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Lymphocyte Subsets and the Role of Th1/Th2 Balance in Stressed Chronic Pain Patients

Ines Kaufmann Christoph Eisner Peter Richter Volker Huge Antje Beyer
Alexander Chouker Gustav Schelling Manfred Thiel

Department of Anesthesiology, Klinikum Grosshadern, Ludwig Maximilians University, Munich, Germany

Key Words
Chronic pain · Stress · Complex regional pain syndrome · Fibromyalgia · Lymphocyte · Intracellular cytokine

Abstract
Background: The complex regional pain syndrome (CRPS) and fibromyalgia (FM) are chronic pain syndromes occurring in highly stressed individuals. Despite the known connection between the nervous system and immune cells, information on distribution of lymphocyte subsets under stress and pain conditions is limited. ***Methods:*** We performed a comparative study in 15 patients with CRPS type I, 22 patients with FM and 37 age- and sex-matched healthy controls and investigated the influence of pain and stress on lymphocyte number, subpopulations and the Th1/Th2 cytokine ratio in T lymphocytes. ***Results:*** Lymphocyte numbers did not differ between groups. Quantitative analyses of lymphocyte subpopulations showed a significant reduction of cytotoxic CD8+ lymphocytes in both CRPS ($p < 0.01$) and FM ($p < 0.05$) patients as compared with healthy controls. Additionally, CRPS patients were characterized by a lower percentage of IL-2-producing T cell subpopulations reflecting a diminished Th1 response in contrast to no changes in the Th2 cytokine profile. ***Conclusions:*** Future studies are warranted to answer whether such immunological changes play a pathogenetic role in CRPS and FM or merely reflect the consequences of a pain-induced neurohumoral stress response, and whether they contribute to immunosuppression in stressed chronic pain patients.

Introduction

Complex regional pain syndrome (CRPS) and fibromyalgia (FM) are chronic pain syndromes which are often preceded by extremely stressful experiences and other major adverse life events [1–7]. CRPS as well as FM have been classified as so-called central sensitization syndromes [8, 9] involving hyperexcitement of the central neurons through various synaptic activities and neurotransmitters leading to dysfunction of brain areas which regulate pain perception and stress responses.

Both disorders, however, differ in type and localization of pain. CRPS is a localized neuropathic pain syndrome usually occurring after a limb trauma without (type I) or with (type II) an injury of a peripheral nerve. Pain in CRPS patients is not proportionally related to the initial injury and does not follow a nerve innervation pattern. It is associated with autonomic dysregulation, swelling, motor and trophic signs [8]. FM is defined according to the classification criteria of the American College of Rheumatology which require the presence of self-report-

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Manfred Thiel, MD, PhD
Department of Anesthesiology, Klinikum Grosshadern
Ludwig Maximilians University
DE–81377 Munich (Germany)
Tel. +49 89 7095 6426, Fax +49 89 7095 8886, E-Mail mthiel@med.uni-muenchen.de

Table 1. Baseline data of healthy volunteers and chronic pain patients (mean ± SEM)

	Healthy volunteers (n = 37)	CRPS patients (n = 15)	FM patients (n = 22)
Age, years	52.2 ± 2.1	53.9 ± 4.1	53.1 ± 2.1
Gender (female/male)	32/5	15/0	17/5
Inflammation parameters			
Leukocytes, 10^3 cells/μl	7.4 ± 0.6	7.1 ± 0.5	7.6 ± 0.5
Leukocyte subpopulations, %			
Neutrophils	59.2 ± 1.2	60.9 ± 2.5	60.6 ± 1.9
Lymphocytes	23.4 ± 1.0	20.7 ± 1.8	20.7 ± 1.3
Monocytes	6.6 ± 0.3	5.9 ± 0.3	6.1 ± 0.4
Eosinophils	2.0 ± 0.2	3.0 ± 0.5	3.1 ± 0.4

ed chronic widespread pain and 11 or more anatomically defined tender points upon palpation [10]. FM patients exhibit an exaggerated pain response upon digital pressure and are hypersensitive to heat, cold, electric stimuli as well as ischemia in many areas of the human body. Initial physical trauma may be involved in the pathogenesis of FM [9]; however, traumatic events are observed much less frequently than in CRPS.

