Sjögren's Syndrome with Myalgia Is Associated with Subnormal Secretion of Cytokines by Peripheral Blood Mononuclear Cells

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ABSTRACT. ***Objective.*** To measure *in vitro* cytokine release from peripheral blood mononuclear cells (PBMC) and serum cytokines in patients with primary Sjögren's syndrome (SS) with and without myalgia, compared to patients with rheumatoid arthritis (RA) and healthy controls.

Methods. Sixteen women with SS (8 with myalgia, 8 without pain), 15 women with RA, and 14 healthy women were studied. PBMC were isolated and cultured. Secretion of interleukin-1ß (IL-1ß), IL-6, IL-10, and tumor necrosis factor-α (TNF-α) was measured in cell supernatants with or without stimulation with phytohemagglutinin, tetanus toxoid, or purified protein derivative (PPD). Enzyme-linked immunospot was used to enumerate interferon-γ (IFN-γ) and IL-4-secreting cells. Serum concentrations of IL-8 and IL-18 were analyzed by ELISA.

Results. PPD-stimulated PBMC from SS patients responded with less production of IL-10, TNF-α, and IFN-γ compared to controls. Patients with SS and pain were hyporesponsive also with respect to IL-1ß and IL-6. The generally subnormal cytokine release was statistically significant in myalgic patients with SS compared to healthy controls. Serum IL-18 was increased in both SS groups as well as in patients with RA, and the highest levels were found in myalgic patients with SS. Serum IL-8 was increased in RA but not in SS.

Conclusion. Patients with SS, especially those with myalgia, had diminished PBMC cytokine release and increased serum IL-18. This finding suggests that impaired cytokine regulation may have pathogenetic importance for myalgia in SS. (J Rheumatol 2004;31:729–35)

Key Indexing Terms:
PRIMARY SJÖGREN'S SYNDROME CYTOKINES INTERLEUKIN 18 MYALGIA

Besides oral and ocular dryness, myalgia is a frequent complaint in patients with primary Sjögren's syndrome (SS). In a previous study we found that 27% of patients with SS had widespread pain and trigger points consistent with fibromyalgia (FM), and a further 17% had myalgia not fulfilling criteria for FM[1]. Histological signs of inflammatory myopathy were also common, but did not relate to the presence of myalgia[1]. Neither were there any differences between myalgic and nonmyalgic patients concerning other organ manifestations, autoantibody pattern, hypergammaglobulinemia, serum creatine kinase, or medication.

The mechanisms underlying pain in FM are a matter of discussion. One view is that it is produced by a combination of peripheral and central mechanisms, the peripheral including changes in microcirculation and energy-rich phosphates, and the central including sensitization of nociceptive nerve cells[2]. The hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system are linked to the cytokine network[3-5], and cytokines have also been implicated in the pathogenesis of FM. Wallace and coworkers reported that peripheral blood mononuclear cells (PBMC) from patients with FM produced more interleukin 6 (IL-6) than controls, and that patients with FM had raised serum concentrations of IL-8[6].

IL-8 promotes sympathetic pain[7], IL-6 induces hyperalgesia[8,9], IL-1ß may produce fatigue and hyperalgesia, and interferon-α (IFN-α) and IFN-γ may cause arthralgia and myalgia, whereas IL-10 can block pain[6]. Herpes zoster-related pain has been associated with increased IL-8 concentrations in cerebrospinal fluid[10].

Mononuclear cells infiltrating the salivary glands of SS patients have been reported to produce T-helper cell type 1 (Th1)-associated cytokines such as IL-2, IL-12, IL-18, and IFN-γ, and the proinflammatory cytokine tumor necrosis factor-α (TNF-α)[11-15]; but as well the mainly antiinflammatory cytokines IL-10 and transforming growth factor-ß (TGF-ß) have been detected[11,13-15]. Analysis of serum and supernatants of cultured PBMC have revealed increased

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concentratons of IL-6 and IL-10 in patients with SS, whereas reports concerning other cytokines are inconsistent[16-19].

