Inflammatory/Stress Feedback Dysregulation in Women with Fibromyalgia

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Abstract

Objective: Although one of the current hypotheses of the aetiology of fibromyalgia (FM) syndrome involves inflammatory and neuroendocrine disorders, its biophysiology still remains unclear. The purpose of the present investigation was to study the systemic inflammatory and stress responses, as well as the innate response mediated by monocytes and neutrophils in FM patients. *Methods:* Twenty-five women diagnosed with primary FM and 20 age-matched healthy women (control group) were enrolled in the study. Circulating 'neuroendocrine-stress' biomarkers (CRH, ACTH, cortisol, NA, eHsp72, serotonin and IGF-1) were evaluated by ELISA. Serum IL-8 and CRP concentrations were also determined by ELISA, and inflammatory cytokine release by monocytes [IL-1β, TNFα, IL-6, IL-10, IL-18, monocyte chemotactic protein-1 (MCP-1) and RANTES] was evaluated by the Luminex BioPlex system. The phagocytic process of neutrophils (chemotaxis, phagocytosis and microbicide capacity) was also evaluated. *Results:* FM patients showed an inflammatory state accompanied by an altered stress response. This is mainly manifested by high circulating levels of IL-8 and CRP (in 100% of the FM group), high circulating levels of cortisol, and increased systemic levels of NA and eHsp72. There is also increased release of inflammatory cytokines (IL-1β, TNFα, IL-6, IL-10, IL-18 and MCP-1) by monocytes, and enhanced activation of the functional capacity of neutrophils (chemotactic, phagocytic and fungicidal activities). *Conclusion:* An inflammatory/stress feedback dysregulation underlies FM. Whether dysregulation of the stress response is the cause of the inflammatory dysregulation or vice versa is also discussed.

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Introduction

The diagnostic criteria of the American College of Rheumatology (ACR) currently define fibromyalgia (FM) as the presence of widespread pain (on both the left and the right sides of the body, both above and below the waist, and obligatorily with axial skeletal pain being present) and tenderness at 11 or more of 18 specific tender point sites [1]. In general FM is associated with such symptoms as reduced physical strength, fatigue, sleep disorders and psychiatric disorders (e.g. depression). Despite this symptom-based definition, the lack of objective criteria has led to doubts about the diagnosis of the pathology and even about its existence. It has been proposed, however, that alterations in the stress and inflam-

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matory responses could be involved, without knowing which of the two is cause and which is consequence. Indeed, the biophysiology of FM remains elusive, and treatment of the syndrome is generally empirical.

In a recent hypothesis proposed for the aetiology of FM, Van West and Maes [2] put forward the idea that inflammatory disorders accompanied by changes in the neuroendocrine-immune system might underlie the syndrome. However, in view of the variety of biophysiological aspects that seem to be implicated in the aetiology of FM, Müller et al. [3] propose that it should not be regarded as a single clinical entity but rather as a syndrome with multiple causes, and that therefore the term FM syndrome (FMS) should always be applied. They establish different subgroups of FMS, with the top of the classification hierarchy being primary or secondary FM. Whilst in secondary FM underlying diseases such as inflammatory rheumatic processes are frequently diagnosed, clinical studies of primary FM do not reveal any organic factor, meaning biochemical markers need to be identified to better characterize the disease. Primary FM is in turn subdivided into four groups [3], the first of which being that into which all the volunteer patients of the present study belonged: FM with extreme sensitivity to pain but no associated psychiatric problems [4]. Although there are conflicting results related to cytokine levels [5, 6], FM might be caused by a mild inflammatory condition, since most FM patients show elevated concentrations of serum IL-8 [5, 7, 8], of IL-6 in the supernatants of PBMC [5], and of IL-1β, TNF-α, IL-6 and IL-10 in the supernatants of isolated monocytes [9].

One methodological problem is that many previous studies have been conducted on only small groups of patients, evaluating either inflammatory or neuroendocrine markers but not both, and comparing the results with the values expected for healthy individuals and not always with the values measured for a sex- and age-matched control group. It is important to unify the criteria for grouping FM patients in accordance with published classifications, above all for distinguishing primary from secondary FM patients since FM patients with other underlying pathologies that may contribute to triggering the syndrome can hinder the interpretation of the results if FM patients are considered as a whole. Also, patients with severe depression should be considered as a separate group [8, 10].

