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Serum levels of interleukin 1-β, tumor necrosis factor-α, soluble interleukin 2 receptor and soluble CD8 in seronegative spondylarthropathies

E. Toussirot [1], P. Lafforgue [1], J. Boucraut [2], P. Despieds [2], A. Schiano [1], D. Bernard [2], P.C. Acquaviva [1]

[1] Service de Rhumatologie, CHU La Timone, boulevard J. Moulin, F-13005 Marseille, France
[2] Laboratoire d'Immunologie, Faculté de Médecine Timone, F-13005 Marseille, France

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Summary. Seronegative spondylarthropathies are disorders with the same predisposing antigen, namely HLA B27, a class I molecule of the HLA system. The mechanisms of the different diseases are unknown, and there is no proof of immune system participation. We have investigated patients with spondylarthropathies in order to search for an immunological component in the pathophysiology of these disorders, by measuring the serum level of two inflammatory cytokines – IL1β and TNFα – by a radioimmunological assay and the serum level of two soluble T cell activation markers – soluble IL2 receptor and soluble CD8 – by an enzyme-linked immunosorbent assay. The choice of soluble CD8 can be explained by the strong link between HLA B27 and spondylarthropathies. Our series compared 24 patients to 24 healthy matched controls. A similar IL1β serum level was observed in both groups, while in the patients there was a nonsignificant increase in the TNFα level, a significant decrease in the soluble IL2 receptor level and a significant increase in the soluble CD8 serum level. The normal or moderately increased serum IL1β and TNFα levels in the disease group do not exclued a local role for these cytokines in the synovium or other inflammatory areas. However, we found a higher soluble CD8 serum level in the patient group. Most of these patients were in clinical exacerbation of their disease. As the serum level of soluble CD8 is well correlated with T CD8 lymphocyte activation, our data suggest that this lymphocyte subset is stimulated and consequently probably involved in seronegative spondylarthropathies.

Key words: Seronegative spondylarthropathies – Cytokines – Lymphocyte activation – CD8

Introduction

The seronegative spondylarthropathies (SS) are disorders with the same predisposing antigen, namely HLA B27, a molecule which belongs to class I of the HLA system [1]. These diseases consist of a classical inflammatory process without immunological participation. However, the link with HLA B27 suggests that T CD8 lymphocytes may play a part, because this lymphocyte subset specifically reacts with cells bearing class I molecules.

The cytokines consist of complex cellular networks and are principally produced by immune cells [2, 3]. In the course of the immune response, cytokine release is involved in the lymphocyte activation and inflammatory reaction. Interleukin-1β (IL1β) [4] and tumor necrosis factor-α (TNFα) [5, 6] are two of these inflammatory cytokines.

Furthermore, immune response induces T cell activation and release of soluble molecules by these activated lymphocytes. Interleukin-2 (IL2) induces T cell expansion [7] by its fixation on a specific receptor which is composed of two chains, p55 and p75 [8]. Of these, p75 is expressed on the unactivated T cell surface while p55, or CD25, is expressed after cellular activation only. After activation a soluble part of CD25 is released, corresponding to a T cell activation marker. This is the soluble IL-2 receptor (sIL2R) which is not specific to a subclass lymphocyte as it is released after T CD4, T CD8 or other T cell activation [9].

The CD8 molecule belongs to the immunoglobulin superfamily and is expressed on the cytotoxic or suppressor T CD8 cell surface. This glycoprotein is composed of two chains – α and β – and is expressed as a heterodimer α/β or as a homodimer α. Activated CD8 lymphocyte also releases a soluble part of the α-chain. This is the soluble CD8 (sCD8) molecule, which is consequently a T CD8 activation marker [10].

To demonstrate that the SS have an immunological component, we measured the serum levels of two inflammatory cytokines which can induce lymphocyte activation – IL1β and TNFα – and of two lymphocyte soluble activation markers – sIL2R and sCD8 – in patients with SS and healthy control subjects.