We investigated factors that distinguish myalgic SS patients from those without myalgia. PBMC cytokine secretion patterns and serum cytokine concentrations were analyzed and related to patients with rheumatoid arthritis (RA) and a group of healthy controls.

MATERIALS AND METHODS

Subjects. Patients and controls were recruited to the study with their informed consent. None had clinical signs or symptoms of ongoing infection at the time of blood sampling. Thirty-six of the subjects had been immunized with bovine Calmette-Guérin (BCG) vaccine and 39 with tetanus toxoid (TT). Nine of the subjects were uncertain about BCG vaccination and 6 about TT vaccination; however, until 1975 BCG vaccination was almost universal in Sweden.

The 16 patients (all were women) with primary SS are described in Table 1. They were randomly selected from a group of patients carefully examined with respect to myalgia in a previous study[1]. All SS patients met the American-European classification criteria, including presence of anti-Ro/SSA ± anti-La/SSB antibodies or typical histopathology as a requirement for diagnosis[20]. All had precipitating anti-Ro/SSA antibodies in serum (Immunoconcepts, Sacramento, CA, USA) and 13 (81%) also had anti-La/SSB antibodies. Assessed by indirect immunofluorescence microscopy (HEp-2 cells; Immunoconcepts) 13 of 16 had antinuclear antibodies (ANA) in a titer ≥ 1:100. Eight of the SS patients had chronic myalgia (the Pain group; mean age 59 yrs, range 41–73), 6 of whose signs were consistent with FM according to the American College of Rheumatology (ACR) criteria[21]. Details of the FM features in these SS patients have been described[1]. The remaining 8 SS patients had no myalgia (the No pain group; mean age 59 yrs, range 37–74). Ten of the 16 SS patients complained of arthralgia.

The 15 patients with RA (all women) are described in Table 2. They were randomly selected from the outpatient clinic at the Division of Rheumatology. All fulfilled the 1987 ACR criteria[22]. Disease activity was evaluated by a Disease Activity Score including the 28 joint count of swollen and tender joints (DAS-28)[23]. None fulfilled criteria for FM. The mean age of patients with RA was 57 years (range 35–74); 13 (87%) were seropositive for agglutinating rheumatoid factor (RF) and one had antinuclear antibodies (ANA).

The 14 healthy controls (all women) were recruited from the staff at the rheumatology unit (mean age 50 yrs, range 41–69). None of the controls were taking medication.

Cell preparation. Mononuclear cells were separated from heparinized peripheral blood by density gradient centrifugation on Lymphoprep® (Axis-Shield PoC AS, Oslo, Norway) according to Bøyum[24], followed by washing 3 times with Hanks' balanced salt solution (HBSS; Gibco BRL, Paisley, Scotland). The mononuclear cells were resuspended in tissue culture medium (TCM) supplemented with L-glutamine (Sigma, St. Louis,

Table 1. Clinical characteristics of 16 patients (all female) with primary SS.