The objective of the present study was to evaluate systemic inflammatory (CRP and IL-8) and neuroendocrine stress (CRH, ACTH, cortisol, NA, eHsp72, serotonin and IGF-1) markers and the functional capacity of isolated monocytes [release of IL-1β, TNF-α, IL-6, IL-10, IL-8, IL-18, monocyte chemotactic protein-1 (MCP-1) and RANTES (Regulated upon Activation, Normal T cell Expressed and Secreted)] and neutrophils (phagocytic process) in a group of primary FM patients (all women belong to the Fibromyalgia Associations of Don Benito and Badajoz, Spain), comparing the results not only with the general values expected in healthy individuals, but also with those of an age-matched control group of healthy women (HW). It is perhaps remarkable that, to the best of our knowledge, this is the first investigation to evaluate the circulating concentration of eHsp72 and the release by monocytes of IL-8, IL-18, MCP-1 and RANTES, and the chemotactic and fungicidal capacity of neutrophils in women with FM.

Materials and Methods

Study Design and FM Patients
FM volunteers: 25 women between 30 and 60 years old, diagnosed with FM by a rheumatologist (according to the ACR criteria for the syndrome) were enrolled in the study. These FM patients belonged to the Fibromyalgia Associations of Don Benito, Badajoz Province, Spain, and Badajoz, Spain. They were requested to fill out a questionnaire about their lifestyle (diet, habits, etc.), medication and other previous or current concomitant illnesses. All procedures were carried out with the written consent of the subjects. Exclusion criteria consisted of neoplastic illness (diagnosed from the medical history), infection, cardiopulmonary, vascular or other internal medical conditions, or use of oral or local corticosteroids or anticytokine therapy that could influence the level of cytokines. All these FM volunteers were classified as belonging to the primary group (with no definitive organic factor triggering the syndrome) according to the classification criteria of Müller et al. [3]: Group I – FM with sensitivity to pain but no diagnosis of depression or other relevant psychiatric disorder.

Twenty HW (who had no pain disorders or infectious illness at the time of blood sampling) served as the age-matched control group (age range 28–55 years). The same exclusion criteria were applied to the control group as for the FM volunteers.

Sampling was carried out between 8.00 a.m. and 9.00 a.m. local time. Peripheral blood samples were drawn by antecubital vein puncture. The study was approved by the Ethical Committee of the University of Extremadura (Spain) according to the guidelines of the European Community Council Directives and the Declaration of Helsinki.

Serum and Plasma
After extraction, blood samples for serum isolation were maintained for 15–20 min at room temperature. For plasma isolation the blood was anticoagulated with citrate during the extraction. For noradrenaline (NA) determination 40 μl of a solution containing 900 mg of EGTA and 700 mg of glutathione in 100 ml of 0.1 M NaOH was added to 2 ml of each blood sample before plasma separation. All the samples were centrifuged at 700 g for 10 min and finally the serum and plasma samples were aliquoted and stored at –80°C until assay.

Inflammatory Cell Isolation

Blood samples were centrifuged in a density gradient (Histopaque, Sigma), obtaining a first halo containing monocytes and lymphocytes, and a second containing neutrophils. These two cell suspensions were washed in PBS. Isolated neutrophils were adjusted to 10^6 cells/ml in Hanks' medium in order to evaluate their phagocytosis performing capacity (chemotactic, phagocytic and microbicidal capacities).

Monocytes were purified from the pool of mononuclear cells using the Monocyte Isolation Kit II (Miltenyi Biotec GmbH). With this method, one obtains a suspension of monocytes with purity higher than 90%, as determined by flow cytometry. They were adjusted to 10^6 cells/ml of medium (Iscove Medium, GIBCO) supplemented with 10% foetal bovine serum, 1% penicillin/streptomycin and 1% L-glutamine) in order to evaluate their inflammatory cytokine releasing capacity.

Determination of Systemic Inflammatory and Neuroendocrine Markers

The serum concentrations of IL-8 (Diaclone), CPR (Cyclex Co. Ltd.), CRH (Cusabio), serotonin (DRG), IGF-1 (DRG) and the plasma concentrations of ACTH (DRG), NA (LDN), and eHsp72 (Stressgen) were determined using commercial ELISA kits. The plasma concentration of cortisol was determined by electrochemiluminescence (Roche Elicys).

Determination of Inflammatory Cytokines Released by Monocytes

Monocytes were cultured for 24 h at 37°C, 5% CO_2 and 100% RH in flat-bottom 48-well cell culture plates (Falcon, Becton Dickinson Labware). Cell viability was checked by the Trypan Blue Exclusion Test, finding at least 98% viable cells. Supernatants were aliquoted in Eppendorf tubes and stored at –80°C until assay. The constitutive release by monocytes of IL-1β, TNFα, IL-6, IL-10, IL-8, IL-18, MCP-1 and RANTES was evaluated using the Bio-Plex® system (Luminex, BioRad). This system uses fluorescently dyed beads, a flow cytometer and associated optics, and a high-speed digital signal processor to detect of up to 100 different types of molecules in a single well of a 96-well microplate.