Correspondence to: P.C. Acquaviva

Table 1. Disease characteristics of 24 patients with seronegative spondylarthropathies

Patient no.	Age/sex	Diagnosis	Disease activity	Inflammatory syndrome	HLA B27
1	44/M	AS	+	+	+
2	78/F	Uveitis	−	+	+
3	24/F	AS	−	+	+
4	60/M	AS	+	+	+
5	31/M	AS	+	−	+
6	44/M	AS	+	−	+
7	46/F	AS	+	−	+
8	30/M	AS	−	−	+
9	20/M	AS	+	+	+
10	19/M	AS	+	−	+
11	41/M	Crohn's disease	+	−	−
12	46/M	AS	−	+	−
13	33/M	AS	+	−	−
14	46/M	AS	−	+	+
15	63/M	AS	−	−	+
16	40/M	AS	−	−	+
17	43/M	AS	+	+	+
18	41/M	AS	+	−	+
19	64/M	Psoriatic arthropathy	+	+	+
20	45/M	AS	+	−	−
21	24/F	AS	−	−	+
22	44/M	AS	+	−	+
23	37/F	AS	−	−	−
24	59/M	AS	−	−	−

AS, Ankylosing spondylitis
Disease activity: −, inactive disease; +, clinical exacerbation
Inflammatory syndrome: −, absent; +, present

Table 2. Serum levels of soluble CD8 (sCD8), soluble interleukin-2 receptor (sIL2R), tumor necrosis factor-α (TNFα) and interleukin-1β (IL1β) in the controls

Age	sCD8 (IU/ml)	sIL2R (pM/ml)	TNFα (pg/ml)	IL1β (pg/ml)
20–40 years	350	95	2.3	15
	350	102	5.2	<15
	660	75	3	<15
	440	62.5	1.5	58
	370	82.5	2.8	31
	270	120	3.5	22
	550	94	2.1	<15
	390	70	4.5	21
	140	50	5.1	38
	350	135	20	22
40–60 years	450	70	3.5	19
	760	152.5	2.5	15
	310	95	5.4	<15
>60 years	540	75	4.2	19
	330	112.5	6.1	<15
	370	155	4.2	25
	570	105	2.1	25
	880	95	1.5	27
	550	107.5	3.1	48
	380	112.5	4.2	31
	640	110	18	36
	660	105	17	31
	550	207.5	19	27
	720	145	19	58
Mean	482.5	105.5	6.6	
Standard deviation	173.1	34.2	6.2	
Normal value	336±197	75±45	6.3±5.0	≤15

Patients and methods

Patients

Seronegative spondylarthropathy patients. Twenty-four hospitalized patients (19 Caucasian men and 5 Caucasian women) aged between 19 and 78 years were enrolled. All were in agreement with the AMOR criteria [11]. This group was composed of: 21 patients with ankylosing spondylitis (AS), 16 HLA B27 positive and 5 negative; one patient suffering from Crohn's disease with articular manifestations, HLA B27 negative; one patient with a peripheral form of psoriasic arthropathy, HLA B27 positive; and one patient suffering from recurrent uveitis with articular manifestations, HLA B27 positive.

Disease activity was examined: 14 patients were in clinical exacerbation of their disease at the time of investigation, and 9 exhibited an inflammatory biological syndrome (Table 1). Clinical exacerbation of SS was defined as inflammatory spine or sacroiliac pain with morning stiffness for more than 2 h or inflammatory peripheral joint involvement. Biological inflammatory syndrome was defined by an erythrocyte sedimentation rate of more than 30 mm in the first hour. No patient in this study was feverish. All patients were treated with nonsteroidal anti-inflammatory drugs, nine with sulfasalazine and none with corticosteroids.

Control subjects. This group was composed of age- and sex-matched healthy blood donors (Marseille blood transfusion center). No HLA tissue determination was performed in this group. The probability that a control subject was HLA B27 positive was the same as the frequency of this antigen in the white Caucasoid population, i.e. 8%.