Patient	Age, yrs	Myalgia	Anti-SSB Antibody*	Duration of Sicca Symptoms, yrs	SS Manifestations	Intercurrent Diseases	Medication			
							HCQ	Pred, mg/day	Analgesics	NSAID or Coxibs
1	74	No	No	23	—	—	—	—	—	—
2	67	No	No	17	Leukopenia, Raynaud's	—	—	—	—	—
3	67	No	Yes	14	—	Op. lumbar disc hernia 1992	—	—	—	As needed
4	65	No	No	6	Lung involvement, RTA	—	—	—	—	—
5	62	No	Yes	11	Leukopenia	—	—	—	—	Daily
6	54	No	Yes	11	Hypothyreosis, lymphadenopathy	Gonarthrosis	—	As needed	Daily	Daily
7	45	No	No	5	—	—	Yes	—	—	As needed
8	37	No	Yes	9	RTA, child with AV block, leukopenia	—	—	—	—	—
9	73	Yes	Yes	7	Hypothyreosis, pernicious anemia, Raynaud's	Spondylosis	—	—	Daily	—
10	72	Yes	Yes	24	Skin vasculitis, RTA, neuropathy, leukopenia, lymphadenopathy	Atrial fibrillation, asthma	—	2.5	—	—
11	65	Yes	Yes	31	Myositis, RTA	Hypertension, heart infarction, low back pain, gyn. cancer 1986	—	7.5	As needed	—
12	62	Yes	Yes	18	Hypothyreosis, purpura, RTA, leukopenia, Raynaud's	Neurinoma, hypertension	—	—	—	As needed
13	59	Yes	No	11	Pulmonary involvement, neuropathy	—	Yes	—	Daily	—
14	50	Yes	Yes	7	—	—	—	—	Daily	—
15	49	Yes	Yes	8	RTA, pernicious anemia, leukopenia	Hypertension	Yes	As needed	As needed	—
16	41	Yes	Yes	8	Leukopenia, child with AV block	Asthma	—	—	—	As needed

* All SS patients had anti-SSA antibody serum tests. RTA: renal tubular acidosis; NSAID: nonsteroidal antiinflammatory drugs; HCQ: hydroxychloroquine; AV: atrioventricular.

Table 2. Clinical characteristics of 15 patients with RA.

Patient	Age, yrs	RF	Disease Duration, yrs	Das-28	Intercurrent Diseases	DMARD	Medication		
							Prednisolone mg/day	NSAID/Coxib	Analgesic
1	74	+	1	4.8	Chronic obstructive lung disease, depression, Grave's disease, intermittent atrial fibrillation	MTX 12.5 mg/wk	7.5	—	As needed
2	72	+	22	4.6	Melanoma 1990, cancer *in situ* breast 1995, cancer *in situ* vulva 1999	Sodium aurothiomalate 50 mg/3wks	5	—	As needed
3	70	+	13	2.7	—	Sulfasalazin 2 g/day	—	—	—
4	67	+	2	5.4	—	MTX 10 mg/wk	—	Daily	—
5	64	+	12	6.1	—	MTX 12.5 mg/wk	—	As needed	—
6	60	–	16	5.2	—	—	—	Daily	—
7	59	+	18	3.9	—	MTX 12.5 mg/wk	5	As needed	—
8	58	+	7	6.2	—	Sodium aurothiomalate 50 mg/2 wks	5	Daily	—
9	58	–	20	3.2	—	Leflunomide 20 mg/day	—	Daily	—
10	56	+	8	4.2	Hypertension, gold induced proteinuria 1999	MTX 15 mg/wk	2.5	Daily	—
11	55	+	20	5.6	Low back pain, hypertension, cholecystectomia 1 mo prior to sampling	—	10	Daily	Daily
12	50	+	15	4.6	Hypertension	MTX 7.5 mg/wk	—	Daily	Daily
13	41	+	23	3.4	Pernicious anemia, recurrent lymphadenopathy	Sulfasalazine 1.5 g/day	—	—	—
14	40	+	1	1.3	—	MTX 15 mg/wk	5	Daily	—
15	35	+	2	4.9	—	MTX 15 mg/wk	5	Daily	—

DMARD: disease modifying antirheumatic drug; MTX: methotrexate; NSAID: nonsteroidal antiinflammatory drug; Coxib: selective cyclooxygenase-2 inhibitor; DAS-28: 28 joint disease activity score.

MO, USA) 292 mg/l; penicillin 50 U/ml and streptomycin 50 µg/ml (BioWhittaker Europe, Verviers, Belgium); 100× minimal essential medium (MEM), nonessential amino acids 20 ml/l (Gibco BRL), and 5% heat inactivated fetal bovine serum (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany). The cells were counted by phase contrast microscopy and diluted to a lymphocyte concentration of 10^6/ml.