Study of the Neutrophils' Phagocytic Process

Chemotaxis, phagocytic and fungicidal capacities against *Candida albicans*, phagocytic capacity against inert particles, and oxygen-dependent microbicidal capacity (production of O_2^-) were evaluated in neutrophils as follows.

Chemotaxis Assay

Neutrophil chemotaxis was evaluated in a Boyden chamber with a modification [11] of the original technique [12], using an isopore polyvinylpyrrolidone-free polycarbonate filter with a pore diameter of 3 μm (Millipore) placed between the two compartments of the chamber. N-formyl-methionyl-leucyl-phenylalanine peptide (10^{-8} M, Sigma) diluted in PBS was used as a chemoattractant in the lower compartment of the chamber. This technique is appropriate for the quantitative evaluation of chemotaxis in isolated phagocytes [11]. Aliquots of 300 μl neutrophil suspension (10^6 cells/ml of Hanks' medium) were put into the upper compartment of the Boyden chamber and allowed to migrate through the filter for 90 min at 37°C in a 5% CO_2 atmosphere. After incubation, the filters were removed from the chambers, fixed and stained. The Chemotaxis Index was determined as the number of neutrophils counted at random (under phase contrast microscopy × 100) in sixteen fields of the lower face of the filter.

Phagocytic and Fungicidal Capacities

The neutrophils' phagocytic and fungicidal capacities against *C. albicans* were evaluated ex vivo using a quantitative technique previously validated for isolated neutrophils [13, 14]. Briefly, 0.5 ml of *C. albicans* suspension (10^6 cells/ml) and 50 μl of serum were added to 0.5 ml of the neutrophil suspension (10^6 cells/ml), followed by incubation in a thermostatic bath at 37°C for 60 min with shaking. The samples were then centrifuged at 300 *g* for 10 min, discarding two-thirds of the supernatant. The remainder of the supernatant was shaken and an aliquot was taken to count the number of *C. albicans* ingested by 100 neutrophils (Phagocytosis Index) in a Neubauer haemocytometer under phase contrast microscopy.

To evaluate the killing of phagocytosed *C. albicans*, a similar procedure to that used for the Phagocytosis Index was followed, but adding 1.5 ml of methylene blue (0.01%; which stains the dead *C. albicans*) at 50 min of incubation. The samples were then centrifuged at 300 *g* for 10 min, and the number of phagocytosed and dead *C. albicans* determined. The results are expressed as the percentage of dead *C. albicans* of the total phagocytosed by 100 neutrophils (Candidicide Index).

Phagocytosis of Inert Particles and Production of O_2^-

Two hundred microlitres of cell suspension (10^6 neutrophils/ml) were incubated (37°C and 5% CO_2) on culture plates for 30 min, and 20 μl of latex beads (1.09 μm, diluted to 1%; Sigma) were then added. After 30 min of incubation, the plates were washed, fixed and stained, and the number of particles ingested by 100 neutrophils was determined (Latex Bead Phagocytosis Index) under phase contrast microscopy [15].

The neutrophils' oxygen-dependent microbicidal capacity (production of O_2^-) was evaluated by the Nitroblue Tetrazolium (NBT) Reduction Test [15]. An aliquot of 250 μl of neutrophil suspension (10^6 cells/ml) was incubated for 30 min with an equal volume of NBT (Sigma, 1 mg/ml in PBS solution) in the presence of 20 μl of latex beads (1.09 mm 1% in PBS). Aliquots of neutrophil suspension incubated in the absence of latex beads were used as non-stimulated samples and a solution with the same reactants and medium without neutrophils was used as blank. In all cases, the reaction was stopped with 2.5 ml hydrochloric acid (0.5 N) after 30 min of incubation. The samples were centrifuged for 30 min at 600 *g* and 4°C, the supernatant discarded, and the reduced NBT (formazan) extracted from the cell pellet with 1 ml of dioxan. The tubes were then centrifuged for 30 min at 600 *g*, and the absorbance of the supernatant was determined in a spectrometer at 525 nm.

Statistical Analysis

The values are expressed as mean ± SEM. The normality of the variables was checked, followed by Student's t test for normally distributed samples or Mann-Whitney test for nonparametric samples. The significance level was set at $p < 0.05$.

