Complex regional pain syndrome related movement disorders : studies on pathophysiology and therapy.

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Citation

Analysis of cerebrospinal fluid inflammatory mediators in chronic complex regional pain syndrome related dystonia

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Abstract

There is compelling evidence of central nervous system involvement in neuropathic pain and movement disorders in patients with complex regional pain syndrome (CRPS). Previously, elevated cerebrospinal fluid (CSF) levels of interleukin-1β and interleukin-6 were found in CRPS patients with and without movement disorders. The aim of the present study was to replicate these findings and to search for additional CSF biomarkers in chronic CRPS patients with dystonia. CSF samples of 20 patients and 29 subjects that underwent spinal anaesthesia for surgical interventions were used. We measured interleukin-1β, interleukin-6, interferon-γ inducible protein-10, RANTES (regulated upon activation, normal T-cell expressed and secreted), complement C3, mannose-binding lectin, complement C1q, soluble intercellular adhesion molecule-1, endothelin-1, nitric oxide, human lactoferrin and hypocretin-1 levels in these samples. No differences in the CSF levels of these effector mediators between patients and controls were found. Our CSF findings do not support a role of a variety of inflammatory mediators or hypocretin-1 in chronic CRPS patients with dystonia.

Introduction

Complex regional pain syndrome (CRPS) is a disorder that usually occurs after trauma and is more common in women.[1-3] The initial clinical features of CRPS, which include persistent pain, changes in skin colour and temperature, sweating and swelling, have led several investigators to suggest an aberrant inflammatory response to trauma in these patients.[3,4] Various studies have reported involvement of a perturbed function of both C and Aδ fibres of sensory nerves (neurogenic inflammation) and the local immune system in the skin.[5-8] Following the acute phase of CRPS, patients may develop chronic pain, allodynia, hyperalgesia and movement disorders, which may include dystonia, myoclonus and tremor.[2,9] There is compelling evidence that these clinical features are associated with aberrant processing of spinal and supraspinal sensorimotor neural networks.[10]

In recent years evidence was obtained indicating that the immune system influences central sensitisation. A wide range of inflammatory mediators including cytokines, chemokines, adhesion molecules, endothelins, nitric oxid and complement are involved in the cascade of central events that play a role in the development and maintenance of pain.[11,12] Furthermore, several lines of evidence have implied involvement of lactoferrin and hypocretins in nociceptive processing.[13,14]

Because cerebrospinal fluid (CSF) is in close proximity of the central nervous system, it may reflect biochemical changes that are associated with mechanisms that underlie immune system involvement in central sensitisation. In this perspective, increased levels of interleukin-1β (IL-1β) and interleukin-6 (IL-6) have been found in CSF of patients with chronic CRPS.[15]

In the present study we first aimed to confirm our earlier findings of increased levels of IL-1β and IL-6 in patients with CRPS and secondly searched for additional inflammatory mediators involved in chronic CRPS with dystonia.

Materials and methods

Patients and controls

We used CSF from patients with CRPS-related dystonia who participated in clinical trials with intrathecal administration of medication. CRPS was diagnosed if patients met the CRPS type 1 criteria of the International Association for the Study of Pain,[16] either at the time of disease onset or at the time of presentation at the clinic. Because we focussed on CRPS type 1, nerve conduction studies were performed in those cases where on the basis

of history or the distribution of sensory abnormalities, dystonia was possibly associated with CRPS type 2. Additionally, imaging studies were performed in those cases where history or neurological exam yielded atypical findings.

CSF acquisition and processing

CSF samples (5 mL) of 20 patients were collected prior to the administration of intrathecal medication. Control CSF samples (1-2 mL) were obtained from subjects who underwent spinal anaesthesia for surgical interventions including urologic (e.g. transurethral resection of urinary bladder tumour), orthopaedic (e.g. total knee prosthesis), vascular (femoropopliteal bypass), gynaecologic (vulvectomy) and general (e.g. lipoma excision) surgery. Otherwise, controls did not suffer from pain or neurological diseases. CSF was always sampled before surgery had started. Neither patients nor controls had any ongoing or recent infection at the time of the sample collection.