Mononuclear cell suspension was applied to microtiter plates (100 µl/well) together with TCM (100 µl/well) for nonstimulated cells, or with 10 LF/ml tetanus toxoid (SBL Vaccin AB, Stockholm, Sweden), 20 µg/ml purified protein derivative (PPD; Statens Seruminstitut, Copenhagen, Denmark), and 40 µg/ml phytohemagglutinin (PHA; Sigma), respectively (all diluted in TCM, 100 µl/well), for stimulated cells. As a negative control TCM alone was applied (200 µl/well). The cells were cultured at 37°C in a humidified atmosphere with 5% CO_2. After 48 and 144 h, respectively, the samples were centrifuged (200 g at 4°C for 10 min) before the supernatants were removed and immediately frozen at –70°C.

Detection of in vitro secreted cytokines by ELISA. IL-1ß, IL-6, IL-10, and TNF-α were measured in cell supernatants from the cultured PBMC, nonstimulated and stimulated *in vitro*, by ELISA performed essentially as described[25]. Microtiter plates (Costar 3690, Corning Inc., Corning, NY, USA) were coated with 10 µl/ml anti-IL-1ß, anti-IL-6, anti-IL-10, or anti-TNF-α antibodies (PeliPair™ reagent set; CLB, Amsterdam, The Netherlands), all diluted in carbonate buffer, pH 9.6 (50 µl/well). Anti-IL-1ß, IL-6, and IL-10 antibodies were incubated at room temperature and anti-TNF-α antibodies at +4°C overnight. The plates were washed 4 times with PBS-Tween in a microtiter plate washer, blocked with 2% low fat milk in phosphate buffered saline (PBS; 100 µl/well), incubated 60 min on a plate shaker at room temperature, and washed as above. Standards (PeliPair reagent set) and supernatants were diluted in TCM. Standards, blanks, and supernatants (undiluted/diluted) were added to the plates in duplicates (50 µl/well). The plates were incubated and washed as above. Biotin anti-IL-1ß, anti-IL-6, anti-IL-10, and anti-TNF-α antibodies diluted 1:100 in high performance ELISA buffer (HPE; CLB) were added (50 µl/well). The plates were incubated and washed as above. Streptavidin-conjugated horseradish peroxidase (CLB) diluted 1:10,000 in HPE was added (50 µl/well). The plates were incubated 30 min on a plate shaker and washed as above. Then 3,3',5,5'-tetramethylbenzidine (TMB; Sigma) was added (50 µl/well) and incubated 30 min in the dark on a plate shaker. The reaction was stopped by adding 1.8 M H_2SO_4 (50 µl/well). The optical density (OD) was measured spectrophotometrically at 450 nm.

Enumeration of cytokine-secreting cells. IFN-γ and IL-4 secreting cells were visualized by enzyme linked immunospot (ELISPOT) as described[26]. The spots were counted in an ELISPOT reader (AID; Autoimmun Diagnostika GmbH, Strassberg, Germany) and evaluated by the same person. The median of the samples was calculated, and the value of the negative control was subtracted. Then the value of the nonstimulated cells was subtracted from the value of the stimulated cells.

Detection of IL-8 and IL-18 in serum. IL-8 and IL-18 were measured in serum using the Human IL-8 US Ultrasensitive kit (Biosource International, Camarillo, CA, USA) and Human IL-18 ELISA kit (MBL, Nagoya, Japan). The analyses were performed in duplicate according to the manufacturer's instructions. Serum samples were kept frozen at –70°C until analyzed.

Statistical analysis. The Kruskal-Wallis test was used for comparison of more than 2 groups. Mann-Whitney U test was used for comparison between 2 groups. A p value < 0.05 was considered statistically significant.

The study was approved by the local ethics committee.

