

BACKGROUND: The pathophysiology of complex regional pain syndrome type 1 (CRPS 1) is still a matter of debate. Peripheral afferent, efferent and central mechanisms are supposed. Based on clinical signs and symptoms (e.g. oedema, local temperature changes and chronic pain) local inflammation is suspected.

Aim: To determine the involvement of neuropeptides, cytokines and eicosanoids as locally formed mediators of inflammation.

Methods: In this study, nine patients with proven CRPS 1 were included. Disease activity and impairment was determined by means of a Visual Analogue Scale, the McGill Pain Questionnaire, the difference in volume and temperature between involved and uninvolved extremities, and the reduction in active range of motion of the involved extremity. Venous blood was sampled from and suction blisters made on the involved and uninvolved extremities for measurement of cytokines interleukin (IL)-6, IL-1 β and tumour necrosis factor- α (TNF- α), the neuropeptides NPY and CRGP, and prostaglandin E₂.

Results: The patients included in this study did have a moderate to serious disease activity and impairment. In plasma, no changes of mediators of inflammation were observed. In blister fluid, however, significantly higher levels of IL-6 and TNF- α in the involved extremity were observed in comparison with the uninvolved extremity.

Conclusions: This is the first time that involvement of mediators of inflammation in CRPS 1 has been so clearly and directly demonstrated. This observation opens new approaches for the successful use and development of immunosuppressives in CRPS 1.

Key words: Complex regional pain syndrome, Neurogenic inflammation, Pain, Oedema, Temperature, Neuropeptide, Cytokine, Eicosanoid

Evidence for local inflammation in complex regional pain syndrome type 1

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Introduction

Complex regional pain syndrome type 1 (CRPS1) is a disease characterized by spontaneous pain, allodynia and hyperalgesia. The symptoms are not limited to the region of a single peripheral nerve and are generally disproportional to the inciting event. There is (or has been) evidence of oedema, disturbed blood flow, or abnormal sudomotor activity in the affected limb. CRPS 1 diagnosis is excluded by the existence of conditions that would otherwise account for the degree of pain and dysfunction.¹ CRPS 1 is a major cause of disability, with a reported incidence of about 10% after various fractures, an incidence of 7–35% in Colles' fracture and, in 10–26% of cases, there is no precipitating factor.² The pathophysiology of CRPS 1 remains unclear. In our recently published review, we summarized studies in which peripheral afferent, efferent and also central mechanisms may play a role.³

Based on various clinical aspects of CRPS 1, such as oedema, local temperature changes and chronic pain, we assumed a neurogenic inflammation. The involvement of an activated immune system could implicate the subsequent release of neuropeptides, cytokines or eicosanoids, which, in turn, would lead to a complex interaction of primary and secondary generated mediators of inflammation. Until now, measurement of blood plasma levels at the site of CRPS 1 showed no change in pro-inflammatory and anti-inflammatory cytokines.⁴ However, this could be because all patients presented in the latter study had a mean disease history of 10 years. Furthermore, it remains questionable whether systemic cytokine levels with or without *in vitro* stimulation by lipopolysaccharide reflect local inflammation. Because there is evidence that substance P induces the release of interleukin-1 β (IL-1 β) and tumour necrosis factor- α (TNF- α) in human skin,⁵ studies should focus on local formation

Table 1. Demographic data

Sex (male/female)	1/8
Age (years)	50 (48–51)
Duration of CRPS 1 (months)	8 (4–12)
CRPS 1, hand/foot	7/2
CRPS 1, trauma (accident, fracture)/surgery	3/6
Smoking history	9
Medical history	1 patient, hypertension; 1 patient, NIDDM
Medication	
Corticosteroids and/or immunosuppressives	None
Non-steroidal anti inflammatory drugs	5 patients
Metoprolol	1 patient
Metformine, glibenclamide	1 patient
Blood chemistry	No deviations from normal values
Blood haematology	No deviations from normal values
Blood sedimentation (BSE) (mm/h)	9 (6–15)
White blood cells ($\times 10^9/l$)	5.7 (4.9–7.1)

Data are for the nine patients and are presented as median \pm interquartile range.

BSE; CRPS 1, complex regional pain syndrome type 1; NIDDM, Non-insulin-dependent diabetes mellitus.

of cytokines in CRPS 1. To test our hypothesis that mediators of inflammation play a role in CRPS 1, we applied the suction blister technique to determine the presence of pro-inflammatory cytokines in involved local tissue.