After CSF was obtained, a small amount was used for leukocyte and erythrocyte count. Subsequently, CSF was centrifuged at 1790 x*g* for 5 min and the supernatant collected. Thereafter, supernatants were frozen in aliquots and stored at -80° C. The complete procedure was performed within 2 h. CSF containing >1000 erythrocytes/µL was excluded from the study. The medical ethics committee approved the study (MEC P01.098 and P03.027) and all subjects gave written informed consent.

Assays

Each test was performed on thawed aliquots, which were not re-frozen for further testing. If available, commercial assays were used and performed following the manufacturer's protocol. *IL-1β* and *IL-6* concentrations were measured using enzyme-linked immunosorbent assays (ELISA; respectively R&D Systems, Minneapolis, MN, USA, high sensitivity assay, and BioSource, Nivelles, Belgium, ultrasensitive assay). Furthermore, ELISA (R&D Systems) were used to determine CSF levels of chemokines *interferon-γ inducible protein-10 (IP-10)* and *RANTES (regulated upon activation, normal T-cell expressed and secreted)*. Complement *C1q* and *C3* levels were determined by radial immunodiffusion using monospecific polyclonal rabbit antisera. Concentrations of *mannose-binding lectin (MBL)*, involved in the lectin pathway of the complement system, were measured using ELISA as described in an earlier study.[17] *Soluble intercellular adhesion molecule-1 (sICAM-1)* as well as e*ndothelin-1 (ET-1)* levels were measured by ELISA (R&D Systems) and *nitric oxide (NO)* levels were measured using a colorimetric activity assay (R&D Systems). Lactoferrin concentrations were quantified with a human

lactoferrin-specific ELISA as described by Van Berkel et al.[18] using a microplate reader (BioTek Instruments, Winooski, VT, USA). Detection limits of these assays are reported in the table. *Hypocretin-1* was measured with a standardised radioimmunoassay with a detection limit of 100 pg/mL (Phoenix Pharmaceuticals Inc, Belmont, CA, USA); levels were measured in unextracted samples.

Statistics

Group differences were analysed using a Mann-Whitney *U* test (SPSS version 12.0). *P* values <0.05 were considered significant.

Results

Twenty female patients with a mean (range) age of 42 (22-57) years and mean (range) disease duration of 10 (2-20) years were included. They reported mean visual analogue scale pain scores of 8 on a scale of 0-10 (range 4-9) and most of them used analgesics as medication. Allodynia and hyperalgesia were present in 13/20, hypesthesia and hypalgesia in 13/20; spread of CRPS to other limbs occurred in 18/20 and spread of dystonia in 16/20 patients. Controls (13 females, 16 males) had a mean age of 59 (range 31-78 years).
Median leukocyte count was 1/µL (range 0-10) in patients and 0/µL (0-9) in controls; median erythrocyte count was 11/µL (0-587) in patients and 1/µL (0-501) in controls.