Fig. 1. Serum concentration of IL-8 (**a**) and CRP (**b**) in HW (n = 20) and in FM patients (n = 25). Columns represent the mean ± SEM of independent experiments performed in duplicate in each HW volunteer and each FM patient. *** $p < 0.001$ with respect to HW (Student's t test). 100% of FM patients presented IL-8 and CRP values higher than those of HW and the expected values according to the manufacturer (<28 pg/ml for IL-8 and <0.5 μg/ml for CRP).
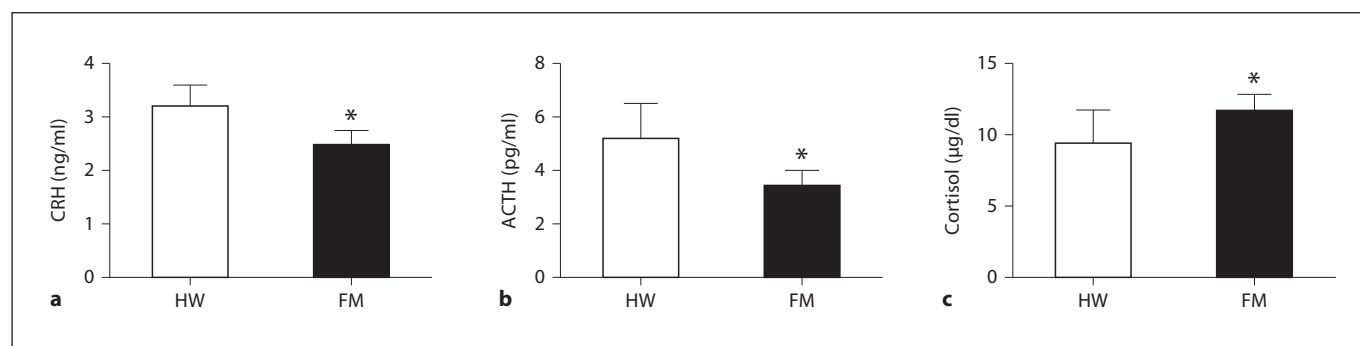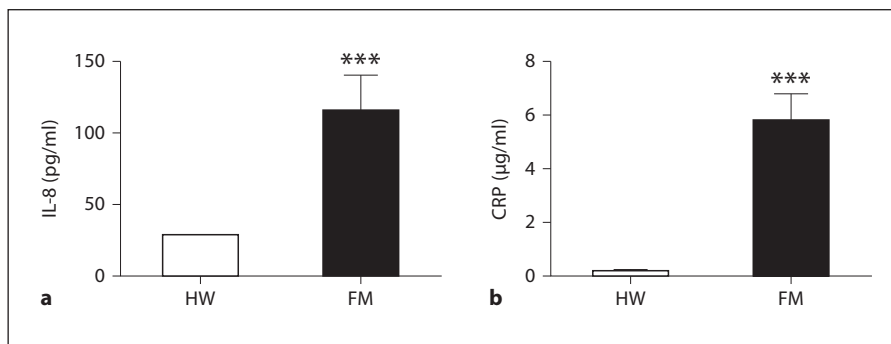

Fig. 2. HPA axis activity: circulating concentration of CRH (**a**), ACTH (**b**) and cortisol (**c**) in HW (n = 20) and in FM patients (n = 25). Columns represent the mean ± SEM of independent experiments performed in duplicate in each HW volunteer and each FM patient. The circulating concentration of cortisol is higher in FM patients than in HW. * $p < 0.05$ with respect to HW (Student's t test).

Fig. 3. Plasma concentration of NA (**a**) and eHsp72 (**b**) in HW (n = 20) and in FM patients (n = 25). Columns represent the mean ± SEM of independent experiments performed in duplicate in each HW volunteer and each FM patient. Circulating concentrations of NA and eHsp72 are higher in FM patients than in HW. ** $p < 0.01$, *** $p < 0.001$ with respect to HW (Student's t test).
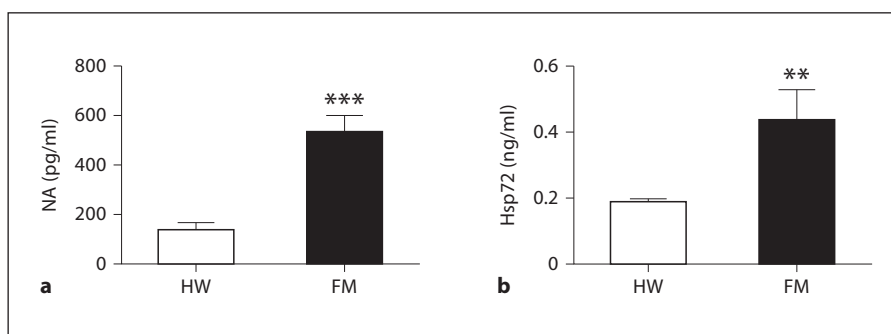
Results