Subjects and methods

Patients

The study was approved by the medical ethical committee of the University Hospital Rotterdam (MECnr. 198.780/2001/24). All patients had to fulfil the CRPS 1 criteria as described by Bruhl *et al.*⁶ and all gave informed consent. Patients using corticosteroids or other immunosuppressives were excluded. In April and May 2001, we studied nine patients (for demographic data, see Table 1).

Registration of pain

The intensity of pain was assessed by means of a visual analogue scale (VAS)⁷ and the McGill Pain Questionnaire Dutch Language Version (MPQ-DLV).^{8–10} The VAS was recorded in millimetres and the MPQ-DLV score was measured by counting the total number of words chosen.

Temperature

Temperature was monitored by local infrared temperature measurement using a tympanic probe thermometer (First Temp Genius®; Sherwood Medical, Crawley, Sussex, UK).¹¹ Temperature was measured on the dorsal aspect of the hand or foot in five points on a standard matrix. Differences in temperature were calculated as the mean difference of these five points of measurement between the involved and uninvolved sides.

Oedema

Difference in volume between the involved and uninvolved extremity was assessed with a volumeter, which measures the amount of water displaced by the immersion of a body part.^{12,13} For hand volume measurements, we used a container with a volume of 15 l and a crosswise bar 10 cm from the bottom. The web between digits 2 and 3 was positioned over the horizontal bar. For foot volume measurements, we used a container with a volume of 40 l. The foot was placed as flat as possible on the bottom of the container, the overflow was collected and the volume determined with a weighing balance (accuracy of 1 g).

Mobility

The active range of motion (AROM) is defined as the arc of motion with muscle power to achieve the motion of a joint. In the upper extremity, the AROM was measured for the dorsal/palmar flexion in the wrist, and for the flexion/extension in the metacarpophalangeal and proximal interphalangeal joints of the two most restricted digits.¹⁴ In the lower extremity, the AROM was measured for the flexion/extension in the ankle. The position of the patient and the method of measurement were standardized for each joint, conforming to the American Society of Hand Therapists clinical assessment recommendations.¹⁵ The extent of reduced AROM was determined by intra-individual comparison with the total AROM. The total AROM was measured in the involved and uninvolved extremity. The AROM on the impaired side (multiplied by 100) was divided by the AROM on the uninvolved side; this resulted in a percentage. Each joint was given points for the percentage of normal mobility: 1 point, $\geq 95\%$; 2 points, 94–85%; 3 points, 84–65%; 4 points, 64–25%; and 5 points, $< 25\%$.

Table 2. Signs and symptoms of impairment

Visual Analogue Scale (0–100 mm, no pain–most pain)	49 (30–52)
McGill Pain Questionnaire (number of counted words that describe pain)	11 (11–12)
Difference in temperature between involved and uninvolved sides (ΔT , (C)	-0.24 (-0.02 to -0.9)
Difference in volume between involved and uninvolved sides (ΔV , % volume of uninvolved extremity)	2.9 (1.4–4.5)
Active range of motion (sum of numbers that express the percentage of normal mobility extremity; 5–55, normal mobility–most abnormal)	18 (11–20)

Data are for the nine patients and are presented as median \pm interquartile range.

normal mobility.¹⁶ The sum of points of the measured joints was calculated.

Plasma collection.

Venous blood was obtained from both the involved and uninvolved extremities for determination of haematological and blood chemistry parameters. In the upper extremity, blood was collected via a vena cubita; in the lower extremity, via the vena femoralis. For measurement of plasma mediators of inflammation, 10 ml of blood anti-coagulated with 0.15% ethylenediamine tetraacetic acid was drawn into endotoxin-free vacutainers (Vacutainer Systems; Becton-Dickinson, Plymouth, UK) and centrifuged for 10 min at 20°C and $2800 \times g_{\max}$. Thereafter, the plasma was stored at -80°C until analysis.

Blisters

Blisters were induced using the suction technique.^{17,18} The blisters were formed on both the involved and uninvolved extremity: on the upper extremity on the dorsal side of the hand and the flexor side of the forearm, and on the lower extremity on the dorsal side of the foot and the frontal or lateral side of the lower leg. We initially applied a negative pressure of 300 mmHg, which was reduced after 15 min to 250 mmHg and again, 15 min later, reduced to 200 mmHg. This negative pressure was maintained for 2–2.5 h until seven blisters per site (each 6 mm in diameter) had emerged. This procedure produced approximately 300 μ l blister fluid per site by puncture of the blister. All samples were stored at -80°C until analysis.

