine production is differentially regulated in the peripheral and central compartments (Feldmann and Maini, 2008), the compartments are integrated, thus cytokine/chemokine levels in one compartment influence the concentrations in the other compartment (Szelenyi and Vizi, 2007). Our findings of higher IL-1β and IL-8 concentrations in the CSF compared to serum as well as the lack of correlations between the CSF and serum levels in both groups does not favor simple transport or leakage of the cytokines across the blood–brain-barrier (BBB) (Gutierrez and Kastin, 1993; Banks et al., 1995; Watkins et al., 1995; Quan and Herkenham, 2002). Rather our data would indicate central cytokine/chemokine production. Notably, circulating cytokines can affect the CNS indirectly by activating the brain endothelium to produce pro-inflammatory cytokines (Reijerkerk et al., 2012), such as IL-1β (Szelenyi, 2001) or IL-8 analogue (Zidovetzki et al., 1999; Chen et al., 2001) thus permitting the signal to be transduced from the blood stream into the CNS without the need to cross the BBB (Szelenyi, 2001). In addition, peripheral injections of pro-inflammatory cytokines/chemokines have the potential to activate glia cells (Watkins and Maier, 2005) and activated glia can produce IL-1β in response to inflammatory stimuli (Pinteaux et al., 2002; Guo et al., 2007) and also have the potential to produce IL-8 (Milligan and Watkins, 2009). Our results indicate that the pattern of the release of pro-inflammatory substances in the CNS could reflect peripheral inflammatory mechanisms, and thus result in specific patterns for different conditions.

While the communication between peripheral tissues and CNS is well established, the reverse, i.e., if central inflammation mediated by activated glia or neurons affects efferent signaling remains highly speculative. However, the inverse correlation between CSF IL-1β and parasympathetic activity

in our RA patients, as well as the symmetric distribution of arthritis in RA with reports of remission of RA in hemiparetic limbs following ischemic cerebral infarcts (Keyszer et al., 2004), suggests that also an efferent communication, i.e., CNS to periphery might be of relevance. The concentrations of TNF were higher in the serum than in CSF in both groups and IL-6 levels were not statistically different between the serum and CSF in either group. The lack of significant up-regulation of these pro-inflammatory cytokines in CSF compared to the serum is in line with previous data demonstrating low levels of these cytokines in CNS (Vladic et al., 2002).

4.1. Limitations The present study suffers from several limitations. For ethical reasons we had to keep to a low number of subjects in the patient groups and we had no healthy CSF controls. Furthermore, patients were recruited from specialized clinics and thus may not be representative for FM or RA populations as such. For ethical reasons the RA patients could not be taken off DMARDS, which most likely affected the serum assessments and possibly also the CSF through indirect mechanisms. Furthermore, the serum samples were not available to compare IL-1Ra, IL-4, IL-10 and CCL-2 in all groups.

5. Conclusions

In conclusion, we found differential CSF cytokine profiles with higher IL-1β and lower IL-1Ra, IL-4 and IL-10 in the CSF of the RA patients, compared to FM and higher IL-8 in the CSF of the FM patients compared to RA. Our results indicate different profiles of central cytokine release, i.e., IL-1β in the patients with inflammatory, prostaglandin associated pain (RA) and IL-8 in the patients with dysfunctional, possibly sympathetically mediated pain (FM). Furthermore, RA was associated with decrease in vagus activity, which correlated with elevated cerebral IL-1β levels in accordance with the cholinergic anti-inflammatory pathway, whereas autonomic disturbances in FM were characterized by sympathetic over-activity but did not correlate to the CSF IL-8 levels. Further studies assessing the usefulness of pro-inflammatory cytokines for diagnostic purposes and to increase the understanding of chronic pain mechanisms are needed. Our results indicate that a bi-directional communication between peripheral tissues and CNS involving the immune system and glia could be of outmost importance in chronic pain conditions. Increased understanding of these mechanisms could open up for truly new treatment approaches.

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Figures and Tables

Table 1

Descriptive data for fibromyalgia (FM) patients, rheumatoid arthritis (RA) patients and healthy controls. SD = standard deviation, NA = non-applicable, NS = non-significant, VAS = visual analogue scale, MFI-20 = Multidimensional Fatigue Inventory 20 item general, PSQI = Pittsburg Sleep Quality Inventory, SF-36 = Short Form-36; phys = physical components; ment = mental components (original 0–100 scoring algorithms based on the summated rating method).