Despite the known connection between the nervous system and immune cells [11–16], information on distribution of lymphocyte subsets under stress and pain conditions is limited [17]. In rodents, acute stress reduces CD4+ cells [18], and chronic social stress induces a decrease in CD4+ and CD8+ lymphocytes [19]. Acute human stress is known to increase CD8+ lymphocytes [20–22] as well as to decrease CD4+ cells and the ratio of CD4+/CD8+ cells [23]. In contrast, in prolonged human stress responses such as posttraumatic stress disorder, lymphocyte subsets are not deranged [24]. With respect to chronic pain, information on alterations of lymphocyte subsets is inconsistent; cluster headache and low back pain lower the CD4+ subpopulation [25], while migraine is characterized by decreased levels of CD8+ T lymphocytes [26].

T cells may be functionally defined by their cytokine profile. With regard to the CD4+ T helper (Th) cells, an adequate host immune response against infection is largely dependent on the activation of their two functionally distinct subsets, Th1 and Th2 cells [12, 13]. The Th1/Th2 balance is a prerequisite for the full functionality of immunity. Th1 cells secrete more interferon-γ and interleukin (IL)-2 and stimulate (cellular) type 1 immunity. Th1 responses promote production of opsonizing antibodies (e.g., IgG1) and induce cellular cytotoxicity by activation of macrophages with powerful phagocytic activity. Th2 lymphocytes produce more IL-4 and IL-10 and induce humoral type 2 immunity with promotion of IgE and IgG4 production, thereby fine-tuning B cell antibody production. Autoimmunity induces an imbalance between the Th1 and Th2 response with a Th1 dominance. Trauma and cancer-bearing states are characterized by an increase in the Th2 cytokine production. To our knowledge, there are no studies in the literature examining Th1/Th2 balance in CRPS or FM, respectively.

Therefore, this study intended to test the hypothesis that lymphocyte subsets and, especially, percentages of T cell subsets producing proinflammatory and anti-inflammatory cytokines are different in CRPS and FM patients and related to the levels of self-perceived emotional stress.

Materials and Methods

The study was approved by the local ethics committee (protocol No. 324/02) and conducted in accordance with the guidelines of the declaration of Helsinki and its amendment in Tokyo, 1975, and Hong Kong, 1989. Data protection met the standards set by the German law.

Patients, Definitions and Entry Criteria

Fifteen CRPS type I patients classified according to the criteria of the International Association for the Study of Pain (IASP) [27] and Bruehl et al. [28] and 22 patients with FM defined according to the American College of Rheumatology [10] admitted to the Interdisciplinary Pain Clinic of the University Clinic of Munich, Germany were included in this study (table 1). FM patients had undergone a thorough evaluation by experienced rheumatologists in the setting of a university-affiliated center during which the diagnosis of FM was confirmed; other inflammatory or noninflammatory conditions mimicking FM were systematically excluded. Exclusion criteria were pregnancy, infection or other inflammation disorders, cancer, concurrent muscle or joint diseases, motor trauma or immunosuppressive medication (e.g., glucocorticoids). Patients with comorbid major depressive disorders or anxiety disorders were excluded. CRPS and FM patients abstained from intake of their current medication (tramadol, amitriptyline, diclofenac, ibuprofen, metamizole, γ-aminobutyric acid, or acetaminophen) for a period of 24 h. Age- and sex-matched healthy subjects (n = 37) served as controls.

Quantification of Pain and Stress Severity in CRPS and FM Patients

The mean pain score at the time of the assessment was determined by using a visual analogue scale with a range of 0–10 (VAS). The intensity of emotional stress symptoms were measured using the German Version of the Post-traumatic Stress Symptom 10-Questionnaire (PTSS-10). This instrument records the presence and intensity of 10 common stress symptoms: (1) sleep distur-

Table 2. Severity of pain and stress (mean ± SEM) in healthy subjects and chronic pain patients

	Healthy volunteers (n = 37)	CRPS patients (n = 15)	FM patients (n = 22)
VAS	–	4.0 ± 0.9	6.4 ± 1.4
PTSS-10	21.3 ± 7.0	29.4 ± 3.1*	46.7 ± 3.5**

* $p < 0.05$; ** $p < 0.0001$.

bance, (2) nightmares, (3) depression, (4) hyperalertness, (5) withdrawal (emotional numbing and inability to care for others), (6) generalized irritability, (7) frequent changes in mood, (8) guilt, (9) fear and avoidance reactions when reminded of hospitals and diseases and (10) increased muscle tension. Patients rate their symptoms, using a scale from 1 (never) to 7 (always) [29, 30].