RESULTS

Unstimulated PBMC. In the composite group of SS patients (with and without pain) unstimulated PBMC secreted less IL-6 than healthy controls after 48 h culture: median 7.5 pg/ml (range 1.7–15.0) versus 10.4 pg/ml (range 5.6–210) (p = 0.0232). No difference was detected for other cytokines when the different groups were compared. Considering IL-1ß, IL-10, and TNF-α, only 2, 0, and 0 patients or controls had levels above the detection level, respectively.

After 144 h, PBMC from the composite group of SS patients with and without pain secreted less IL-6 than controls: median 11.7 pg/ml (range 3.1–60) versus 29.3 pg/ml (range 8.7–200) (p = 0.01). Further, PBMC from the Pain group secreted only 9.0 pg/ml (range 3.1–50), which was significantly less than controls (p = 0.0185). The No pain group secreted 15.2 pg/ml (range 3.2–60).

PPD stimulation. The median values and range for each cytokine after 48 h stimulation of PBMC with PPD are shown in Table 3 and Figure 1. When PPD-stimulated samples were compared for 4 groups (RA, SS Pain, SS No pain, controls), Kruskal-Wallis test showed significant differences for TNF-α and IFN-γ (TNF-α, p = 0.0016; IL-1ß, p = 0.0646; IL-6, p = 0.0885; IL-10, p = 0.0758; IFN-γ, p = 0.0417; IL-4, p > 0.1). Compared to PBMC from controls, those from myalgic SS patients secreted significantly less IL-1ß, IL-6, IL-10, TNF-α, and IFN-γ (p = 0.0265, p = 0.0478, p = 0.0406, p = 0.0021, and p = 0.0406, respectively). PBMC from SS patients without pain were more like those from controls, but secreted less IL-10, TNF-α, and IFN-γ (p = 0.0478, p = 0.0026, and p = 0.0185, respectively).

There were trends for decreased release of IL-1ß (p = 0.0587) and TNF-α (p = 0.0528) in SS patients with myalgia compared to those without pain. Compared to controls, RA patients secreted less TNF-α after PPD stimulation (20 vs 56 pg/ml; p = 0.0270). After 144 h of PPD stimulation, PBMC from SS patients with pain still secreted less IL-1ß and IL-6 than controls (p = 0.0154, p = 0.0265, respectively). TNF-α, IL-4, and IFN-γ were not analyzed after 144 h.

Tetanus toxoid stimulation. After stimulation with TT for 48 h, PBMC from SS patients with pain were significantly less responsive with respect to IL-6 compared to PBMC from controls or SS patients without pain: median values 18 pg/ml (range 5.3–280) vs 100 pg/ml (19.3–6000) and 165 pg/ml (7.7–1800), respectively (p = 0.0298 and p = 0.0406). PBMC from SS patients with pain as well as the composite group of SS patients were significantly less responsive with respect to IFN-γ compared to PBMC from healthy controls: median 3.5 spots (range –14 to 6) and 4.5 spots (–14 to 115) versus 11 spots (–2 to 104) (p = 0.0169 and p = 0.0145, respectively). The median value for RA patients was 4 spots (range –1 to 24), which was not different from controls. No differences were found concerning TNF-α, IL-1ß, IL-4, or IL-10 when the different groups were compared. After 144 h stimulation with TT, a pattern similar to that after 48 h was found for IL-6 (corresponding p values 0.0007 and 0.0742, respectively). TNF-α, IL-4, and IFN-γ were not analyzed after 144 h.

PHA stimulation. No differences were found after PHA stimulation of the PBMC.

Serum cytokines. Serum concentrations of IL-18 were highest in SS patients with pain (median 327 pg/ml), were similar in RA and SS patients without pain (median 230 and 225 pg/ml), and were lowest in controls (median 150 pg/ml). SS patients with pain as well as those without pain and RA patients had significantly higher values than the controls (p = 0.0019, p = 0.0441, p = 0.0028, respectively). IL-8 in serum was increased in RA patients compared to SS patients and controls (31 vs 9 and 9 pg/ml; p = 0.0088, p = 0.0272, respectively).