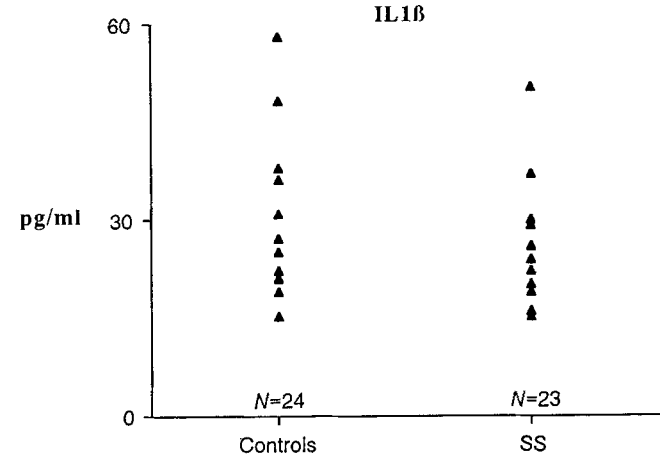
Methods

Serum samples were deep frozen (−20°C) and stored for 1–6 months. Cytokines and soluble activation markers were measured using commercially available assays.

IL1β was measured with a radioimmunological assay (RIA; IRMA Medgenix, Brussels, Belgium) using different monoclonal antibodies directed against distinct epitopes of IL1β. Briefly, the first monoclonal antibody was attached to the surface of the tube, capturing IL1β from the serum sample. The second monoclonal antibody, labeled with iodine-125, revealed the reaction. After washing, the remaining radioactivity bound to the tube reflected the IL1β level. A standard curve was prepared from IL1β standard. Results were given in pg/ml. The normal value by this assay was inferior or equal to 15 pg/ml.

TNFα was also measured with an RIA (IRMA Medgenix), with different monoclonal antibodies directed against distinct epitopes of TNFα. The assay protocol was the same as for the RIA of IL1β. Results were also given in pg/ml. The normal value by this assay was 6.3±5 pg/ml.

sIL2R was measured with a sandwich enzyme-linked immunosorbent assay (ELISA; Immunotech, Marseille, France) using different monoclonal antibodies directed against different epitopes. Well microtiter plates were coated with a first monoclonal antibody raised against the p55 chain. Sera were added, and after washing the second alkaline phosphatase-conjugated antibody revealed the reaction by its fixation on a second epitope of the molecule. A substrate solution was added to the wells and the absorbance was determined at 490 nm. A standard curve was prepared from sIL2R standard. Results were given in pM/ml. The normal value by this assay was 70±45 pM/ml.



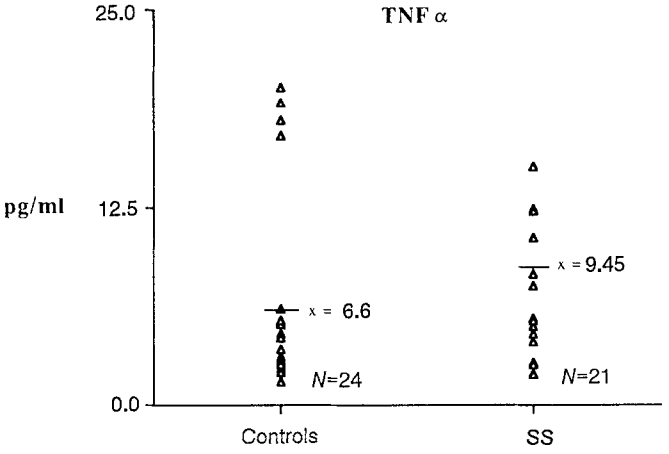
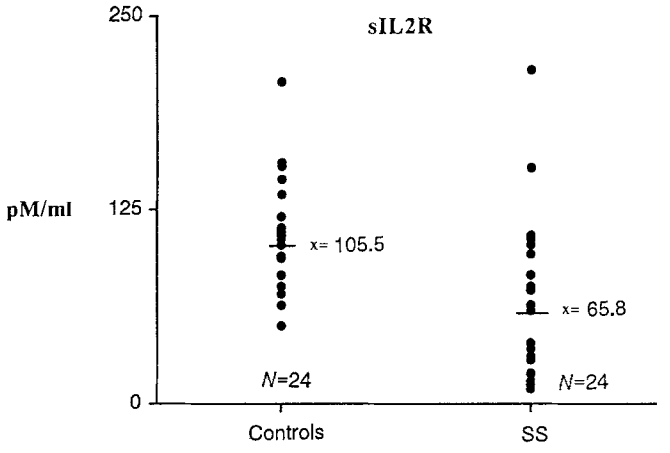