Biochemical Measurements

Reagents. Fluorescence-activated cell sorting (FACS) permeabilizing solution, SimulTest IMK plus® kit, bovine serum albumin (BSA) and monoclonal antibodies [peridinin-chlorophyll-protein complex (PerCP)-conjugated anti-CD3, anti-CD4 and anti-CD8; fluorescein isothiocyanate (FITC)-conjugated anti-human IL-2 and anti-human interferon-γ; R-phycoerythrin (PE)-conjugated anti-human-IL-4 and anti-human-IL-10] were obtained from Becton Dickinson (Heidelberg, Germany). Hanks' buffered salt solution (HBSS), phosphate-buffered saline (PBS) and 1% paraformaldehyde were manufactured from soluble ingredients by the hospital's own pharmacy. RPMI 1640, ionomycin and brefeldin A were purchased from Sigma-Aldrich (Taufkirchen, Germany).

Lymphocyte Populations. For immunophenotyping of lymphocyte populations a SimulTest IMK plus was used. Venous heparinized blood samples (heparin activity 10 IU/ml) were incubated with each of six antibody reagents from the SimulTest IMK plus kit for 20 min at room temperature. Subsequently, the stained samples were treated with lysing solution to eliminate erythrocytes for 10 min, washed with cold HBSS solution and put on ice until flow-cytometric analysis.

Intracellular Cytokine Synthesis. Heparinized blood (heparin activity 10 IU/ml) was diluted with RPMI 1640 (ratio 1:1), and incubated for 4 h at 37°C, 7% CO_2, with 7.5 nmol/l phorbol myristate acetate and 1.34 nmol/ml ionomycin. After 1 h of incubation, 1.4 μmol/l brefeldin A was added. Subsequently, cells were incubated for 20 min at room temperature with PerCP-labeled monoclonal antibodies with specificity for surface antigens (CD3, CD4, CD8). Afterwards, erythrocytes were lysed with FACS lysing solution for 60 min. After washing of cells with a buffer (PBS, BSA 0.5%, NaN_3 0.1%), they were permeabilized by incubation with permeabilizing solution for 10 min and stained with monoclonal antibodies against cytokines (FITC-marked: anti-interferon-γ, anti-IL-2; PE-marked: anti-IL-4, anti-IL-10). After a 2-fold washing, cells were fixed with paraformaldehyde 1% and put on ice until analysis by flow cytometry as described below. However, for each sample in the assay an unstimulated and activated intracellular isotype staining control was run.

Control of Changes in Intracellular Cytokine Production for Changes in a Lymphocyte Subpopulation of Interest. Since an immunomodulating factor, like a stress hormone, might not only affect the capacity of a given lymphocyte subset to produce a regulatory cytokine, but also the blood concentration of the respective lymphocyte subset, we determined the ratio between the observed change in the cytokine-producing cells and the change of the lymphocyte subset. Specifically, we looked at whether a decrease in the IL-2-producing CD8+ cells was as strong as the reduction in the number of CD8+ lymphocytes.

Flow Cytometry. Samples were quantitatively analyzed by the use of a Becton Dickinson FACScan (Becton Dickinson, San Jose, Calif., USA) equipped with an argon laser emitting light at 488 nm. During the study period, the instrument was intermittently calibrated and standardized by Calibrite beads (Becton Dickinson, Erembodegen-Aalst, Belgium).

Lymphocyte subpopulations were obtained following gating of lymphocytes on SSC/FSC dot plots and referred to the SimulSET Software User's Guide.

For intracellular cytokine analyses, a total of 10,000 events was collected. Lymphocytes were identified by means of their light SSC/FSC characteristics. The dual staining of samples allowed for discrimination of different CD-positive lymphocyte T cell subpopulations (CD3, CD4, CD8) by live gating on the SSC/FL3 dot plots followed by analyses of the percentage of cells producing IL-2 and interferon-γ and on the SSC/FL1 dot plots or IL-4 and IL-10 on the SSC/FL2 dot plots (fig. 1). The specific response of cells was obtained by subtraction of the percentage of the isotype-corrected response of the unstimulated sample from the percentage of the isotype-corrected activated sample.