C-reactive protein (CRP). Serum concentrations of CRP

Table 3. Cytokine production from PBMC after 48 h stimulation with PPD. Median (range) are given. ELISPOT was used for IL-4 and IFN-γ, and ELISA in cell supernatants for the other cytokines.

	All SS, n = 16	SS Pain, n = 8	SS No Pain, n = 8	RA, n = 15	Healthy Controls, n = 14
TNF-α, pg/ml	5*** (3.9–160)	3.9** (3.9–160)	12** (4–23)	20* (3.9–160)	56 (16–220)
IL-1ß, pg/ml	31 (13.8–280)	22.4* (13.8–280)	90 (17.8–210)	60 (21.1–260)	85 (17.1–180)
IL-6, pg/ml	3500 (900–18,000)	2000* (900–14,000)	4250 (2000–18,000)	8500 (1100–26,000)	5750 (1700–11,000)
IL-10, pg/ml	16.2* (3.1–39.9)	15.2* (3.1–39.3)	16.0* (7.1–39.9)	25.4 (7.2–99.3)	27.4 (10–54.6)
IL-4, no. spots	1 (–1 to 6)	1.5 (0 to 6)	0.5 (–1 to 3)	3 (–2 to 14)	0.5 (–6 to 7)
IFN-γ, spots, n	2** (–11 to 24)	4* (–11 to 24)	0* (–10 to 16)	7 (–4 to 109)	29.5 (–8 to 208)

Statistical significance vs controls: * p < 0.05, ** p < 0.01, *** p < 0.001.