Fig. 1. IL1β and TNFα serum levels in patients with seronegative spondylarthropathies (*SS*) and in controls. *x*, mean



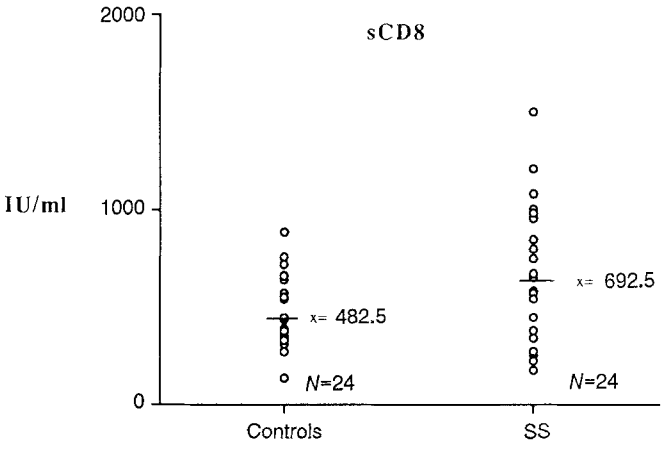


Fig. 2. sIL2R and sCD8 serum levels in patients with seronegative spondylarthropathies and in controls

sCD8 was also measured with an ELISA (T cell Science, Cambridge, UK) utilizing two noncompeting monoclonal antibodies which react to the α-chain of the CD8 protein. Well microtiter plates coated with the first monoclonal antibody reacted to the CD8 α-chain of the serum sample. A second horseradish peroxidase antibody recognized a different epitope of the CD8 molecule. A substrate was then added and the absorbance was determined at 490 nm. A standard curve was prepared from sCD8 standard. Results were given in IU/ml. The normal value by this assay was 336 ± 197 IU/ml.

Statistical analysis. Statistical significance was determined using Student's *t*-test. *P* values lower than 0.01 were considered significant.

Results

IL1β and TNFα (Table 2, 3). The IL1β serum level was equivalent in the two groups (Fig. 1), while a moderately increased TNFα level was observed in the patients. This latter difference was nonsignificant [patients: mean 9.4 pg/ml, standard deviation (SD) 4.6 pg/ml; controls: 6.6 pg/ml, SD 6.2 pg/ml; $P < 0.1$]. The mean IL1β value was not determined, as many results were inferior or equal to 15 pg/ml.

sIL2R (Tables 2, 3). We observed a significantly lower serum value in the patients than in the controls (patients: mean 65.8 pM/ml, SD 47.9 pM/ml; controls: mean 105.5 pM/ml, SD 34.2 pM/ml; $P < 0.001$) (Fig. 2). However, these mean values are within the normal range.

sCD8 (Tables 2, 3). In the patients, the serum level was higher than in the control group (patients: mean 692.5 IU/ml, SD 330.9 IU/ml; controls: mean 482.5 IU/ml, SD 173.1 IU/ml). This difference is significant ($P < 0.01$) (Fig. 2).

Serum sCD8 level was lower in patients with active disease than in patients with inactive disease (active: mean 667.1 IU/ml, SD 331 IU/ml; inactive: mean 728 IU/ml, SD 327.6 IU/ml). This different was not significant ($P < 0.6$) (Fig. 3, Table 4).

However, if we take 530 IU/ml as elevated sCD8 value (i.e. mean of normal population +2 SD given by protocol assay) we find among our patient group 17 subjects with markedly elevated sCD8. Among these 17 patients, 11 suffered from clinical exacerbation; 14 had an axial form of the disease, 3 a peripheral form; 11 were HLA B27 positive and 6 exhibited an inflammatory syndrome. Finally, there is a moderate but nonsignificant increase of sCD8 in the HLA B27 negative patients (HLA B27 negative: mean 875 IU/ml, SD 117.4 IU/ml positive: mean 631.6 IU/ml, SD 335.8 IU/ml; $P < 0.1$) (Table 4, Fig. 3).