Systemic Inflammatory and Neuroendocrine Markers

As expected, the circulating levels of IL-8 were markedly higher in patients with FM compared with the control group of HW ($p < 0.001$; fig. 1a). It is important to note that not only did we find a higher IL-8 concentration in the FM group as a whole with respect to the healthy control group, but also that the concentration was higher in each FM patient independently. The case was similar for CRP ($p < 0.001$; fig. 1b) in which we found a marked difference between the control group and the FM group, the concentration of this acute phase protein being nearly 30 times higher in the latter, with the value also being higher in each FM patient independently.

Figure 2 shows the results relating to HPA axis activity. Women with FM presented lower systemic concentrations of CRH ($p < 0.05$) and ACTH ($p < 0.05$), and high-
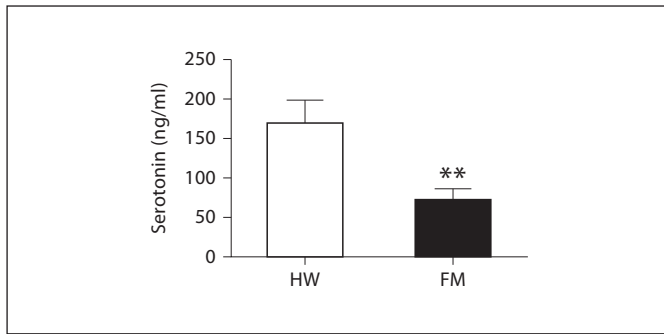
Fig. 4. Serum concentration of serotonin in HW (n = 20) and in FM patients (n = 25). Columns represent the mean ± SEM of independent experiments performed in duplicate in each HW volunteer and each FM patient. Concentration of serotonin is lower in FM patients than in HW. ** p < 0.01 with respect to HW (Student's t test).
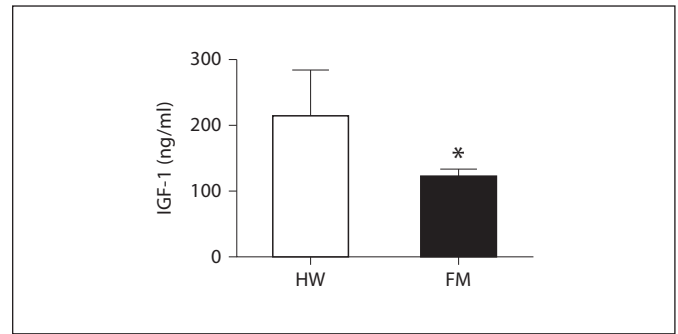

Fig. 5. Serum concentration of IGF-1 in HW (n = 20) and in FM patients (n = 25). Columns represent the mean ± SEM of independent experiments performed in duplicate in each HW volunteer and each FM patient. Circulating concentration of IGF-1 is lower in FM patients than in HW. * p < 0.05 with respect to HW (Student's t test).

Fig. 6. IL-1β, TNF-α, IL-6, IL-10 (left scale), IL-18, MCP-1 and RANTES (right scale) release by monocytes of HW (n = 8) and FM patients (n = 17). Columns represent the mean ± SEM of independent experiments performed in duplicate in each HW volunteer and each FM patient. The monocytes from FM patients released more IL-1β, TNFα, IL-6, IL-10, IL-18 and MCP-1, and less RANTES, than those from HW. * p < 0.05 compared with HW (Mann-Whitney test). Determinations were made using the BioPlex System (LUMINEX).
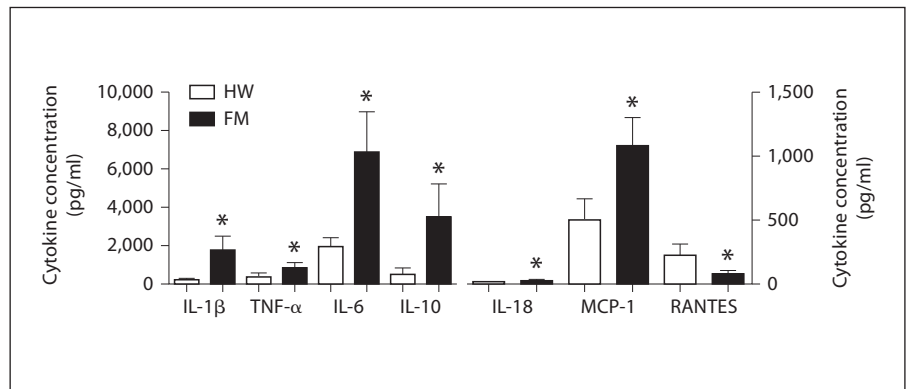
er concentrations (p < 0.05) of cortisol than the age-matched control group.