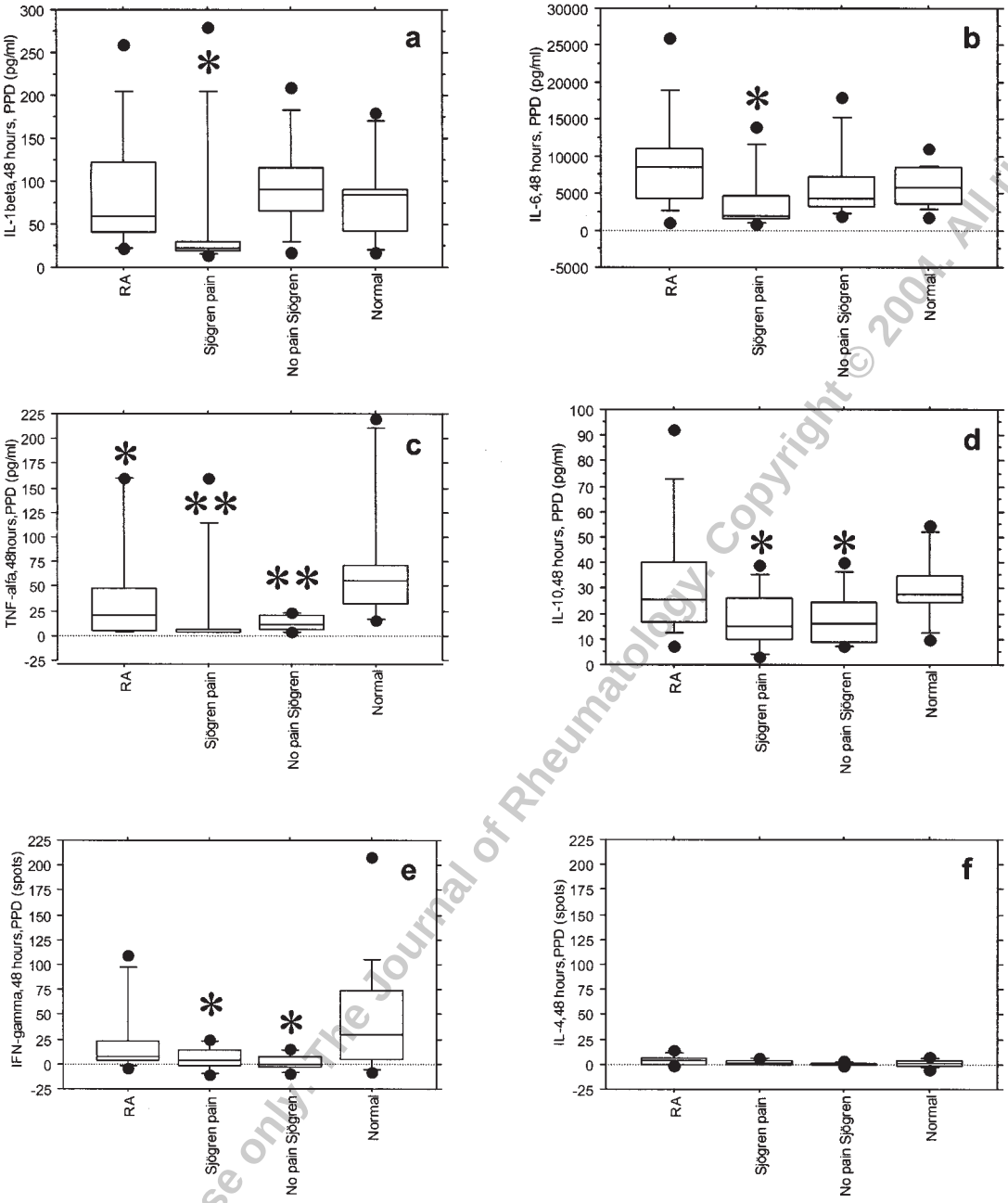

Figure 1. Cytokine release from PBMC after 48 h stimulation with PPD. Box plots show median values and 10th, 25th, 50th, 75th, and 90th percentiles. Statistically significant differences compared to healthy controls, *p < 0.05; **p < 0.01.

were normal in all controls and all SS patients except one with pain (CRP 15 mg/l). The RA patients had a median value of 20 mg/l (range < 10–68 mg/l).

DISCUSSION

Based on the finding of a high rate (44%) of myalgia in patients with SS[1], we wished to identify pathogenetically important factors that distinguish myalgic SS patients from those without muscle pain. To our knowledge, this is the first investigation where cytokine secretion patterns have been compared between SS patients with and those without muscle symptoms. The results were compared with those in healthy controls and patients with RA. FM has been reported to occur in 14% of RA patients[27] and in 0.5–5% of a reference population[28].

Since IL-8 promotes sympathetic pain[7], it has been hypothesized to be of pathogenetic importance in the development of FM[6]. In SS, IL-8 has been studied in salivary

glands[29] and airways[30], but not in serum. Both these studies found increased expression of IL-8 in subjects. However, we found that serum concentrations of IL-8 in patients with SS, regardless of pain, did not differ from healthy controls. Thus, our results do not support a role of IL-8 in the development of myalgia in SS. We also chose to analyze serum IL-18 because of its proinflammatory and antibody-inducing properties, and since expression of IL-18 has been described in glands of patients with SS[11]. The highest values were seen in SS patients with pain, but significantly elevated levels of serum IL-18 were also found in SS patients without pain, as well as in RA patients. This contrasts with the results of Kawashima, *et al*, who found no increased serum IL-18 in SS patients compared to healthy controls[31]. We believe the elevated serum levels of IL-18 in our SS and RA patients indicate a continuing inflammatory process in the target organs. Although high values of IL-18 were associated with myalgia in SS, further investigations are required to establish whether this is of pathogenetic importance for the pain.

In addition to unstimulated cytokine release from isolated PBMC, we analyzed the ability of PBMC to release cytokines after antigen-specific stimulation with recall antigens, i.e., PPD and TT. PPD-stimulated PBMC from patients with primary SS responded with less *in vitro* secretion of IL-10, TNF-α, and IFN-γ compared to healthy controls. Further, patients with SS and myalgia were hyporesponsive also for IL-1ß and IL-6 secretion, whereas nonmyalgic SS patients showed normal responses for both these cytokines.