Laboratory assays

Sample preparation

Undiluted plasma samples (100 μ l) were assayed for cytokines IL-1 β , interleukin-6 (IL-6) and TNF- α without prior sample preparation. Blister fluids were twofold to fourfold diluted in assay buffer for the direct measurement of cytokines. For the determination of neuropeptides CGRP and NPY, 400 μ l of undiluted plasma and diluted blister fluid was applied to an

Oasis® HLB extraction cartridge (Waters Ass., Etten-Leur, The Netherlands). According to the assay protocol, all samples were acidified with 1% trifluoroacetic acid (TFA) and eluted with 60% acetonitrile in 1% TFA in 39% distilled water. The eluant was evaporated to dryness in a centrifugal concentrator (SpeedVac, Savant, Switzerland) and the dried extract reconstituted with peptide assay buffer. In parallel, sample elution of prostaglandin E₂ from the Oasis® HLB column was completed by the subsequent elution of 100% of methanol. Evaporation and reconstitution was similar to the procedure followed for neuropeptides.

Immunoassays

For the determination of cytokines, assays were performed following the manufacturer's protocol (PeliKine-compact™ human ELISA kits; CLB, Amsterdam, The Netherlands). The standard curve ranges and detection limits, respectively, were: for IL-1 β , 0–300 pg/ml and 0.2 pg/ml; for IL-6, 0–450 pg/ml and 0.3 pg/ml; and for TNF- α , 0–1000 pg/ml and 1 pg/ml. The peptide enzyme immunoassays used for the determination of CGRP and NPY were EIAH-6006 and EIAH-7180, respectively (Peninsula Laboratories Inc., Belmont, CA, USA). Standard curve ranges from 0 to 10 ng/ml were included. The minimal detectable concentration was 0.06 ng/ml.

Prostaglandin E₂ was determined by enzyme immunoassay (Biotrak EIA, RPN 222; Pharmacia Biotech, Amersham, UK). The standard curve range was 0 to 6.4 ng/ml.

All assays were performed in 96-well microtitre plates. The absorbance per well was measured at 450 nm with a Medgenix EASIA reader (Fleurus, Belgium). Sample concentrations were calculated using the appropriate standard calibration lines and the Softmax® software of the reader.

Statistical analysis

First, data were analysed for linearity. If non-linear, frequencies were described as median and interquartile ranges. Correlation was measured with the Spearman' test, and the difference in parameters was tested with the Wilcoxon rank sum test. Significance was accepted with $p < 0.05$.

Table 3. Mediators of inflammation, and levels in plasma and blister fluid in the involved and uninvolved extremities

	Plasma		Blister	
	Involved side	Uninvolved side	Involved side	Uninvolved side
IL-6 (pg/ml)	0.8 (0.6–1.4)	0.8 (0.6–1.4)	54 (19–68)*	17 (4.9–20)
IL-1 β (pg/ml)	1.5 (0.4–28)	1.1 (0.5–24)	1.7 (1.0–1.8)	1.7 (1.5–1.9)
TNF- α (pg/ml)	4.6 (2.2–17.6)	2.3 (0.8–14.6)	18 (11–37)*	7 (4.7–8.2)
NPY (ng/ml)	0.4 (0.3–0.5)	0.4 (0.3–0.4)	1.7 (1.1–2.3)	1.9 (1.5–2.3)
CGRP (ng/ml)	0.2 (0.2–0.3)	0.4 (0.2–0.4)	2.8 (1.7–2.9)	3.4 (1.8–4)
PGE ₂ (ng/ml)	6.1 (5.6–7)	5.8 (5.5–6.6)	23 (14–29)	27 (21–28)

Data are for the nine patients and are presented as median \pm interquartile range.

IL, Interleukin; TNF- α , tumor necrosis factor- α ; PGE₂, prostaglandin E₂.

*Wilcoxon rank sum test, $p < 0.05$.

Results

The clinical signs and symptoms of impairment from the patients are described in terms of median and interquartile VAS, number of words counted in the McGill Pain Questionnaire, differences in temperature, differences in percentage volume and AROM classified as a number. The results are presented in Table 2.

Data on cytokines, neuropeptides and prostaglandin E₂ levels in plasma and blister fluid in the involved and uninvolved extremities are presented in Table 3.