Other stress markers such as NA (fig. 3a) and eHsp72 (fig. 3b) were also higher (p < 0.001 and p < 0.01, respectively) in FM patients. Conversely, this group presented lower serum levels of serotonin (p < 0.01; fig. 4) and of the main mediator of the anabolic effects of growth hormone (GH), the 'insulin-related growth factor-1' IGF-1 (p < 0.05; fig. 5).

The Monocytes' Functional Capacity

Figure 6 shows the results relating to the capacity of monocytes to release inflammatory cytokines. The monocytes from FM women released constitutively more IL-1β, TNFα, IL-6, IL-10, IL-18 and MCP-1 than those from HW, but less RANTES. No inter-group significant differences were found in the release of IL-8

(5,521 ± 2,374 pg/ml for FM vs. 4,260 ± 1,501 pg/ml for HW).

*The Neutrophils' Functional Capacity*

The chemotaxis (fig. 7a), phagocytosis against *C. albicans* (fig. 7b) and fungicidal (fig. 7c) capacities of neutrophils were greater in the FM patients than in the healthy control group, but the latex bead phagocytosis index was lower (fig. 7d), and no differences were found in the production of O_2^- (fig. 7e).

Discussion

Systemic Inflammatory and Neuroendocrine Markers

To the best of our knowledge, the present work represents the first complete study of both the functional ca-

Fig. 7. Functional capacity of neutrophils: chemotactic (**a**), phagocytic (**b**) and fungicidal (**c**) capacities, phagocytosis of latex beads (**d**), and O_2^- production (**e**) in HW (n = 7) and FM patients (n = 18). Columns represent the mean ± SEM of independent experiments performed in duplicate in each HW volunteer and each FM patient. The neutrophils from FM patients presented higher chemotactic, phagocytic and fungicidal capacities, and lower latex bead phagocytosis than those from HW. * $p < 0.05$, *** $p < 0.001$ with respect to HW (Student's t test).
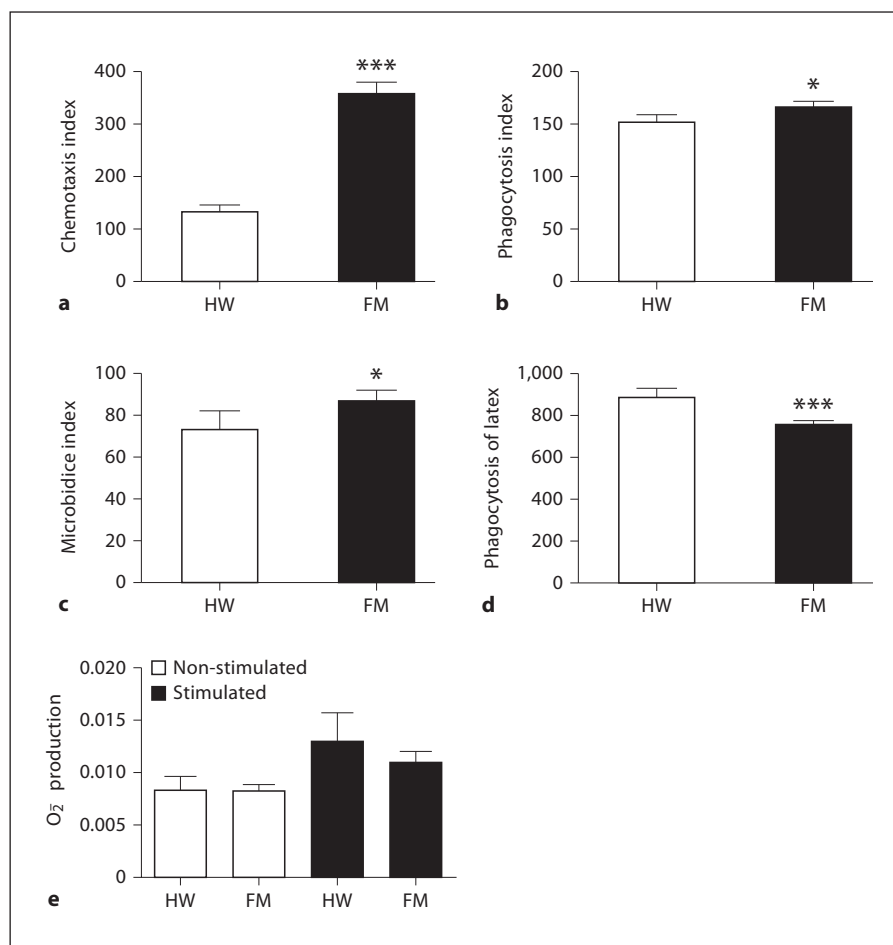
pacity of inflammatory cells and the systemic inflammatory and neuroendocrine status of a single group of women diagnosed with primary FM, compared with a control group of age-matched healthy women. IL-8 can promote sympathetic pain [16] and, therefore, must be considered as playing a potential role in FM [5]. As expected, we found elevated serum concentrations of IL-8 in the patients with FM compared to the control group. Most of the few previous studies also report increased serum levels of IL-8 in FM patients [5, 6, 8, 17, 18] irrespective of differences in the aetiology of the illness [8]. In the present study, it is important to note that each FM patient had a higher concentration of IL-8 than not only the expected value in healthy people, but also the control group of age-matched HW. Taking this into account, the absence of elevated levels of IL-8 could contribute as an exclusion criterion for FM diagnosis. With respect to CRP (a generic inflammatory marker), the results confirm both a previous report that FM patients present levels above the