The small numbers of SS patients with and without myalgia make reliable statistical comparisons between these groups difficult. However, there were trends for decreased PPD-stimulated release of IL-1ß ($p = 0.0587$) and TNF-α ($p = 0.0528$) in the myalgic SS patients compared to those without muscle pain; and after TT stimulation the reduced secretion of IL-6 by PBMC from myalgic SS patients did achieve statistical significance compared to controls ($p = 0.0298$) and also compared to the nonmyalgic SS patients ($p = 0.0406$). Our results are in accord with those of Halse, *et al*, who found that IFN-γ production by PBMC after PHA stimulation was similar in SS patients and healthy controls[32].

We believe that the low stimulated cytokine secretion by PBMC in myalgic SS patients, but not in the SS patients without myalgia, indicates genuine differences, since we found no bias concerning serum autoantibody tests, duration of sicca symptoms, occurrence of other manifestations of SS, or medication. This suggests that differences in cytokine response patterns may be involved in the pathogenesis of myalgia in SS. How is it, then, that a general unresponsiveness of circulating PBMC correlates to the occurrence of muscle symptoms in SS? In contrast to the low cytokine secretion by circulating PBMC in SS patients found in this study, Fox and coworkers reported at least 40-fold greater mRNA levels for IL-1α, IL-2, IL-6, IL-10, and IFN-γ in salivary glands from SS patients, compared to findings in glands from healthy controls and from blood lymphocytes from the same SS patients[13]. They suggested that T cell-derived IFN-γ promotes salivary gland epithelial cells to produce IL-1 and TNF-α, eventually perpetuating homing of lymphocytes to the salivary glands[13]. Brookes, *et al* found that CD4+ T cells from SS salivary glands produced 15 times more IL-10 than CD4+ T cells from peripheral blood[33]. Thus, data exist to support the concept of active recruitment of certain (autoreactive?) circulating lymphocytes to the salivary glands, with subsequently reduced circulating quantities of these pathogenetic cells, in SS. Similarly, it has been reported that circulating lymphocytes in SS have a subnormal frequency of t(14;18) translocations in PBMC[34], whereas hypermutated V genes were found in B cells located in the inflamed salivary glands[35], which also supports a compartmentalization of active and adaptive immune responses. We hypothesize that compartmentalization of active PBMC to inflammatory tissues (glandular and extraglandular) contributes to our findings of hyporesponsive PBMC in SS, especially in our patients with pain. Additional or alternative mechanisms may, of course, also be operative. For instance, the low level of cytokine secretion from PBMC in SS could be explained by a general state of systemic suppression/hyporesponsiveness, possibly due to disturbances in the HPA axis[36-38].

In this study, the generally lower cytokine production by circulating PBMC contrasts to the higher level of circulating IL-18 in these patients. A possible explanation for the elevated concentration of IL-18 could be production and release in the inflamed organs (e.g., salivary glands, muscle, etc.), whereas cytokine release from isolated PBMC does not necessarily indicate the situation in the affected organs, as discussed above. To determine the pathogenetic mechanisms in SS, we advocate further studies on lymphomononuclear cytokine production in inflamed organs combined with analyses of cytokine production from circulating cells of the same patients. We also believe that careful subclassification of SS patients with different clinical manifestations, as in our study, is imperative.

We found increased serum IL-18 and a pattern of depressed cytokine response in PBMC from patients with SS, especially in those with pain. These data indicate that cytokines may be involved in the pathogenesis of pain in SS. We hypothesize that cytokines originating from the inflamed tissues, apart from local actions, may act at a distance via spillover to the circulation. Further studies are necessary to elucidate whether cytokines from compartments such as salivary glands, from muscles, or even from the central nervous system are important in the pathogenesis of myalgia in SS.

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