There was no correlation between the clinical signs and symptoms of impairment and the mediators of inflammation.

There was a significant difference ($p < 0.05$, Wilcoxon rank sum test) in IL-6 and TNF- α levels between blisters on the involved and uninvolved extremities. There was no difference in IL-1 β , NPY, CGRP and prostaglandin E₂ levels between blisters on the involved and uninvolved extremities. There is no significant difference between any measured value in plasma from involved and uninvolved extremities (see Table 3).

Discussion

To our knowledge, this is the first time that increased local levels of the pro-inflammatory cytokines IL-6 and TNF- α have been detected locally in CRPS 1.

Until now, the involvement of a neurogenic inflammation remains debatable. Results of a large prospective study suggested that the early signs and symptoms of CRPS 1 resembled regional inflammation,² and a scintigraphic study on CRPS 1 demonstrated vascular leakage of macromolecules.¹⁹ These latter results also suggest the presence of local inflammation. A ³¹P nuclear resonance spectroscopy study showed impairment of high-energy phosphate metabolism in muscles of patients with CRPS 1; this may have been caused by cellular hypoxia or diminished oxygen utilization, which is classically seen in inflammation.²⁰ Substance P and CRPG are neurope-

tides that play an important role in oedema formation and inflammation.²¹ In blood sampled from patients with CRPS 1, bradykinin, NPY, CGRP and vasoactive intestinal peptide were considerably increased compared with healthy volunteers, whereas substance P, neurokinin A and B were unchanged.²² Infusion of substance P in the CRPS 1 extremity has been shown to potentiate signs and symptoms.²³ Depletion of substance P from primary afferent neurons by topical application of capsaicin effectively diminished signs and symptoms in CRPS 1 and comparable diseases.^{24,25} In contrast, another study showed significantly lower levels of NPY in blood drawn from CRPS 1 extremities compared with the uninvolved sides.^{26,27} CGRP, substance P and NPY were unchanged in samples of hyperalgesic skin from dorsal hand and foot.²⁸

In our study, there was no paired increase in local neuropeptide formation, as measured by NPY and CGRP. Although substance P can induce the release of IL-1 α and TNF- α in human skin,⁵ our findings of increased pro-inflammatory IL-6 and TNF- α in blister fluids may not necessarily be the direct result of a primary neurogenic inflammation. In view of the lack of a clear contribution of neuropeptides, in our patients a late phase reaction could be considered in which not only mononuclear cells, but also polymorphonuclear cells could be involved. Therefore, a wide range of immunocompetent cells, such as activated T lymphocytes, monocytes and macrophages, and skin resident cells, such as keratinocytes, fibroblasts, endothelial cells and mast cells, could contribute to this complex interplay of mediators of inflammation formed at the site of the CRPS 1. In our patients, the levels of cytokines found in blister fluid were comparable with those seen in psoriasis where cytokines clearly correlate with psoriasis severity.^{29,30} Capsaicin-treated normal skin showed minor, but not significant, effects on the diminished release of inflammatory mediators.³¹ On the contrary, pharmacological intervention by betamethasone or salicylic acid showed a clear disease improvement and significant decreases in cytokine levels in blister fluid.³²

Generally, involvement of inflammatory mediators in a disease may be considered when generation at the site of injury can be confirmed. Furthermore, local injection of these mediators should provoke or increase clinical signs and symptoms. Treatment with specific antagonists or synthase inhibitors of inflammatory mediators that are involved should have beneficial effects on disease activity.

Until now, evidence that inflammatory mediators are involved has only indirectly been shown by injection of substance P, which results in a deterioration of the signs and symptoms.

Our data indicate, in a direct way, that inflammation plays an important role in CRPS 1. The challenge now is to investigate the entire time-dependent cascade of inflammatory mediators that could be involved in CRPS 1 and then develop antagonists or synthesis inhibitors that are beneficial in the treatment of this disabling disease.

ACKNOWLEDGEMENTS. The authors thank Wilma de Bruijn and Tanja Hamm, Nurse Practitioners of the Pain Treatment Centre, for their support in data management; Dr Ilse Beckmann, Department of Obstetrics and Gynaecology, for laboratory facilities; Dr Ingrid M. Garrelds, Department of Pharmacology, for assistance in enzyme-linked immunosorbent assay measurements; and Laraine Visser-Isles for English-language editing. This study was sponsored by the Algesiological Institute Foundation of the University Hospital Rotterdam.

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Accepted 30 November 2001.