expected normal range in healthy people (<0.5 µg/ml) [17], and our own findings in an earlier study on a small group of FM patients [8]. In particular, the present results show the FM patients to have higher circulating concentrations of CRP than the age-matched control group of HW (also below 0.5 µg/ml), and that this held for each of the FM patients considered separately.

The results relating to the HPA axis showed a higher circulating concentration of cortisol paralleled by low ACTH and CRH levels in the FM patients. This corresponds to a disrupted HPA axis, as has been previously reported in studies evaluating circadian variations of ACTH and cortisol in these patients [19]. Nevertheless, a limitation of our study may be that we measured cortisol and ACTH only once. This is because another study assessing the feedback sensitivity of the HPA axis in FM patients by conducting low-dose dexamethason suppression tests revealed normal feedback sensitivity in FM patients [20]. In any case, elevated circulating cortisol levels

together with the increased systemic levels of NA denote a higher stress response in patients with FM, probably due to dysregulation of the inflammatory and stress feedback mechanisms. The mild chronic increase in cortisol found in FM patients, with a chronic low-grade inflammatory status, indicates that the HPA axis failed to control the increase of pro-inflammatory cytokines.

Hsp72 plays an important role in physiology and human health. Under normal physiological conditions, Hsp72 is expressed at low levels. However, a wide variety of pathological stressful stimuli can induce a marked increase in its intracellular synthesis [21] and extracellular release [22, 23]. Inflammation also results in the release of eHsp72 [24] and high circulating levels of eHsp72 may reflect a 'chronic low-grade inflammatory status'. To the best of our knowledge, this is the first time that an elevated circulating concentration of eHsp72 has been reported in FM patients. NA can act as a 'stress signal' for the release of eHsp72 [25]. Thus, increased circulating levels of NA and eHps72, together with cortisol, CRP and IL-8 reflect an alteration in the inflammatory and stress feedback mechanisms in FM.

In addition, circulating levels of serotonin are related to pain in FM patients [26, 27]. Indeed, one of the pharmacological treatments currently used in FMS is a serotonin re-uptake inhibitor [28]. Coherent with reports in the literature [28], we found the circulating serotonin levels to be lower in the FM group than in the healthy control group, confirming the role serotonin plays in the neuroendocrine disorders associated with FMS. Treatment of GH deficiency in adults has also been reported to reduce pain and improve quality of life [29]. The main mediator of the effects of GH is somatomedin C, also called 'insulin-related growth factor-1' (IGF-1). This has a half-life of approximately 21 h and no diurnal variations, so that its plasma levels are considered to reflect an integration of the pulses of GH secretion during the previous 48 h. For these reasons, studies of FM patients have primarily evaluated IGF-1. Although it has been reported that there are no differences in the systemic levels of IGF-1 connected with FM [30], most investigations report evidence for deficient GH function in FM patients as reflected in the serum levels of IGF-1, and that this could be secondary to some dysfunction in the hypothalamic-hypophyseal-GH axis [31, 32]. Our results are coherent with the latter and the majority of studies, given that we found a significantly lower concentration of IGF-1 in FM patients compared with the control group, thus confirming that IGF-1 is involved in some alterations associated with this syndrome. Nevertheless, since GH deficiency is not always associated with FM, it has been suggested that it is an epiphenomenon in this pathology, although treatable and with clinical relevance, since GH appears to improve symptoms of FM including pain, and hence also the patient's quality of life [32].