Table 3. Serum levels of sCD8, sIL2R, TNFα and IL1β in patients with SS

Patient no.	sCD8 $n=336\pm197$ (IU/ml)	sIL2R $n=70\pm45$ (pM/ml)	TNFα $n=6.3\pm5$ (pg/ml)	IL1β $n\leq5$ (pg/ml)
1[a]	170	102	ND	30
2	250	60	ND	20
3	220	20	ND	50
4[a]	340	82.5	7.5	26
5[a]	650	15	7.5	ND
6[a]	270	12.5	12.4	15
7[a]	670	12.5	8.3	19
8	450	10	15	16
9[a]	1500	215	10.5	<15
10[a]	380	62.5	15	19
11[a,b]	840	82.5	15	15
12[b]	960	60	15	37
13[a,b]	670	75	15	29
14	1200	152	15	24
15	800	96	15	22
16	1080	35	4.5	<15
17[a]	580	30.5	2.7	<15
18[a]	950	39	5	<15
19[a]	570	105	4	<15
20[a,b]	1000	27.5	2.6	<15
21	540	30	5.5	<15
22[a]	750	72.5	5.4	<15
23[b]	980	75	5.4	<15
24[b]	800	108	12.3	<15
Mean	692.5	65.8	9.4	ND
Standard deviation	330.9	47.9	4.6	ND

N, Normal value; ND, not done
[a] Clinical exacerbation
[b] HLA B27 negative

Table 4. Serum levels of sCD8 in SS patients positive or negative for HLA B27 and with currently active or inactive disease

B27− ($n=6$)	B27+ ($n=18$)	Active disease ($n=14$)	Inactive disease ($n=10$)
840	170	170	250
960	250	340	220
670	220	650	450
1000	340	270	960
960	650	670	1200
800	270	1500	800
	670	380	1080
Mean 875	450	840	540
SD 117.4	1500	670	980
	380	580	800
	1200	950	
	800	570	Mean 728
	1080	1000	SD 327.6
	580	750	
	540		
	750	Mean 667.1	
	570	SD 331	
	950		
	Mean 631.6		
	SD 355.8		

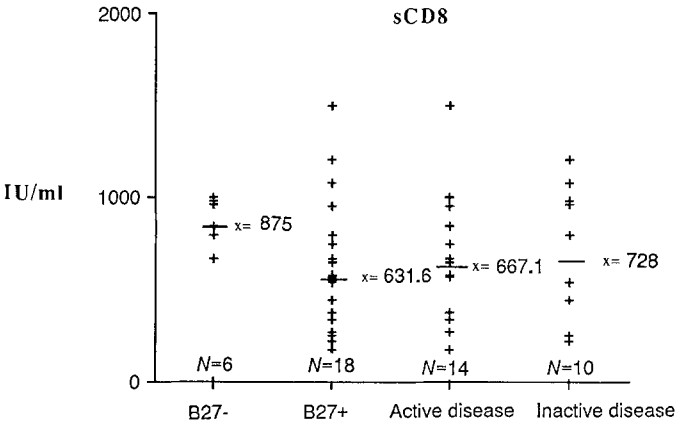


Fig. 3. sCD8 serum levels in patients with seronegative spondylarthropathies with or without HLA B27 and with active or inactive disease

Discussion

The group of SS includes AS and diseases such as psoriasic arthropathy, reactive arthritis and inflammatory bowel disease. These different disorders all have in common HLA B27, which is present in 90% of patients with AS. The pathophysiology of these classical inflammatory entities is unknown, and there is no proof of an immunological process [1]. Thus, there is no abnormality in the lymphocyte subclass rate and there are no circulating immune complexes or self-antibodies. Furthermore, histological analysis of the synovium shows moderate inflammation without specificity [12].

We searched for an immunological component of SS by measuring serum levels of inflammatory cytokines and soluble markers of lymphocyte activation. The serum levels of these different biological markers have been measured in connective tissue diseases such as rheumatoid arthritis (RA) in many studies [13, 14]. However, cytokines and soluble activation markers have not been previously evaluated in SS. Inflammatory cytokines are produced by immune cells and are responsible for many biological activities [13]. Thus, IL1β and TNFα are principally produced by macrophages [4, 5]. These two cytokines are able to induce T lymphocyte activation and are involved in the inflammatory process though liver protein production or prostaglandin synthesis or by vasodilatation [4, 5, 15]. In RA, a disease with immunological aspects, serum levels of IL1β and TNFα are higher than in control subjects [13]. It is therefore also interesting to evaluate these two cytokines in SS.