Statistics

Data analyses were performed with commercially available software (SPSS 13.0; SPSS, Chicago, Ill., USA). Normal distribution of data was tested by the Kolmogorov-Smirnov test. Comparisons between independent groups were performed by one-way ANOVA followed by a post hoc correction for multiple comparisons using the Bonferroni method. Correlations were analyzed using Pearson's correlation coefficient for normally distributed data. Data are considered to be significantly different at $p < 0.05$. Results are expressed as mean ± SEM.

Results

Baseline Characteristics

There were no significant differences between healthy volunteers, CRPS and FM patients for baseline characteristics, leukocyte numbers and their subpopulations (table 1).

Quantification of Disease Severity in Chronic Pain Patients

Both CRPS and FM patients had evidence of severe chronic pain and stress as shown by scores on the VAS and the PTSS-10 instrument (table 2). PTSS-10 stress symptom scores in both CRPS and FM patients were

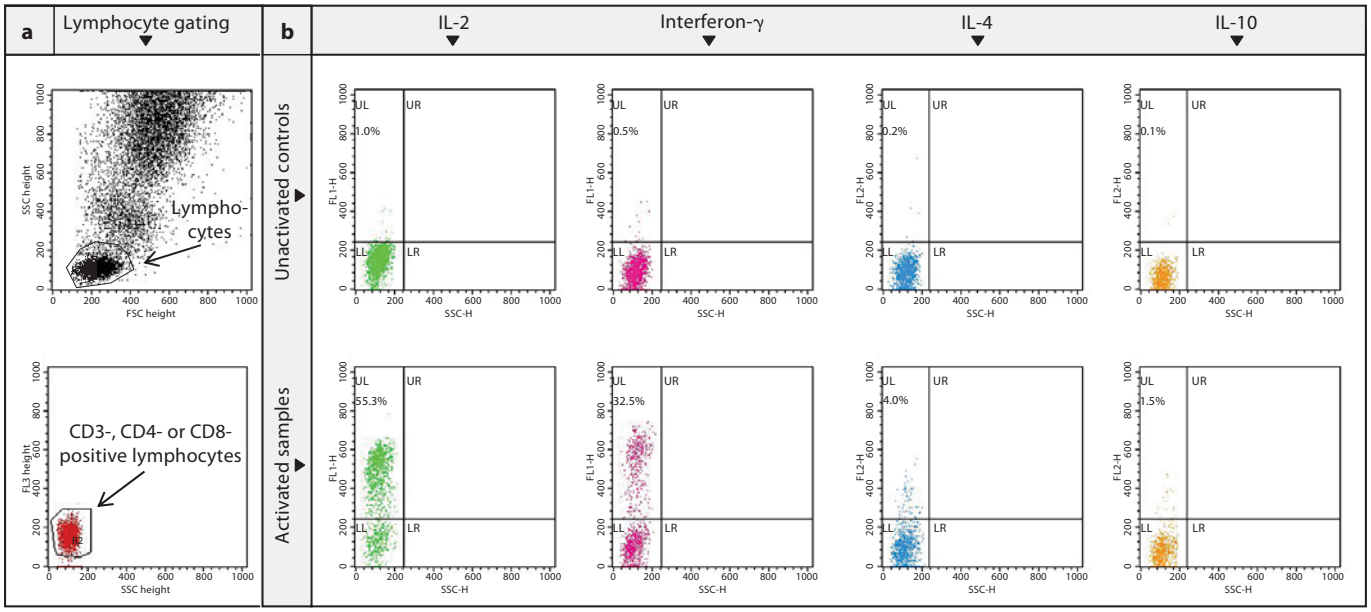

Fig. 1. a The FSC/SSC dot plot shows the gating of lymphocytes. The live gate in SSC/FL-3 dot plot identifies CD3+, CD4+ or CD8+ lymphocytes depending on the PerCP-labeled surface antibody used (anti-CD3, anti-CD4, anti-CD8). **b** Dot plots show IL-2-, interferon-γ-, IL-4- and IL-10-positive T lymphocyte subpopulations (as immunophenotyped by the respective cell surface marker gated in **a** (CD3+, CD4+ or CD8+ cells). Comparison was made between cells in a resting state or after activation by a combination of phorbol myristate acetate (7.5 nmol/l) and ionomycin (1.34 nmol/ml).