Functional Capacity of Monocytes

During a local inflammatory response, TNFα, IL-1β and IL-6 are the main cytokines released by monocytes, together with the anti-inflammatory cytokine IL-10, which is released later. The monocytes from FM patients released more IL-1β, TNFα, IL-6, IL-10 (as well as IL-18 and MCP-1, but less RANTES) than those from the control group, as determined using an innovative sensitive technique (Bio-Plex system, Luminex). This confirms our previous results for IL-1β, TNFα, IL-6 and IL-10 determined by ELISA in a small number of FM patients [9]. To the best of our knowledge, there have been no previous publications on the role of IL-18 and RANTES in FMS. IL-18 was initially described as the induction factor of IFN-γ. It is a pro-inflammatory cytokine that contributes to the organism's systemic and local defences, and thus has been proposed as being one of the main pro-inflammatory cytokines involved in inflammatory diseases [33, 34]. MCP-1 is a cytokine secreted by antigen-presenting cells. It participates in the recruitment of neutrophils (and monocytes), and is required for their early accumulation at points of inflammation [35]. The increased release of MCP-1 in the FM group may explain, at least in part, the reduced local release of RANTES by the monocytes of the women in that group, since it has been shown that treating macrophages with MCP-rm-1 selectively suppresses RANTES expression [35]. Although previous results concerning the systemic levels of MCP-1 in this syndrome have been controversial [36, 37], it seems clear that the release of this cytokine by monocytes is altered in FMS. The results presented here confirm the hypothesis of a dysregulated inflammatory response in FMS that involves monocytes, however, no significant differences were found between women with FM and HW in the release by monocytes of IL-8, which seems to indicate that, surprisingly, monocytes are not the main source of the elevated systemic concentration of IL-8 in women with FM. One might speculate, therefore, that the origin could be endothelial cells. Further investigation in this line may shed some light on this question.

Functional Capacity of Neutrophils

The existence of sexual dimorphism in the immune response in humans, particularly in the innate and in-

flammatory response, is well known. Thus, the level of neutrophil activity is higher in women than men, with elevated circulating concentrations of IL-8, cortisol, NA and eHsp72 seeming to be involved. While this can give women increased resistance to infection compared to men, this enhanced 'inflammatory status' can also contribute to a greater incidence of inflammatory diseases [38]. In the evaluation of the neutrophils' phagocytic process those from FM patients showed a greater chemotactic activity than those from HW. The FM patients' elevated circulating levels of IL-8 and greater release of IL-18 and MCP-1 by monocytes could contribute to this phenomenon. FM patients are more prone to *C. albicans* infections than HW [39], which seems consistent with the increased phagocytic and microbicidal capacities against *C. albicans* found in the neutrophils from our FM patients. This greater phagocytic and fungicidal capacity (together with a greater chemotactic capacity) of neutrophils could be mediated by the raised circulating concentration of NA and eHsp72 in the FM patients, since it has been reported that increased systemic levels of NA and eHsp72 mediate the stimulation of chemotaxis, phagocytosis and the fungicidal activities of neutrophils [22, 40–42]. However, the FM group's decreased latex bead phagocytic capacity (a less specific form of phagocytosis because it is not mediated by pre-opsonization through antibodies) is suggestive of an immunosuppression mediated by the less specific innate immune response carried out by neutrophils.

No differences were found in O_2^- production, so it seems that FM patients have no altered oxygen-dependent microbicidal capacity, especially considering that neutrophils from FM patients are reported as presenting elevated spontaneous H_2O_2 production [43].

Conclusion

The present study has confirmed in a group of 25 primary FM patients (compared with a group of age-matched HW) that this syndrome can be categorized as an inflammatory and stress-related disease. This was mainly manifested in raised circulating levels of IL-8 and CRP (in 100% of the FM volunteers), high circulating levels of cortisol, and increased systemic levels of NA and eHsp72. There was also an increased release of pro-inflammatory cytokines by monocytes, and increased activation of the neutrophils' functional capacity.

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