Control levels for IL-1β ranged from <0.125-0.83 pg/mL (median <0.125). This was not significantly different from that published earlier (Figure 5.1A). While in our earlier study a significant increase of IL-1β in CRPS patients was found, in the present study these differences between patients and controls were not obvious (*P*=0.10). In a similar fashion, IL-6 was analysed and again not significantly different between the two groups (Figure 5.1B). Elevated CSF levels of IL-1β (4.6 and 7.4 pg/mL) were measures in two patients and in none of the controls. CSF IL-6 was elevated in one other patient (5.9 pg/mL) and in none of the controls. The clinical features of these three patients were similar to those of the other patients.
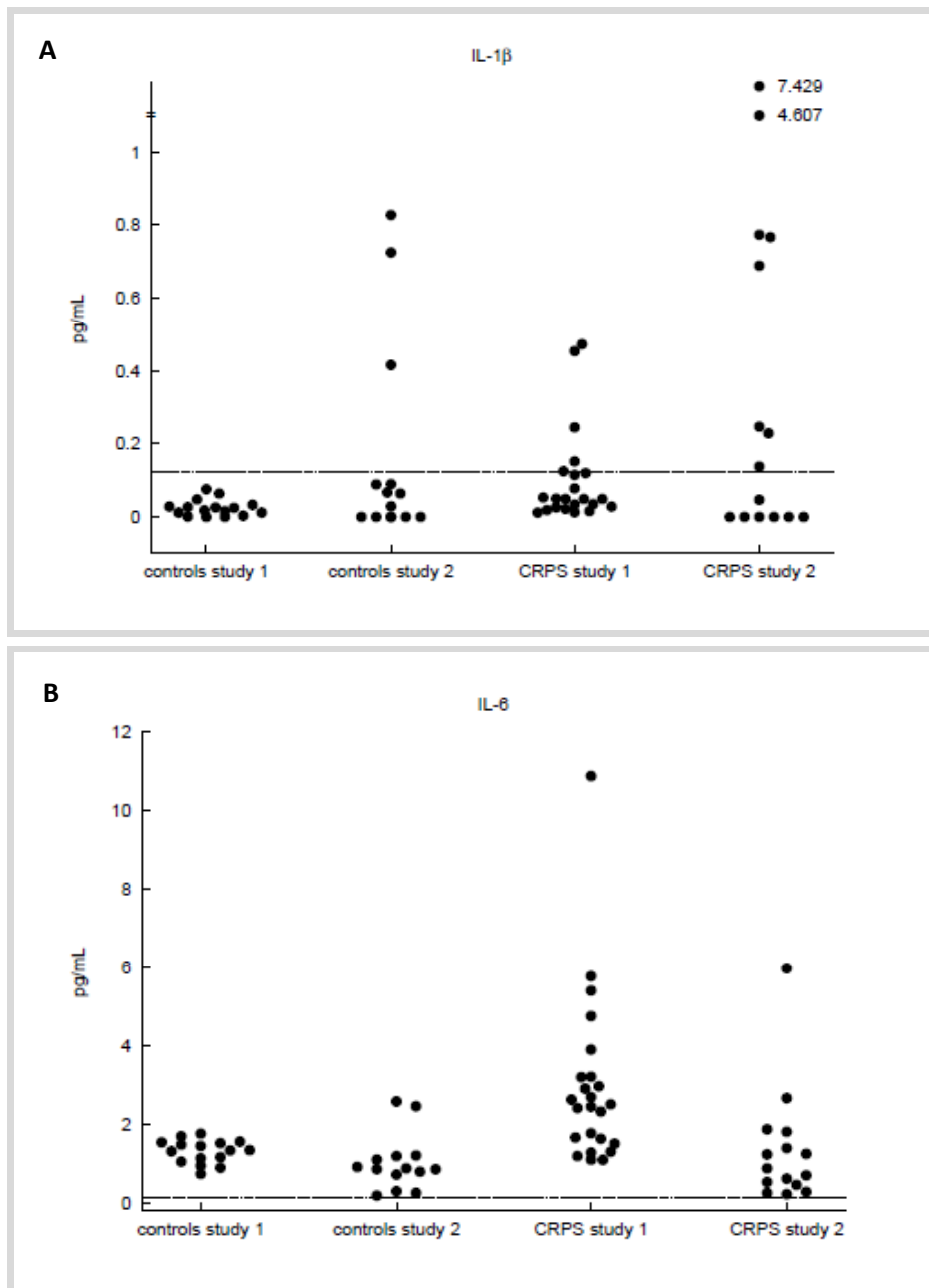
Figure 5.1. Dot plots of CSF levels of IL-1β (A) and IL-6 (B) in CRPS patients and controls in the current study (study 2). For comparison, levels measured in an earlier study[15] (study 1) are also shown. According to the ELISA manuals, inter-assay coefficient of variation is 8.2-19.2% for IL-1β and 6.7-10.0% for IL-6. The dotted lines mark the lowest detectable levels of the ELISAs.