Furthermore, we cannot exclude the possibility that lymphocytes have a role in SS due to the normality of this cell subset rate in the blood compartment. To investigate a lymphocyte component in connective tissue disorders, the serum levels of soluble lymphocyte activation markers such as sIL2R can be easily measured [14]. This marker reflects T cell activation well and is elevated in many autoimmune diseases, such as systemic lupus erythematosus (SLE) [16], RA [17, 18] and systemic sclerosis [19, 20]. Elevated levels are also found in infectious diseases such as bacterial endocarditis and acquired immunodefi-

ciency syndrome (AIDS). sIL2R is a useful marker of disease activity and is clearly related to clinical exacerbation or clinical improvement of the autoimmune diseases. However, it is a nonspecific lymphocyte activation marker, as T CD4 or T CD8 activated cells can produce it.

To investigate specific activation of T CD8 lymphocytes in SS, the serum level of sCD8 can be measured. This molecule is a good marker of the T CD8 subset activation. sCD8 is released after the T CD8 activation by proteolytic cleavage of the CD8 glycoprotein or by alternative splicing of the primary CD8 transcript [10]. Elevated serum levels of sCD8 are observed in T CD8 leukemia [21], infectious mononucleosis (an infectious disease with strong CD8 activation) [22] and connective tissue diseases such as polymyositis, SLE [22] or in the cerebrospinal fluid of multiple sclerosis patients [23]. In RA, evaluation of serum sCD8 has given conflicting results [24–26].

We observed a moderate but nonsignificant elevation of TNFα in the spondylarthritis group, and serum levels of IL1β were equivalent in the control and patient groups. Despite these results, we cannot exclude a possible role of these two cytokines in SS. Indeed, cytokines are low-molecular-weight proteins which act at low concentrations. They are produced locally, acting in an autocrine rather than a paracrine or endocrine manner. There are also cytokine inhibitors or proteinases which can inhibit biological activity of cytokines [27, 28]. For these different reasons, cytokine serum levels are problematic. We cannot exclude a local role of these cytokines at the site which display inflammation in SS, such as the synovium or the entheses. In situ hybridization is a helpful method for direct investigation of the role of cytokines in tissue such as the synovium. Such a study has been performed in RA [29] but not in AS or other SS. Another explanation for our results could be the low number of patients in our study.

The results concerning the soluble activation markers were interesting. sIL2R was lower in the patients than in the control group. Our results are in agreement with those of a prior study by Wendling et al. [30]. These authors investigated the sIL2R serum level in a group of 36 patients with AS by means of an ELISA and found no difference between the patients and the controls. They found no difference in sIL2R level between those AS patients with evidence of inflammation and those with noninflammatory disease, and none between those with axial and those with peripheral forms of the disease. They concluded that there was no lymphocyte activation in AS.

We completed this study by evaluating the sCD8 serum level in SS. We observed significantly higher sCD8 in the patients. Most of these patients were HLA B27 positive and in clinical exacerbation of their disease. Thus, this result suggests specific participation of the T CD8 lymphocyte in the pathophysiology of SS. The lack of an associated sIL2R increase in the patient group may result from degradation of this molecule subsequent to local production. Another hypothesis is T CD8 suppressive cell activation, which may explain the lack of total T lymphocyte activation and thus the low sIL2R level.

We can hypothesize an immunological component in SS through a specific CD8 cell activation. This is in good agreement with the immunogenetics of the HLA B27-related disorders.

In conclusion, serum inflammatory cytokines such as IL1β and TNFα are not elevated in SS, unlike RA, and the synovial fluid levels of these two cytokines must be evaluated. Serum sIL2R does not seem to be a helpful biological marker in SS, unlike sCD8, which is elevated. The clinical or biological usefulness of this molecule must be clarified by further studies.

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