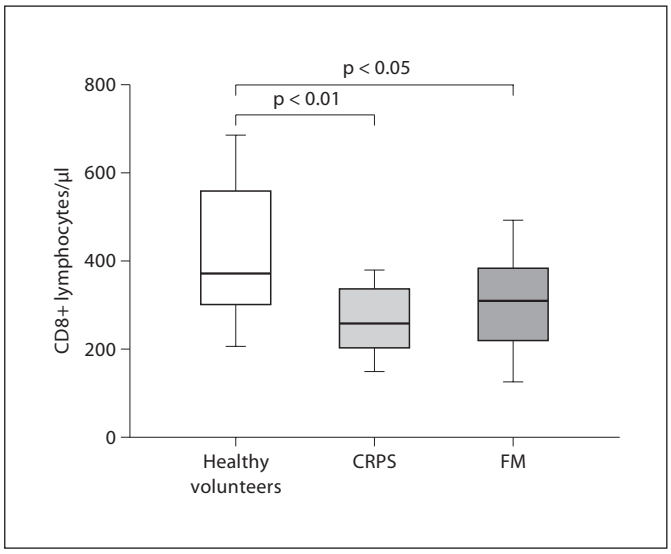

Fig. 2. Plots of the absolute numbers of CD8+ lymphocytes per microliter in healthy volunteers (n = 37), patients with CRPS (n = 15) and FM (n = 22). The top of the box represents the 75th percentile and the bottom the 25th percentile. The horizontal line in the box represents the median value, and the lines above and below the box are whiskers to the 95th and 5th percentiles, respectively.

massively increased and significantly higher than values for healthy individuals (CRPS: $p < 0.05$; FM: $p < 0.0001$).

Lymphocyte Subpopulations

The number of cytotoxic T cells (CD8+) was significantly diminished in patients with CRPS ($p < 0.01$) and FM ($p < 0.05$) as compared with healthy volunteers (fig. 2). However, the CD4/CD8 ratio increased only in CRPS patients to a significant extent (healthy volunteers: 2.2 ± 0.1; CRPS patients: 2.8 ± 0.2; $p < 0.01$).

The proportion of CD8+ subset correlated negatively with the PTSS-10 score in FM patients ($r = -0.55$; $p < 0.05$; fig. 3), but not in CRPS patients. No correlation was found for CD8+ subsets and VAS scores in both CRPS and FM.

With regard to the numbers of B cells (CD19+), T cells (CD3+), helper T cells (CD4+), natural killer cell population (CD3–CD16+CD56+) and CD3+CD16+CD56+ lymphocytes, no differences were observed between chronic pain patients and healthy volunteers, respectively (data not shown).

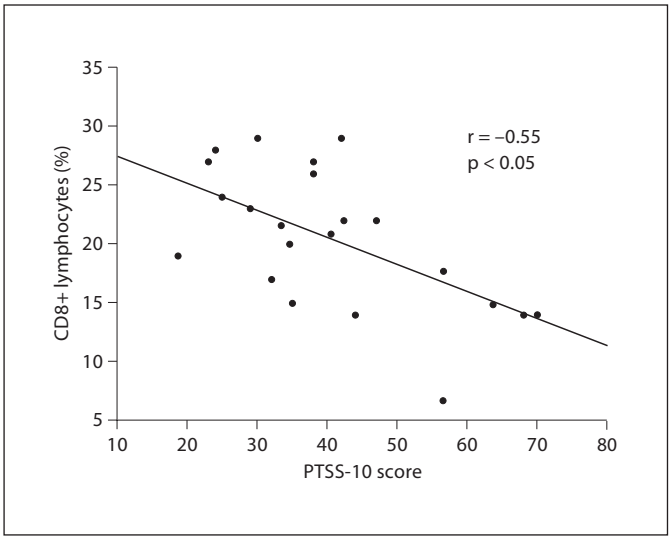

Fig. 3. Correlation between PTSS-10 scores and the proportion of CD8+ lymphocytes in FM patients. Using Pearson's correlation, the correlation coefficient, Pearson's r, also called linear or product-moment correlation, was determined.

Intracellular Cytokine Production

With regard to the percentages of IL-2-producing CD3+, CD4+ and CD8+ lymphocytes (fig. 4), lower values were obtained in CRPS patients (CD3+, $p < 0.05$; CD4+, $p < 0.05$; CD8+, $p < 0.01$), but not in FM patients (CD3+, $p = 0.13$; CD4+, $p = 0.36$; CD8+, $p = 0.12$). When the decrease in IL-2-producing CD8+ cells was controlled for the decrease in the CD8+ cells, the decline of IL-2 positive cells was 1.6-fold stronger than that of CD8+ cells.