Means ± SD	FM patients	RA patients	Healthy controls	Group differences
Age (years)	46.2 ± 11.1 n = 15	51.1 ± 7.2 n = 14	44.4 ± 10.7 n = 15	NS
Duration FM/RA (years)	2.9 ± 2.7 n = 15	8.4 ± 8.7 n = 14	NA	$p < 0.028$
Pain (mm VAS)	65.8 ± 13.2 n = 15	24.0 ± 18.0 n = 14	NA	$p < 0.001$
Fatigue (MFI-20)	18.1 ± 1.4 n = 15	14.0 ± 4.2 n = 14	5.1 ± 1.0 n = 15	$p < 0.001$
Sleep (PSQI)	13.2 ± 3.7 n = 15	6.6 ± 3.0 n = 13	1.8 ± 1.7 n = 15	$p < 0.001$
SF-36phys	26.4 ± 7.6 n = 15	62.4 ± 18.6 n = 14	97.5 ± 2.7 n = 15	$p < 0.001$
SF-36ment	40.3 ± 21.2 n = 15	72.5 ± 21.6 n = 14	90.4 ± 6.3 n = 15	$p < 0.001$

Table 2

Serum cytokine and chemokine concentrations in fibromyalgia (FM) patients, rheumatoid arthritis (RA) patients and healthy controls. Overall group differences are shown. Statistically significant differences between FM and RA patients are marked † and significant differences between controls and patients are marked ‡. $p < 0.05$ is regarded as a statistically significant difference. SD = standard deviation. IL = interleukin, TNF = tumor necrosis factor.

Serum levels (pg/mL) Means ± SD	FM	RA	Controls	Group differences
IL-1β	0.59 ± 0.08 ‡ n = 15	0.02 ± 0.06†‡ n = 13	0.83 ± 0.24 n = 15	p < 0.001
IL-8	21.36 ± 5.54 ‡ n = 15	10.42 ± 6.68†‡n = 12	16.58 ± 6.20 n = 15	p < 0.001
TNF	2.77 ± 1.61 ‡ n = 14	1.41 ± 0.96†‡ n = 13	4.42 ± 2.29 n = 15	p < 0.001
IL-6	1.45 ± 0.76 n = 14	7.50 ± 16.07 ‡ n = 14	1.21 ± 0.70 n = 15	p = 0.054

Table 3

Concentrations of cytokines and chemokines in cerebrospinal fluid (CSF) of fibromyalgia (FM) and rheumatoid arthritis (RA) patients. Overall group differences are shown. $p < 0.05$ is regarded as a statistically significant difference. SD = standard deviation. IL = interleukin, TNF = tumor necrosis factor, CCL-2 = chemokine (C–C motif) ligand 2, IL-1Ra = interleukin 1 receptor antagonist.

CSF levels (pg/mL) Means ± SD	FM	RA	Group differences
IL-1β	2.58 ± 1.98 n = 14	8.83 ± 7.21 n = 14	p = 0.002
IL-8	62.35 ± 26.26 n = 14	26.92 ± 14.07 n = 12	p < 0.001
TNF	0.38 ± 0.22 n = 14	0.26 ± 0.09 n = 14	NS (p = 0.056)
IL-6	1.80 ± 0.69 n = 14	1.60 ± 0.73 n = 14	NS
CCL-2	439.03 ± 114.54 n = 12	491.43 ± 134.74 n = 13	NS
IL-1Ra	27.50 + 4.96 n = 14	17.06 + 9.82 n = 14	p = 0.002
IL-4	0.25 + 0.20 n = 14	0.04 + 0.05 n = 14	p < 0.001
IL-10	0.43 + 0.19 n = 14	0.13 + 0.08 n = 14	p < 0.001

Table 4

Time and frequency domains of heart rate variability (HRV) in fibromyalgia (FM) patients, rheumatoid arthritis (RA) patients and healthy controls. Overall group differences are shown. Statistically significant differences between patients and controls are marked ‡. SD = standard deviation. Bpm = beats per minute, RMSSD = the square root of the mean squared differences between adjacent NN intervals, SDNN = the standard deviation of the NN interval, NN interval = the normal-to-normal interval; all intervals between adjacent QRS complexes resulting from sinus node depolarizations, LF = low frequency power; HF = high frequency power; LF/HF = ratio LF (ms^2)/HF (ms^2).

Means ± SD	FM	RA	Healthy controls	Group differences
Heart rate (bpm)	78 + 10 ‡ n = 15	75 + 6 ‡ n = 14	68 + 5 n = 15	p = 0.003
RMSSD (ms)	30.9 + 12.2 ‡ n = 15	29.2 + 8.1 ‡ n = 14	50.7 + 24.8 n = 15	p = 0.002
SDNN	124 + 24.9 ‡ n = 15	127.8 + 27.6 ‡ n = 14	152.9 + 33.0 n = 15	p = 0.02
LF	836 + 541 n = 15	530 + 213 ‡ n = 13	948 + 523 n = 15	NS
HF	410 + 259 n = 15	313 + 280 ‡ n = 13	759 + 657 n = 15	p = 0.018
LF/HF	3.41 + 1.30 ‡ n = 15	2.65 + 0.71 n = 13	2.23 + 1.0 n = 15	p = 0.036