Table 5.1. CSF assays in chronic CRPS patients and healthy controls

Assay	Measurement unit	Lowest detectable level	CRPS patients		Healthy controls		P value (Mann-Whitney U test)
			median	range (n)	median	range (n)	
IL-1β	pg/mL	0.125	0.14	<0.125 − 7.43 (n=15)	< 0.125	<0.125 − 0.83 (n=14)	0.10
IL-6	pg/mL	0.16	0.89	0.23 − 5.98 (n=15)	0.88	0.20 − 2.59 (n=14)	0.74
IP-10	pg/mL	7.8	70.7	29.4 − 385.6 (n=20)	140.2	41.1 − 293.9 (n=19)	0.08
RANTES	pg/mL	31.2	ND	ND (n=20)	ND	ND (n=19)	-
C3	ng/mL	10	2213	1511 − 3271 (n=15)	2534	1775 − 2953 (n=6)	0.59
C1q	ng/mL	0.1	184	132 − 293 (n=15)	231	148 − 322 (n=6)	0.16
MBL	ng/mL	0.1	ND	ND (n=15)	ND	ND (n=6)	-
sICAM-1	ng/mL	0.35	2.1	1.3 − 3.6 (n=15)	2.8	1.1 − 6.7 (n=14)	0.08
ET-1	pg/mL	0.064	<0.064	<0.064 − 0.3 (n=15)	<0.064	<0.064 − 0.3 (n=13)	0.71
NO	μmol/L	0.54	3.0	0 - 15.0 (n=15)	3.5	1.7 - 7.0 (n=13)	0.68
Lactoferrin	ng/mL	0.4	4.9	<0.4 − 15.8 (n=15)	3.8	0.6 − 8.8 (n=11)	0.62

ND = not detectable.

CSF levels of C3 and C1q in patients were not significantly different from controls. MBL was undetectable in both groups. sICAM-1, ET-1, NO and lactoferrin CSF levels were not significantly different between patients and controls (Table 5.1). Hypocretin-1 (orexin A)

levels were measured in 15 patients and were all in the normal range (197-391 pg/mL, median 346 pg/mL).

Discussion

The clinical spectrum of CRPS is heterogeneous and most likely reflects a mixture of symptoms and signs that are linked to differentially involved peripheral and central biological pathways. Identification of biomarkers that are related to particular biological pathways may provide clues to the pathogenesis of CRPS and perhaps contribute to improving therapeutic strategies. In this study we found no differences between patients and controls for any of the evaluated mediators of inflammation or hypocretin-1. Although patients and controls had a different age and gender distribution, these variables were not controlled because they were not related to the various mediators and could therefore not have acted as confounders.

Recently, in a collaborative study, we found elevated CSF levels of IL-1β and IL-6 in chronic cases of CRPS.[15] In the current study we could not confirm these findings, which may have at least two reasons. First, IL-1β and IL-6 levels in the controls of the present study were more heterogeneous (Figure 5.1A and B). This finding was unexpected because, contrary to the controls in the previous study, our controls did not have a history of neurological disease. Review of the medical records of the three controls with an elevated level of IL-1β (Figure 5.1A) revealed no explanation for these findings. Controls in the present study did not have neurological symptoms or signs and consequently are unlikely to have CSF abnormalities; therefore they better represent a normal population than those in the previous study. Second, inter-assay variation (IL-1β 8-19%; IL-6 7-10%) may have contributed to the different findings. Nonetheless, inspection of the IL-1β and IL-6 data of both studies shows that the majority of patients have values in the same range as controls.

We additionally evaluated the presence of several other inflammatory molecules in CSF of patients and controls, because of their presumed role in neuropathic pain.[7,12,15,19-21] Unfortunately, the results again revealed no difference in CSF levels between both groups. RANTES and MBL were not detectable in both patients and controls. Hence, our study does not support a role of a variety of inflammatory mediators in these patients, but absence of evidence is not evidence of absence.[22] Because our patients represent an

extreme dystonic phenotype with long disease duration, we cannot exclude a role of these inflammatory mediators in the early inflammatory phase where they may be a prerequisite to develop CRPS[23,24] For both neuropathic pain and dystonia aberrant neuroplasticity is considered to be the pivotal underlying mechanism.[25-28] Hence, a search for CSF biomarkers involved in molecular pathways that play a role in the ability of the CNS to re-organize its neural circuits may be more fruitful in chronic cases with the dystonic phenotype.[29]

Finally, neuroplasticity may involve a coordinated up and down-regulation of multiple protein complexes within the activated circuits.[30] As a consequence, in future research a more global proteomics-based approach may be more informative than studies that focus on changes in CSF levels of inflammatory proteins.

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