When compared to healthy volunteers, no significant differences were observed for percentages of CD3+, CD4+ and CD8+ lymphocytes of CRPS and FM patients producing proinflammatory interferon-γ. However, CRPS patients showed enhanced anti-inflammatory IL-4 and immunoregulatory IL-10 production (IL-4: CD3+, $p = 0.45$; CD4+, $p = 0.37$; CD8+, $p = 0.45$; IL-10: CD3+, $p = 0.05$; CD4+, $p = 0.17$; CD8+, $p = 0.47$) without reaching significance (table 3). Similarly, FM patients were also characterized by a tendency of increased IL-4 (CD3+, $p = 0.05$; CD4+, $p = 0.05$; CD8+, $p = 0.12$) and IL-10 production (CD3+, $p = 0.06$; CD4+, $p = 0.45$; CD8+, $p = 0.97$).

The Th1/Th2 ratios calculated on the basis of the percentages or the absolute number of Th cells producing cytokines were significantly different between healthy volunteers and CRPS patients (both $p < 0.05$).

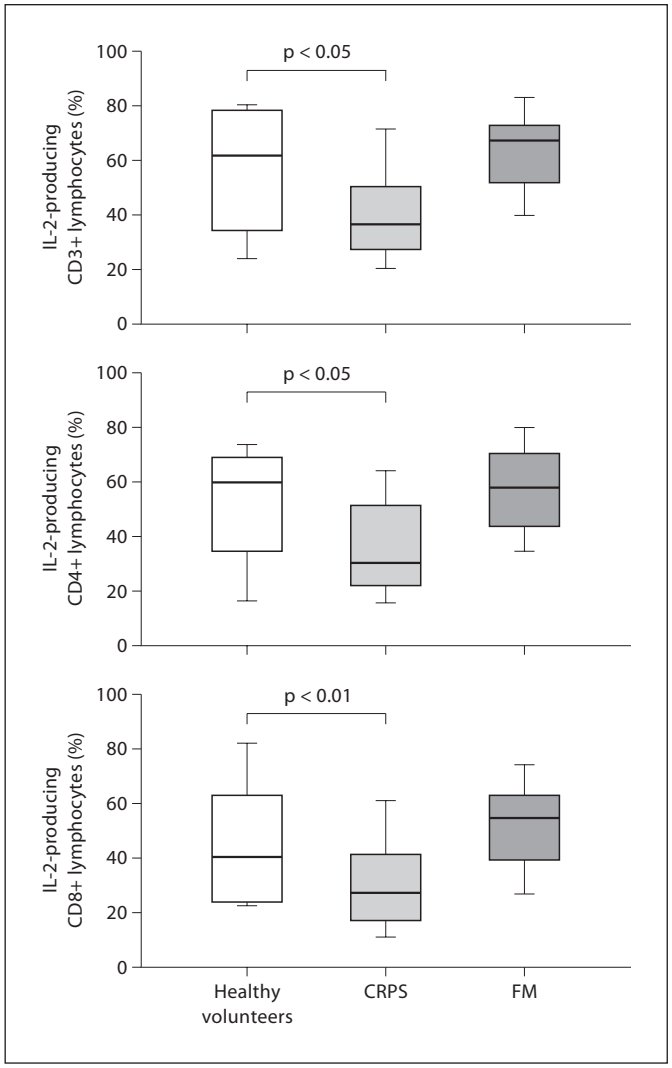

Fig. 4. Plots of the percentages of IL-2-producing T lymphocytes (CD3+), Th cells (CD4+) and cytotoxic T cells (CD8+) in healthy volunteers (n = 37), patients with CRPS (n = 15) and FM (n = 22). The top of the box represents the 75th percentile and the bottom the 25th percentile. The horizontal line in the box represents the median value, and the lines above and below the box are whiskers to the 95th and 5th percentiles, respectively.

Discussion

This study compared lymphocyte numbers, subpopulations and their intracellular cytokine production in patients with CRPS, FM and healthy controls. CRPS and FM patients were selected because these patients suffer from chronic pain syndromes accompanied by a high degree of emotional stress. Major findings of this study are: (1) total lymphocyte numbers did not differ between

Table 3. Intracellular cytokine production by T lymphocyte subpopulations (mean ± SEM)

	Healthy volunteers (n = 37)	CRPS patients (n = 15)	FM patients (n = 22)
Interferon-γ-producing lymphocytes, %			
CD3+	32.1 ± 3.3	34.8 ± 3.5	37.7 ± 2.7
CD4+	27.4 ± 5.5	33.3 ± 4.7	35.0 ± 3.8
CD8+	52.0 ± 4.8	52.8 ± 3.7	62.6 ± 3.9
IL-4-producing lymphocytes, %			
CD3+	3.2 ± 0.5	3.8 ± 0.5	5.2 ± 0.5
CD4+	2.8 ± 0.9	3.8 ± 0.8	4.8 ± 0.7
CD8+	6.5 ± 1.3	5.3 ± 1.1	6.8 ± 1.0
IL-10-producing lymphocytes, %			
CD3+	1.4 ± 0.2	2.3 ± 0.3	1.9 ± 0.2
CD4+	1.0 ± 0.2	2.4 ± 0.7	1.6 ± 0.9
CD8+	2.5 ± 0.4	2.9 ± 0.3	2.5 ± 1.5

healthy volunteers and both groups of stressed chronic pain patients, (2) quantitative analyses of lymphocyte subpopulations showed a clear reduction of the cytotoxic CD8+ lymphocytes in both CRPS and FM patients as compared with healthy controls, and (3) CRPS patients were characterized by a lowered percentage of IL-2-producing CD3+/CD4+/CD8+ T cells reflecting a diminished Th1 response in contrast to no changes in the Th2 cytokine profile, an effect which was not seen in FM patients.

Decrease in the CD8+ Lymphocyte Subpopulation in Chronic Pain

Only a few trials on immunity in chronic stress and pain have been published to date and even fewer addressed CRPS and FM. It is known that lymphocytes show an altered subset distribution under conditions of stress and pain [21–23, 25, 26]. We noticed significantly decreased numbers of cytotoxic CD8+ lymphocytes in both chronic pain patient groups as it has been described for chronic tension-type headache [31] and migraine sufferers [26, 31]. However, our study did not confirm either changes in lymphocyte counts or percentages of CD3+ and CD4+ cells [23, 25] in stressed pain patients.

Cytotoxic CD8+ T cells play an important role in the recognition and destruction of cells expressing foreign antigens, e.g. of viral or tumor origin [32, 33]. It is known that pediatric patients with a persistent lack in CD8+ T cells develop numerous infections attributable to bacteria and fungi [34]. However, further studies are needed to determine whether a diminished number of CD8+ T cells leads to increased susceptibility of chronic pain patients towards infections and tumor development.

The decrease in CD8+ lymphocytes is known to result in part from chronic stress [19, 35] mediated through the activation of the sympathetic nervous system. This is corroborated by our finding of a negative correlation between PTSD scores and CD8+ T cell proportion in FM patients. The absence of a correlation in CRPS patients is probably due to the smaller sample size. Furthermore, it should be taken into consideration that PTSS-10 scores in CRPS patients show a small range of variation making detection of statistically significant correlations difficult. Interestingly, pain magnitude did not influence CD8+ subsets suggesting that pain alone is not the all-dominant factor in CD8+ cell decrease.

Decreased Intracellular IL-2 Production by T Lymphocytes in Contrast to an Unchanged Th2 Cytokine Profile in CRPS

Besides cytotoxic CD8+ T cells, the other and most important fraction of CD3+ T lymphocytes is represented by CD4+ Th cells. Naive CD4+ Th cells (Th0) can differentiate into functionally distinct subsets, Th1 and Th2 cells. Th1 cells secrete more interferon-γ and IL-2 and stimulate type 1 immunity participating in cellular immune responses against intracellular pathogens like viruses and some bacteria. Th2 cells produce more IL-4 and IL-10 and induce humoral type 2 immunity [12, 13]. In our study, CRPS patients were characterized by a significant decrease in IL-2-producing percentages of CD3+ T lymphocytes, CD4+ Th cells and CD8+ cytotoxic T cells (fig. 4). Based on these findings one might conclude a reduced Th1 response.

The impaired capacity of CD8+ cells to produce IL-2, however, cannot fully be explained by a decrease in the absolute number of circulating CD8+ cells because the decrease in IL-2-positive cells was much more pronounced than the reduction in the CD8+ lymphocyte blood count. As the decrease in IL-2 production in CD8+ cells was over-proportional to the decline in CD8+ cell counts, one might suggest these changes to be independent phenomena. In this regard it is interesting to note that also CD4+ T lymphocyte exhibited significantly less IL-2 production in CRPS patients, irrespective of the number of CD4+ cells in blood. This might be in further support of a factor or factors able to selectively decrease IL-2 production without suppressing the number of circulating CD4+ lymphocytes.

In contrast, no significant changes were observed for IL-2-producing T cells of FM patients. Both patient

groups showed a slight, nonsignificant increase in their anti-inflammatory IL-4 and IL-10 production suggesting a minimally enhanced Th2 response. The Th1/Th2 ratio was significantly decreased in CRPS patients only.

Although polarization of the immune response has been documented in many other clinical conditions [36–41], the exact cause for a Th1/Th2 imbalance – as observed in our CRPS patients – still remains unknown.

Stress may influence the differentiation of bipotential Th cells away from a Th1 phenotype toward a Th2 phenotype. Early studies in animals revealed that sympathetic stimulation is able to inhibit Th1 responses via β-adrenergic receptor stimulation [42, 43]. It is known that sympathetic activation of the human nervous system is associated with the release of catecholamines and an enhanced Th2 cytokine production [44]. Both CRPS [45] and FM [46] patients were shown to exhibit increased noradrenaline levels. However, it is difficult to explain why the Th1/Th2 imbalance only occurs in CRPS patients and not in FM subjects. It could be that, conversely, predominance of a Th2-mediated immune response may induce antigen-specific B lymphocytes to produce specific antibodies which promote a secondary enhancement of the Th1 response leading to an autoimmune clinical picture, respectively [47]. This hypothesis is supported by an increased frequency of thyroid antibodies in FM [48]. The form of Th1/Th2 imbalance due to catecholamine release could be blocked by β-adrenoceptor antagonists [49] which might be a therapeutic alternative in some of the CRPS patients.

Second, a reduced Th1 response could be elicited by a strong Th2 response induced by low affinity interactions of T cell receptors with relatively low doses of antigen or peptide ligands [50]. In CRPS patients, this syndrome might develop after limb injury as a result of increased (neuron-)peptide levels [51] and additional infections [52]. In FM, trauma or infections occur much less frequently than in CRPS [53]; thus, associated peptide or antigen ligands as a cause for Th2 polarization are rarely possible.

As a third mechanism, glucocorticoids might act directly or indirectly via a decrease in IL-12 or IFN-γ production, thereby attenuating a Th1 response. In this way, an elevation of plasma cortisol could induce a decrease in the Th1 products resulting in an imbalance between Th1/Th2 cytokines [54]. However, no changes of plasma cortisol could be detected in CRPS [52]. Furthermore, in contrast, glucocorticoids are considered as a therapeutic option in early CRPS [55].

In addition, altered dehydroepiandrosterone (DHEA) levels which occur under acute and chronic inflammatory conditions [56, 57] may also exert some influence on Th1 and Th2 cytokines. In vitro DHEA was able to decrease Th1 and increase Th2 cytokine production [58]. The role of DHEA in chronic pain patients is still not characterized and represents an emerging field of investigations.

Study Limitations

Our study has several limitations. A possible confounding factor in our study is the concurrent use of analgetic and antidepressive medication. A prolonged washout period of all analgetic and antidepressive medication in chronic pain patients is difficult to be completely achieved and may be ethically unacceptable. However, patients abstained from intake of drugs for a period of 24 h. A direct interaction between the use of opioids and immunological mechanisms cannot be definitely excluded [59]. Despite an in vitro induction of apoptosis by tricyclic antidepressants [60], clinically used amitryptiline is not known to influence leukocyte functions. The half-life of the other drugs ranging between 1.5 and 6 h is in our opinion too short to affect lymphocyte function because the time interval between the intake of the last dosage and blood withdrawal was longer than 24 h.

Finally, a comparison between intracellular and secreted cytokines, a more detailed analysis of lymphocyte subpopulations such as regulatory T cells, functional assays of such lymphocyte subpopulations and determination of hormone secretion are lacking in our study.

Conclusion

CRPS and FM patients show decreased numbers of CD8+ cells probably due to a stress-associated activation of the sympathetic nervous system. CRPS patients are characterized by a diminished Th1 response, as suggested by an attenuated intracellular IL-2 production. Future studies are warranted to answer whether such immunological changes play a pathogenetic role in CRPS and FM or merely reflect the consequences of a pain-induced neurohumoral stress response.

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